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Genetic linkage map construction and identification of QTLs associated with agronomic traits in bambara groundnut (*Vigna subterranea* (L.) Verdc.) using DArTseq-based SNP markers

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Abstract

Bambara groundnut [*Vigna subterranea* (L.) Verdc.] is an underutilised, protein-rich and self-pollinating legume that can withstand high temperature and drought stress and is mainly grown in semi-arid Africa. In order to dissect the complexity of drought resistance and to use genomic tools for yield enhancement of bambara groundnut in response to drought stress, yield-related and morphological traits under drought-stressed (DS) and well-watered (WW) conditions were evaluated in the F_3 and F_4 segregating generations derived from a cross between two genotypes selected from landraces S19-3 (originally from Namibia) and DodR (originally from Tanzania). Significant quantitative trait loci (QTLs) for *shoot dry weight* (SDW) were mapped on LG10 accounting for 15.5% of the phenotypic variation explanation (PVE) under well-watered conditions and a putative quantitative trait locus (QTL) for the same trait mapped on LG10 with reduced PVE (10.10%) under drought-stressed conditions in the F_3 segregating population. Significant QTLs associated with the *number of seeds per plant* (NS), *number of double-seeded pods per plant* (NDP), *seed weight per plant* (SW) and *pod weight per plant* (PW) were mapped on LG4 (nearest marker: 4181663 and 4175954) with overlapping confidence intervals and explained 21.9%, 21.8%, 23.5% and 19.9% of the PVE, respectively, under well-watered conditions in the F_4 population, which could be considered as the major QTL involved in the control of these traits. Seven consensus QTLs for yield-related and morphological traits were mapped on LG2, LG3, LG4, LG7A and LG10. The study provides fundamental knowledge of QTLs associated with yield-related and morphological traits under drought-stressed and well-watered conditions in bambara groundnut, which is also essential for yield improvement of bambara groundnut in response to drought stress.

1 | INTRODUCTION

Bambara groundnut is an underutilised and drought-resistant leguminous crop with a relatively high protein content (16%–25%). The crop is mainly grown by subsistence farmers and serves as a good source of edible protein in Africa (Atoyebi et al., 2017; Halimi et al., 2020; Massawe et al., 2016). Genetic maps with reliable molecular markers are useful tools to identify quantitative trait loci (QTLs) and potential candidate genes that regulate complex traits, accelerating the marker-assisted breeding process and potentially shortening the breeding cycle (Conson et al., 2018). Understanding the genetic basis of bambara groundnut and the identification of molecular markers for traits of interest are prerequisites for development of superior genotypes in molecular breeding programmes (Kullan et al., 2012). However, to date only a limited number of studies have reported mapping quantitative and qualitative loci to a location on the chromosomes of bambara groundnut (Ahmad et al., 2016; Chai et al., 2017; Ho et al., 2017).

The first genetic map reported in bambara groundnut consisted of 20 genetic linkage groups, which were identified using amplified fragment length polymorphism (AFLP) and simple sequence repeat (SSR) markers in the F_2 segregating population derived from a 'wide' cross between domesticated type (DipC) and wild type (VSSP11), and covered 516 cM (centimorgan) of bambara groundnut genome (Basu et al., 2007). The first intraspecific genetic linkage map consisting of 21 linkage groups and covering 608.3 cM of genetic distance was constructed using 209 diversity arrays technology (DArT) dominant and 29 co-dominant SSR markers in an F_3 segregating population derived from two domesticated landraces, Tiga Nicuru × DipC, in bambara groundnut (Ahmad et al., 2016). Two consensus QTLs were mapped for *internode length* and *growth habit* under controlled environment and field conditions (Ahmad et al., 2016).

The first expression marker-based genetic map using gene expression markers (GEMs), which were developed after cross-hybridisation of bambara groundnut to the Affymetrix Soybean Genome Gene Chip using 65 F_5 segregating population derived from Tiga Nicuru × DipC, was reported to consist of 13 linkage groups containing 218 GEMs and covered 982.7 cM of bambara groundnut genome (Chai et al., 2017). Chai et al. (2017) identified co-localised QTLs mapped on LG11 in the GEM map for agronomic traits including yield-related traits (i.e. pod number per plant, seed number per plant, pod weight per plant, seed weight per plant and harvest index) and morphological traits (i.e. internode length and peduncle length), under well-watered conditions in the F_5 segregating population of bambara groundnut, which may suggest that these traits are controlled by the same underlying pleiotropic gene. QTLs associated with pod number per plant (PW) and harvest index (HI) in the GEM map in bambara groundnut have also been reported to be affected by drought stress (Chai et al., 2017). This GEM map presented the possibility of translating information and resources from major and/or model plants to underutilised crops.

In addition, Ho et al. (2017) demonstrated using bambara groundnut maps to link to well-characterised closely related sequenced legumes, which included common bean (*Phaseolus vulgaris* L.), adzuki bean (*Vigna angularis*), mung bean (*Vigna radiata*) and soybean (*Glycine max*). Furthermore, the conserved syntenic locations of QTLs in the related species could be used to identify candidate genes underlying target traits in bambara groundnut (Ho et al., 2017). A combination of population-specific and pre-selected common informative markers was used to construct two individual intraspecific genetic maps in bambara groundnut from the two crosses: the genetic map of IITA686 × Ankpa4, which was derived from 263 F_2 segregating population, gave spaced markers on 11 linkage groups comprising of 223 DArTseq-based SNP markers and covered 1395.2 cM while a genetic map of Tiga Nicuru × DipC, derived from 71 F_3 segregating population, gave spaced markers across 11 linkage groups consisting of 293 DArTseq-based SNP markers and covered 1376.7 cM in bambara groundnut (Ho et al., 2017). A significant quantitative trait locus (QTL) for *internode length* mapped on LG2 (50.6 cM; flanking markers between 47.6 and 54.4 cM) with 33.4% of the PVE was observed in this cross and showed conserved syntenic blocks at *Pv03* (38.4–39.1 Mbp; common bean), *Va11* (12.5–17.4 Mbp; adzuki bean) and *Vr07* (39.4–43.5 Mbp; mung bean) (Ho et al., 2017). Genetic maps are essential tools for analysing genetic architecture of important traits and for identifying QTLs responsible for phenotypic variation in bambara groundnut (Chai et al., 2017). The first whole genome sequence of bambara groundnut, which was published by the African Orphan Crops Consortium (AOCC) (https://bioinformatics.psb.ugent.be/orcae/aocc/overv

iew/Vigsu), provided a better understanding of candidate genes involved in agronomic trait regulation (Chang et al., 2019). Moreover, this will accelerate the identification of candidate genes underlying QTL through application of molecular marker-assisted selection (MAS) in bambara groundnut breeding programmes.

One of the adverse effects of climate change is the expectation of more regular droughts in many parts of the world, leading to reduced yield and more frequent crop failure. This calls for targeted crop improvement to develop drought-resistant crops, especially capitalising on advances in omics technologies. Various researchers have used genetic mapping approaches to begin to dissect drought resistance traits and utilised MAS to incorporate drought resistance traits in breeding programmes. For example, Varshney et al. (2014) identified nine QTL clusters from two recombinant inbred line (RIL) mapping populations—ICCRIL03 (ICC 4958 × ICC 1882) and ICCRIL04 (ICC 283 × ICC 8261) under drought conditions in chickpea (*Cicer arietinum* L.). QTL Cluster 5 on CaLG04 showed high potential to enhance drought tolerance in chickpea and could be introgressed in elite varieties, as this region contained stable and consistent QTLs, explaining up to 58.20% of the phenotypic variation for morphological and yield-related traits (Varshney et al., 2014). Dramadri et al. (2019) also identified 18 significant QTLs under drought stress and non-stress conditions in a RIL mapping population of common bean. Significant QTLs for *seed yield per plant* co-located with *pod weight per plant* on Pv01 and on Pv02 under drought stress conditions (Dramadri et al., 2019). In the present study, we mapped QTLs for yield-related and morphological traits under drought-stressed (DS) and well-watered (WW) conditions in the F_3 and F_4 segregating populations of a bambara groundnut cross. This study provided critical insights into how genetic features control these traits in bambara groundnut under drought-stressed and well-watered conditions.

2 | MATERIALS AND METHODS

2.1 | Mapping population and DNA isolation

A total of 86 lines of the F_2 segregating population derived from a controlled cross between a drought tolerant genotype (S19-3, collected from Namibia) and presumed drought susceptible genotype (DodR, collected from Tanzania), and two parental lines (S19-3 and DodR) were used in the present study. Genomic DNA was extracted from freeze-dried leaf samples using the DNeasy Plant Mini Kit (Qiagen GmbH, Hilden, Germany) following

manufacturer's instructions. The quantity and quality of DNA was estimated visually on a 1% agarose gel with ethidium bromide staining and restriction enzyme digestion using a restriction enzyme, *Hind*III (NEB, USA). DNA concentration was then adjusted to 50 ng/µl and sent to Diversity Arrays Technology Pty Ltd (Canberra, Australia) for DArTseq genotyping prior to development of genetic map.

2.2 | Plant material and experimental design

A total of 114 lines of the F_3 segregating population derived from S19-3 × DodR were evaluated in a rainout shelter at the University of Nottingham Malaysia (2°56′46.74″N; 101°52′24.35″E) with mean air temperature of 29°C/24°C day/night and relative humidity of 75%/95% day/night from November 2018 to February 2019. This was followed by a subsequent F_4 segregating population in the rainout shelter with mean air temperature of 36°C/25°C day/night and relative humidity of 58%/91% day/night from April to July 2019, respectively.

Both experiments were carried out in a completely randomised design (CRD) with three replicates and two treatments, drought-stressed and well-watered treatments. Each of the replicates was represented by one plant from each of the individual lines. Irrigation for the well-watered treatment was continued throughout the experiment while the drought-stressed treatment was imposed after 100% flowering was observed at 47 days after sowing (DAS) and no further irrigation was applied until early pod-filling stage at 74 DAS, at which irrigation of plants for the drought-stressed treatment was resumed. A trickle tape irrigation system was set to irrigate the plants at 07:00 and 19:00 h for 10 min with a flow rate of 2 L/h, with each tube 6 m in length. A distance of 40 cm ×30 cm was kept between the plants. NPK (nitrogen, phosphorus and potassium) fertiliser was applied at a rate of 20:40:60 kg/ha (133 kg/ha NPK (15:15:15), 44 kg/ha TSP (triple superphosphate) and 67 kg/ha MOP (muriate of potash) at sowing and after emergence. All other agronomic procedures, such as weeding and spraying of pesticides, were carried out when necessary.

2.3 | Trait measurements

Yield-related traits, that is, shoot dry weight (SDW), number of pods per plant (NP), number of seeds per plant (NS), number of double-seeded pods per plant (NDP), pod weight per plant (PW), seed weight per plant (SW), 100-seed weight (100SW), harvest index (HI) and shelling

percentage (SP), as well as morphological traits, that is, days to flowering (DTF), plant height (PH), petiole length (PL), internode length (IL), number of leaves per plant (NL) and petiole internode ratio (P/I), were evaluated based on the bambara groundnut descriptor list (IPGRI, 2000) with minor modification (Table 1).

2.4 | Soil moisture content

Two evenly spaced PR2 profile tubes (Delta-T Devices Ltd., Cambridge, UK) were inserted into the centre of each plot with a distance of 3 metres between two profile tubes in each plot. There were 12 access tubes in total. Three PR2 readings %Vol (volumetric water content as a percentage) were taken twice a week between 09:00 and 11:00 h at soil depth of 100, 200, 300, 400, 600 and 1000 mm from seeds sowing until maturity in 2018 and 2019 planting seasons.

2.5 | Genetic linkage map construction

The presence or absence (0/1) scoring of two alleles in the co-dominant DArTseq-based SNP markers for each individual line in the F_2 segregating population was converted into genotype codes (a, b, h), by comparison with parental lines. The markers were scored as 1:1 and/or 0:0 in parental lines. Markers, which did not fit expected segregation patterns when compared to the parental lines, were filtered out. Table S1 presented the linkage map group,

position, trimmed sequence and SNP of DArTseq-based SNP markers.

A chi-square goodness-of-fit test in JoinMap v4.1 (Ooijen & Kyazma, 2009) was used to evaluate any discrepancy from the expected segregation ratios (1:2:1 for the F_2 segregating population) at a significance level of $p < 0.05$. A total of 843 polymorphic DArTseq-based SNP markers were pre-selected from 6396 markers and a total of 48 from 843 DArTseq-based SNP markers showing distorted segregation ($p < 0.05$) from expected Mendelian ratios were excluded. A total of 795 DArTseq-based SNP markers were selected and 86 F_2 individual lines were used to construct the genetic linkage map using JoinMap v4.1 (Ooijen & Kyazma, 2009). Markers were sorted to linkage groups with the *Create Groups Using the Grouping Tree* function of JoinMap 4 (Van Ooijen, 2006). The grouping of markers was performed between LOD (logarithm of the odds) 2.0 and 10.0 with a step of 0.5 and the Independence LOD option was adopted. The Haldane mapping function with default calculation settings (recombination frequency <0.4 and LOD >1.0, ripple value = 1, jump in goodness-of-fit threshold = 5) was selected to calculate genetic distances based on recombination frequencies. The markers that showed double cross-over events between two neighbouring markers within a map distance of 1 to 3 cM were manually removed. The nearest neighbour fit, the nearest neighbour stress (Fit & Stress) and plausible positions produced by the maximum likelihood mapping (MLM) algorithm were used as indicators to confirm whether a locus fitted well between its neighbouring loci.

TABLE 1 Evaluation of traits in the F_3 and F_4 segregating populations derived from S19-3 × DodR

Traits	Trait abbreviation	Evaluation method	Measurement time
Shoot dry weight (g)	SDW	Above-ground plant parts after drying in oven at 70ºC for 3–5 days	Harvest stage
Number of pods per plant	NP	Pod number per plant	Harvest stage
Number of seeds per plant	NS	Seed number per plant	Harvest stage
Pod weight per plant (g)	PW	Pod weight per plant after drying at 37°C for 14 days	Harvest stage
Seed weight per plant (g)	SW	Seed weight per plant after drying at 37°C for 14 days and deshelled	Harvest stage
Petiole internode ratio	P/I	Petiole length/internode length	Harvest stage
100-seed weight (g)	100SW	Seed weight/number of seeds per plant * 100	Harvest stage
Harvest index	HI	Seed weight/(pod weight + shoot dry weight)	Harvest stage
Shelling percentage (%)	SP	Seed weight/pod weight * 100	Harvest stage
Days to flowering	DTF	From sowing date to the first open flower	Flowering stage
Plant height (cm)	PH	From the ground level to the tip of the highest point, including the terminal leaflet	Harvest stage
Petiole length (cm)	PL	The average length of three petioles	Harvest stage
Internode length (cm)	IL	The average length of three internodes	Harvest stage
Number of leaves per plant	NL	One leaf including three leaflets	Harvest stage

The optimal positions of each marker in the final genetic map were used for QTL analysis.

2.6 | QTL analysis

Genetic linkage map and phenotypic data from drought-stressed and well-watered conditions in the F_3 and F_4 segregating populations were subjected to QTL analysis using MapQTL 6.0 software (Ooijen & Kyazma, 2009). The significant threshold of the Genome-Wide (GM) LOD threshold was obtained from the permutation test using 10,000 repetitions at $p < 0.05$ (5%). Interval mapping (IM) was carried out following the permutation test and the LOD values from IM were compared with the GW LOD threshold at $p < 0.05$ from the permutation test. Significant QTLs were detected if the LOD score was equivalent to or higher than the GM LOD threshold. Putative QTLs were detected if the LOD score was lower than the GM LOD threshold by up to a 1-LOD interval.

The non-parametric Kruskal–Wallis (KW) test was performed to determine the significant level of all marker loci associated with the non-normally distributed quantitative traits in the F_3 and F_4 segregating populations. KW tests ranked all individuals according to their quantitative trait value and tested them for an association with their marker allele genotype (Van Ooijen & Maliepaard, 1996). MapChart 2.3.2 (Voorrips, 2002) was used to depict the linkage groups and QTLs.

2.7 | Data collection and analysis

Normality of trait data was examined using the Shapiro–Wilk normality test and data transformation was performed for non-normally distributed trait data. Two-way analysis of variance (ANOVA) was carried out with 95% confidence intervals (CIs) of the mean (CI = population mean ± 1.96×standard deviation/√sample size), while Pearson's correlation coefficient analysis was conducted to analyse the relationship between yield-related and morphological traits in the F_3 and F_4 segregating populations using the Genstat Statistical package (18th edition, VSN International, UK).

3 | RESULTS

3.1 | Soil moisture content

There was no significant difference for soil moisture content changes at each of soil depth within the treatments between two planting seasons, 2018 and 2019 ($p > 0.01$).

The average of total reduction of soil moisture content in both 2018 and 2019 under drought-stressed treatment was 42.7% from 47 DAS to 74 DAS. On average in 2018 and 2019, soil moisture content declined by 0.44% per day at depth 200 mm and 0.36% per day at depth 300 mm over 28 days of drought (Figure 1). Significant reduction ($p < 0.01$) in soil moisture by 7.9%, 12.4% and 10.0% was observed under drought-stressed compared to well-watered conditions at depth 100 mm, 200 mm and 300 mm, respectively. However, there was no significant difference ($p > 0.01$) for soil moisture content at depth 400 mm, 600 mm and 1000 mm between drought-stressed and well-watered treatment.

3.2 | Variation of yield-related and morphological traits in the F_3 and F_4 segregating populations

Parental lines showed significant differences for 100SW and PH between drought-stressed and well-watered conditions ($p < 0.05$) in both 2018 ($p < 0.05$) and 2019 ($p < 0.05$) planting seasons. DodR had significantly higher ($p < 0.05$) 100SW and PH in the 2018 planting season, and significantly higher ($p < 0.05$) NL, PL and PH in the 2019 planting season compared to S19-3 under drought-stressed condition.

In the F_3 segregating population, the average results showed a significant reduction ($p < 0.05$) of 14.8% in NS, 10.5% in SW, 16.1% in PW, 5.4% in HI and 9.8% in PH under drought-stressed conditions compared to well-watered conditions (Table 2). All yield-related and morphological traits showed significant differences among individual lines ($p < 0.05$), except NDP, 100SW, SP and PH. The interaction between individual lines and treatment was significant ($p < 0.05$) for NDP, SW, PW, SP, DTF, NL and PL.

In the F_4 segregating population, the average results showed a significant reduction ($p < 0.05$) of 41.5% in SDW, 41.2% in NS, 45.8% in NP, 47.9% in SW, 47.6% in PW, 12.5% in HI, 40.5% in NL and 4.9% in PL under drought-stressed conditions compared to well-watered conditions (Table 3). All yield-related and morphological traits showed significant differences ($p < 0.05$) among individual lines, except for NDP. The interaction between treatments and F_4 individual lines was significant ($p < 0.05$) for all traits, except NP, 100SW and DTF.

Positive correlations between yield-related traits, that is, SDW, NS, NP, SW and PW, and morphological traits, that is, NL, PL, IL and PH were observed under drought-stressed and well-watered conditions (Tables S2 and S3). Yield-related traits, that is, NS, NP, SW and PW showed strong positive linear relationships under both water regimes, while the overall correlations under well-watered
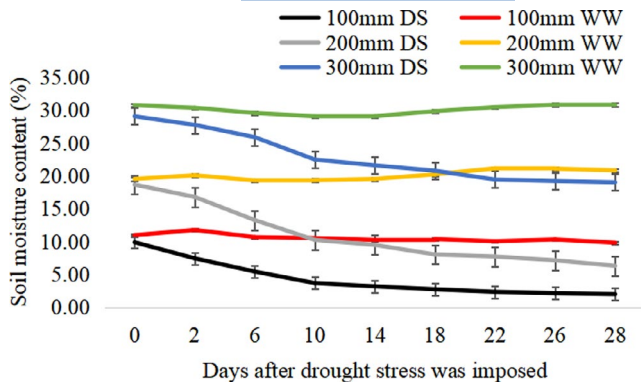
FIGURE 1 Soil moisture content measurements at depth 100 mm, 200 mm and 300 mm based on PR2 reading (% vol) under drought-stressed (DS) and well-watered (WW) conditions. Data represent mean values of average soil moisture content during plant growth season in 2018 and 2019; $n = 12$. Data represent mean values ± standard error

conditions (F_3: $r_{WW} = 0.88$–0.91, $p < 0.01$; F_4: $r_{WW} = 0.90$–0.97, $p < 0.01$) were higher than those under drought-stressed conditions (F_3: $r_{DS} = 0.73$–0.86, $p < 0.01$; F_4: $r_{DS} = 0.73$–0.82, $p < 0.01$) (Tables S2 and S3). HI positively correlated with NS ($r = 0.57$, $p < 0.01$), SW ($r = 0.82$, $p < 0.01$) and PW ($r = 0.80$, $p < 0.01$), and negatively correlated with NDP ($r = -0.64$, $p < 0.01$) under drought-stressed conditions in the F_4 generation (Table S3). A moderate positive correlation was observed among morphological traits, that is, NL, PL, IL and PH under both water regimes in the F_3 generation ($r_{DS} = 0.32$–0.47, $p < .01$; $r_{WW} = 0.40$–0.60, $p < 0.01$) and PL, IL and PH under both water regimes in the F_4 generation ($r_{DS} = 0.63$–0.78, $p < 0.05$ and/or $p < 0.01$; $r_{WW} = 0.70$–0.74, $p < 0.01$) (Tables S2 and S3).

3.3 | Linkage map and marker distribution

At LOD >3.5 of grouping independence in the regression mapping (RM) approach, 795 of 843 polymorphic markers were assigned into 11 linkage groups. The final genetic linkage map was constructed by using 234 DArTseq-based SNP markers after pre-selection and thinning of markers, covering 1040.92 cM of the genome with an average marker density of 5.23 cM (Table S4). Among the linkage groups, LG3 with 27 DArTseq-based SNP markers was the longest group covering 171.67 cM followed by LG2 with a length of 152.07 cM and LG5 with a length of 119.70 cM (Table S4). LG1B with four DArTseq-based SNP markers was the shortest group covering 4.90 cM, followed by LG6B with a length of 8.10 cM. LG7A has the longest average distance of 8.38 cM and

the second-longest distance of 35.45 cM between two adjacent markers (Table S4).

3.4 | Detection of QTLs associated with yield-related and morphological traits under drought-stressed and well-watered conditions in the F_3 and F_4 segregating populations

Significant and putative QTLs for yield-related and morphological traits were detected under both water regimes in the F_3 and F_4 segregating populations (Figure 2). Most QTLs were distributed in LG2, LG3, LG4, LG7A and LG10. Significant QTL for NS, NP and putative QTL for PW under well-watered conditions and putative QTL for IL under drought-stressed conditions in the F_3 segregating population were co-located on LG2 (85.95 cM, nearest marker: 4181165 and 27636104) with overlapping confidence intervals (Table 4). Significant QTLs for NS, NDP, SW, PW and putative QTL for NP and HI under well-watered conditions in the F_4 segregating population were co-located on LG4 (3.29 cM, nearest marker: 4181663 and 4175954) with overlapping confidence intervals (Table 4). In addition to the co-located QTL on LG4, significant QTL for NDP and PW under well-watered conditions in the F_4 segregating population were also mapped on LG6A and LG5, respectively (Table 4). Significant QTL for NP under drought-stressed conditions in the F_4 segregating population was observed to have mapped on LG11 (38.03 cM, nearest marker: 2764162 and 4182072), explaining 16.0% of the phenotypic variation (Table 4). However, putative QTL for NP was detected on LG4 (3.29 cM, nearest marker: 4181663) under well-watered conditions in the F_4 generation, explaining 12.8% of the phenotypic variation. Seven QTLs were found to have overlapping confidence intervals for yield-related and morphological traits, which included 4181165 and 27636104 (85.95 cM) on LG2 (NS, NP and PW under well-watered conditions and IL under drought-stressed conditions in the F_3 generation), 4182352 (100.03 cM) on LG2 (P/I under drought-stressed conditions in the F_3 and F_4 generations), 4183509 (87.10 cM) on LG3 (SDW and PH under drought-stressed conditions in the F_4 generation), 4175954 and 4181663 (3.29 cM) on LG4 (NS, NP, NDP, SW, PW and HI under well-watered conditions in the F_4 generation), 4175814 (35.38 cM) on LG7A (NS, SW and PW under drought-stressed conditions in the F_3 generation), 4178651 (32.66 cM) on LG10 (SDW and NL under drought-stressed conditions in the F_3 generation) and 4181438–1 (43.76 cM) on LG10 (SDW under well-watered conditions and PH

TABLE 2 Comparison of yield-related and morphological traits under drought-stressed (DS) and well-watered (WW) conditions in the F₃ segregating population derived from S19-3 × DodR

Traits	Treatment	Shapiro–Wilk Normality	Median/Mean	Range	95% confidence interval lower	95% confidence interval Higher	Interquartile range/standard deviation	F-probability Treatment	F-probability Genotypes	F-probability G*E	S19-3 Min	S19-3 Max	DodR Min	DodR Max
SDW (g)	DS	**	11.31	24.51	10.86	12.18	7.91	0.79	**	0.09	5.02	20.98	5.04	14.08
	WW	**	11.16	34.32	11.26	12.82	7.30				4.32	30.13	6.30	13.96
NS	DS	**	23.00	68.00	23.44	26.77	18.75	**	**	0.15	9.00	42.00	9.00	23.00
	WW	**	27.00	114.00	27.81	32.92	23.00				11.00	72.00	11.00	18.00
NP	DS	**	31.00	97.00	30.37	34.49	22.00	0.17	**	0.15	17.00	46.00	17.00	28.00
	WW	**	31.00	112.00	32.39	38.16	27.00				11.00	95.00	13.00	33.00
NDP	DS	**	0.00	10.00	0.08	0.28	0.00	0.55	0.35	0.07	0.00	0.00	0.00	2.00
	WW	**	0.00	4.00	0.10	0.26	0.00				0.00	3.00	0.00	0.00
SW (g)	DS	**	7.15	27.43	7.16	8.48	6.90	**	**	*	2.25	11.27	1.48	12.00
	WW	**	7.99	32.35	8.45	10.18	9.47				2.10	18.36	2.80	5.35
PW (g)	DS	**	8.53	34.20	8.76	10.38	8.48	**	**	*	2.98	14.11	1.97	13.33
	WW	**	10.17	40.56	10.76	12.93	11.73				2.60	26.20	3.51	7.00
100SW (g)	DS	ns	29.76	51.93	28.41	31.11	10.84	0.77	0.20	0.28	16.98	28.30	16.44	52.17
	WW	ns	30.02	57.21	28.83	31.22	9.35				10.39	25.50	15.56	43.82
HI	DS	**	0.35	0.52	0.33	0.36	0.13	**	**	0.19	0.20	0.48	0.21	0.44
	WW	**	0.37	0.66	0.35	0.37	0.14				0.16	0.47	0.18	0.33
SP	DS	**	0.82	0.61	0.81	0.82	0.06	**	0.71	0.08	0.71	0.83	0.75	0.90
	WW	**	0.79	0.63	0.77	0.79	0.05				0.64	0.81	0.69	0.84
DTF	DS	**	33.00	12.00	32.89	33.58	3.00	0.29	*	**	31.00	40.00	29.00	37.00
	WW	**	31.00	7.00	31.01	31.55	3.00				30.00	34.00	28.00	33.00
NL	DS	ns	51.71	86.00	49.60	53.81	16.91	**	**	**	36.00	72.00	4.50	85.00
	WW	**	44.50	86.00	45.62	49.97	22.50				31.00	59.00	32.00	59.00
PL (cm)	DS	**	16.17	19.57	15.98	16.82	3.79	**	**	**	13.50	19.50	14.40	21.57
	WW	**	15.41	14.83	15.45	16.22	3.90				13.33	20.00	13.93	19.03
IL (cm)	DS	ns	2.23	4.10	2.21	2.40	0.75	0.08	**	0.51	1.70	2.93	1.90	4.07
	WW	ns	2.24	4.17	2.16	2.34	0.72				1.03	2.30	2.47	3.87
P/I	DS	**	7.16	34.36	7.49	8.44	2.58	0.60	**	0.71	6.00	9.26	4.14	9.65
	WW	**	7.09	21.33	7.31	7.99	2.87				6.78	15.81	4.92	6.82
PH (cm)	DS	**	23.00	34.50	23.34	24.78	7.43	**	0.06	0.23	20.10	29.50	23.00	40.50
	WW	**	25.50	28.00	25.27	26.52	5.90				22.00	26.70	21.00	31.50

Note: Yield-related traits: SDW, shoot dry weight; NS, number of seeds per plant; NP, number of pods per plant; NDP, number of double-seeded pods per plant; SW, seed weight per plant; PW, pod weight per plant; 100SW, 100-seed weight; HI, harvest index; SP, shelling percentage; morphological traits: DTF, days to flowering; NL, number of leaves per plant; PL, petiole length; IL, internode length; P/I, petiole internode ratio; PH, plant height; G*E, interaction between treatment and genotypes; *Significant at (p = 0.05), **Significant at (p = 0.01), ns, not significant. Median (interquartile range) and mean (standard deviation) used for description of non-normally (Shapiro–Wilk normality = significant at p = 0.05 and p = 0.01) and normally distributed data (Shapiro–Wilk normality = ns), respectively.

TABLE 3 Comparison of yield-related and morphological traits under drought-stressed (DS) and well-watered (WW) conditions in the F_4 segregating population derived from S19-3 × DodR

Traits	Treatment	Shapiro–Wilk Normality	Median/Mean	Range	95% confidence interval lower	95% confidence interval higher	Interquartile range/standard deviation	F-probability Treatment	F-probability Genotypes	F-probability G*E	S19-3 Min	S19-3 Max	DodR Min	DodR Max
SDW (g)	DS	**	8.41	31.47	8.63	10.07	5.35	**	**	**	5.25	15.01	5.97	11.71
	WW	**	14.38	44.30	14.65	17.96	11.16				4.68	13.39	12.49	38.39
NS	DS	**	10.00	74.00	10.72	14.34	12.25	**	**	0.05	6.00	30.00	1.00	16.00
	WW	**	17.00	97.00	17.88	23.04	13.75				1.00	30.00	12.00	28.00
NP	DS	**	13.00	70.00	14.66	19.02	16.00	**	**	0.19	9.00	56.00	1.00	16.00
	WW	**	24.00	125.00	24.12	30.39	19.50				3.00	43.00	14.00	41.00
NDP	DS	**	0.00	11.00	0.01	0.27	0.00	0.76	0.20	*	0.00	0.00	0.00	0.00
	WW	**	0.00	2.00	0.10	0.28	0.00				0.00	1.00	0.00	1.00
SW (g)	DS	**	1.55	20.07	2.06	2.88	2.73	**	**	*	1.18	5.37	0.03	3.36
	WW	**	2.98	28.51	3.52	4.88	3.56				0.07	5.70	2.89	7.55
PW (g)	DS	**	2.52	27.08	3.06	4.19	3.74	**	**	*	1.68	11.42	0.16	4.40
	WW	**	4.81	42.08	5.41	7.31	4.88				0.15	10.43	3.52	13.07
100SW (g)	DS	ns	19.08	42.17	17.95	20.22	7.47	0.34	**	*	11.08	19.89	3.00	21.00
	WW	ns	19.62	22.54	18.75	20.48	4.82				6.38	19.00	23.57	26.96
HI	DS	*	0.14	0.47	0.15	0.18	0.17	**	**	**	0.13	0.30	0.01	0.21
	WW	*	0.16	0.72	0.17	0.21	0.11				0.01	0.22	0.08	0.23
SP	DS	*	0.70	0.79	0.63	0.68	0.20	0.21	**	**	0.44	0.70	0.19	0.76
	WW	ns	0.66	0.51	0.64	0.68	0.12				0.38	0.69	0.36	0.82
DTF	DS	**	33.00	18.00	33.70	34.67	4.00	0.57	**	0.96	33.00	41.00	31.00	40.00
	WW	**	33.00	18.00	33.80	34.85	3.00				32.00	42.00	32.00	40.00
NL	DS	**	44.00	125.00	45.48	51.22	22.25	**	**	**	24.00	54.00	24.00	69.00
	WW	**	74.00	386.00	75.21	93.75	46.00				29.00	63.00	61.00	220.00
PL (cm)	DS	**	13.17	18.40	12.79	13.42	2.51	*	**	**	11.17	13.67	12.33	15.03
	WW	ns	13.85	10.94	13.45	14.25	2.23				11.67	14.53	12.47	17.37
IL (cm)	DS	**	1.72	4.50	1.72	1.91	0.78	0.12	**	**	1.07	1.67	1.50	2.83
	WW	**	1.87	4.10	1.87	2.10	0.96				1.00	1.77	1.70	2.77
P/I	DS	**	7.59	14.50	7.52	8.27	2.96	0.46	**	**	8.04	10.75	4.53	10.02
	WW	**	7.07	15.15	7.14	8.04	3.21				7.29	13.47	5.43	8.57
PH (cm)	DS	ns	22.23	16.00	21.70	22.76	3.47	0.10	**	**	17.00	20.70	20.00	26.00
	WW	**	23.00	28.60	22.71	24.38	5.88				19.00	24.50	21.00	32.00

Note: Yield-related traits: SDW, shoot dry weight; HI, harvest index; NS, number of seeds per plant; NP, number of pods per plant; NDP, number of double-seeded pods per plant; SW, seed weight per plant; PW, pod weight per plant; 100SW, 100-seed weight; HI, harvest index; SP, shelling percentage; morphological traits: DTF, days to flowering; NL, number of leaves per plant; PL, petiole length; IL, internode length; P/I, petiole internode ratio; PH, plant height; G*E, interaction between treatment and genotypes; *Significant at (p = 0.05), **Significant at (p = 0.01), ns, not significant. Median (interquartile range) and mean (standard deviation) used for description of non-normally (Shapiro–Wilk normality = significant at p = 0.05 and p = 0.01) and normally distributed data (Shapiro–Wilk normality = ns), respectively.
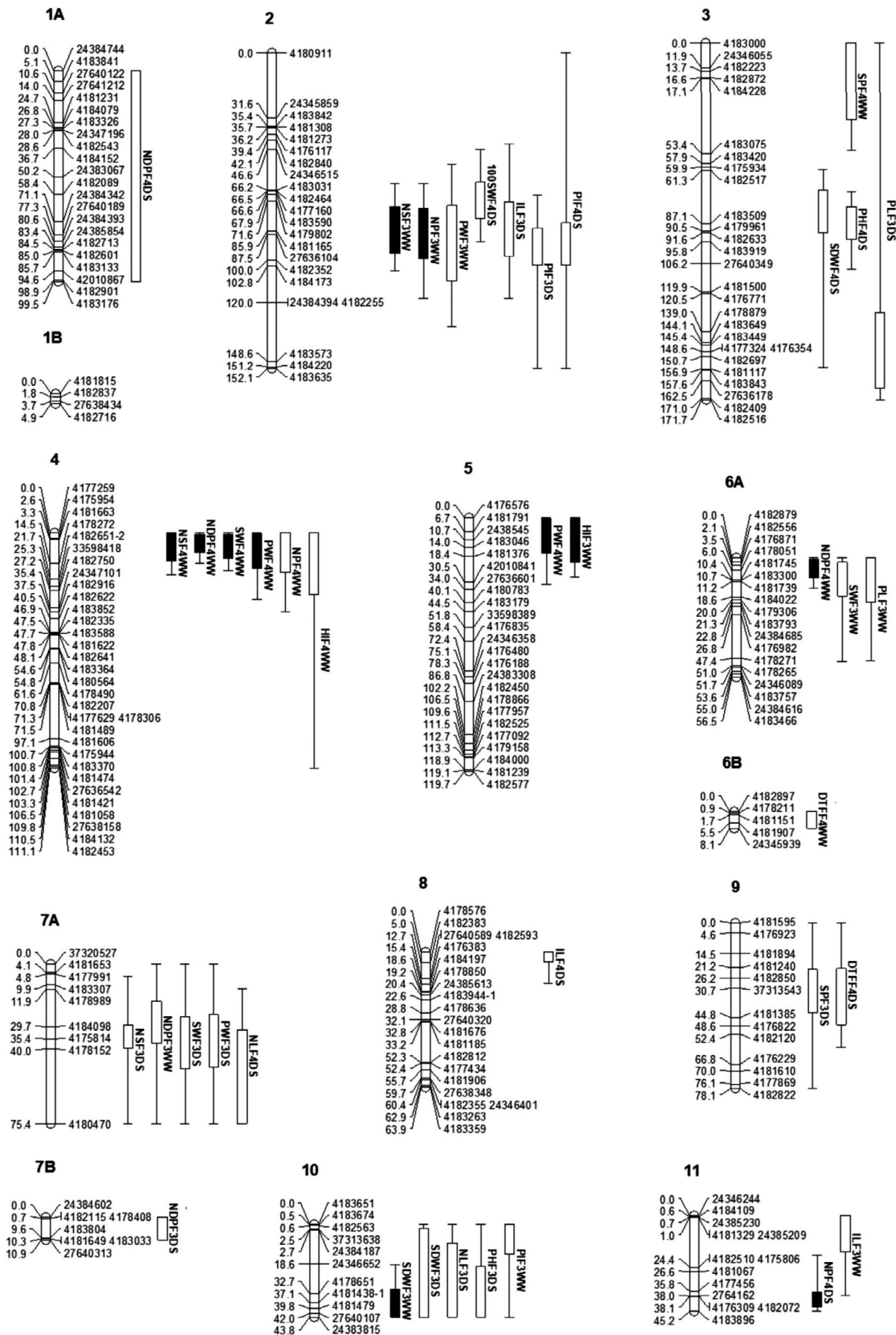
FIGURE 2 Legend on next page

FIGURE 2 Map position of the quantitative trait loci (QTL) under drought-stressed (DS) and well-watered (WW) conditions in the F_3 and F_4 segregating population developed from S19-3 × DodR. Rectangular bars represent the 1- and 2-LOD QTL interval (inner and outer interval). Solid rectangular bars represent significant QTLs, while blank bars represent putative QTLs. LG1, LG6 and LG7 were divided into subgroups '1A' and '1B', respectively, based on the association observed in the maximum likelihood mapping (MLM) due to insufficient linkage to complete the map using regression mapping (RM). SDW, shoot dry weight; NP, number of pods per plant; NS, number of seeds per plant; PW, pod weight per plant; SW, seed weight per plant; 100SW, 100-seed weight; HI, harvest index; SP, shelling percentage; DTF, days to flowering; PH, plant height; PL, petiole length; IL, internode length; NL, number of leaves per plant; PI, petiole internode ratio

under drought-stressed conditions in the F_3 generation) (Table 4).

4 | DISCUSSION

Several molecular and genetic studies (Chai et al., 2017; Redjeki et al., 2013) as well as physiological studies (Basu et al., 2007; Chai et al., 2016; Jørgensen et al., 2010; Muhammad et al., 2016; Vurayai et al., 2011) have been focused on understanding the complexity of drought resistance in bambara groundnut. However, the inheritance and genetic architecture of quantitative traits for drought resistance in bambara groundnut are still not well understood. For the first time, we identified and compared the QTLs under drought-stressed and well-watered conditions in the F_3 and F_4 segregating populations derived from S19-3 × DodR. The present study has also furthered our understanding of the variation of traits in segregating populations of bambara groundnut and the correlation between yield-related and morphological traits, and the impact of drought stress on these traits.

In the present study, significant QTLs were mapped to approximately the same position on LG4 (3.29 cM) for NS, NDP, SW and PW with PVE ranged from 19.9 to 23.5% and putative QTLs for NP and HI were mapped to the same location on LG4 (3.29 cM) with PVE ranged from 11.3% to 12.8% under well-watered conditions in the F_4 segregating population. Such pleiotropism has also been observed in other species, such as soybean, in which QTLs associated with DTF, days to maturity, PH, number of nodes on main stem, *lodging* and *plot yield* mapped to the same chromosomal regions (Zhang et al., 2004). Chai et al. (2017) reported that QTLs controlling NP, NS, IL and *peduncle length* were centred around the same marker in an F_5 segregating population of bambara groundnut, Tiga Nicuru × DipC. QTLs for NS, NP and PW under well-watered conditions and QTL associated with IL under drought-stressed conditions in the F_3 segregating population were co-located on LG2 (85.95 cM) with overlapping confidence intervals. The clustered QTL on the same loci could correspond to a single gene controlling yield and growth habit in bambara groundnut (Chai et al., 2017).

Multiple significant QTLs for NDP under well-watered conditions in the F_4 segregating population were mapped

on LG4 (3.29 cM) and LG6A (43.37 cM), explained 21.80% (LOD 3.85) and 16.10% (LOD 2.75) of the phenotypic variation, respectively, suggesting the inheritance of double-seeded pods was controlled by a major QTL and few minor QTLs. Similar results were also observed for PW under well-watered conditions in the F_4 segregating population mapped on LG4 (3.29 cM) and LG5 (30.51 cM), explained 19.9% (LOD 3.5) and 17.6% (LOD 3.03) of the phenotypic variation, respectively, suggesting the inheritance of pod yield could probably be controlled by few QTLs with minor effect. QTLs identified under well-watered conditions could reflect the intrinsic genetic mechanisms underlying yield-related and morphological traits which vary between the parental lines, although there are also clear differences observed among individual lines and the interaction between genotypes and environment factors for these traits clearly exists, as shown by the difference in QTL between treatments.

Takuno et al. (2012) reported that F_4 and F_3 populations would be almost as useful as RIL populations for QTL mapping. Bradshaw et al. (1998) estimated the accuracy of QTL detection in two different population sizes in interspecific crosses of monkeyflower (*Mimulus* spp.), 12 QTLs of relatively large effect were detected in the smaller population ($n = 93$), while 27 QTLs including 11 of the same QTLs were detected in the larger population ($n = 465$). Although the number of the plants sampled ($n = 3$) and the population size ($n = 86$) are potential limiting factors that could have affected the power of QTL detection, the estimated QTLs with PVE of ≥20% could be considered as major QTLs that control these traits, including NS, NDP and SW.

As the indicators of drought tolerance, PH and SW were located in the same genomic regions on the same chromosomes in soybean (Ren et al., 2020). Ghaffari et al. (2012) reported that PH positively correlated with seed yield in both normal and drought stress conditions and PH is an important determinant of seed yield in sunflower (*Helianthus annuus* L.). In the present study, PH and SW showed a positive correlation under both water regimes in the F_3 and F_4 segregating generations (F_3: $r_{DS} = 0.43$, $p < 0.01$, $r_{WW} = 0.38$, $p < 0.01$; F_4: $r_{DS} = 0.49$, $p = 0.08$, $r_{WW} = 0.33$, $p = 0.27$) (Tables S2 and S3). The significant and positive correlation between PH and SDW were also observed under both water regimes in

TABLE 4 Significant and putative quantitative trait loci (QTLs) for yield-related and morphological traits under drought-stressed and well-watered conditions in the F_3 and F_4 segregating populations derived from S19-3 × DodR

Traits	Treatment	Generation	GW LOD	IM LOD	Linkage group	Position (cM)	Nearest marker	PVE%	Additive effect	Dominance effect	KW value	Significant levels
SDW (g)	WW	F3	2.8	3.05	10	43.76	24383815, 4181438–1	15.5	−2.10	2E−05	13.99	*****
	DS	F3	2.8	1.92	10	32.66	4178651	10.1	−1.42	2.5E−05	7.95	**
	DS	F4	2.8	2.40	3	87.10	4183509	12.9	−1.62	1.5E−05	9.96	***
NS	WW	F3	2.7	3.87	2	85.95	4181165, 27636104	19.1	7.66	0	16.13	*******
	WW	F4	2.8	3.87	4	3.29	4181663, 4175954	21.9	−7.84	−5E−05	12.41	****
	DS	F3	2.8	2.04	7A	35.38	4175814	10.7	−3.78	0	9.99	***
NP	WW	F3	2.9	3.34	2	85.95	4181165, 27636104	16.9	7.93	−5E−05	14.42	*****
	DS	F4	2.8	2.91	11	38.03	2764162, 4176309	16.0	−7.36	−2.5E−05	7.15	**
	WW	F4	2.7	2.16	4	3.29	4181663	12.8	−7.02	0	7.01	**
NDP	WW	F4	2.6	3.85	4	3.29	4181663, 4175954	21.8	−0.23	5E−08	18.92	********
				2.75	6A	43.37	4178271	16.1	−0.22	1.5E−07	23.84	********
	WW	F3	2.6	2.18	7A	29.74	4184098	11.4	−0.20	0	10.37	***
	DS	F3	2.2	1.42	7B	0.68	4182115, 4178408	7.5	−0.20	−5E−08	7.17	**
	DS	F4	1.3	0.87	1A	85.03	4182601	5.1	−0.21	4E−07	6.15	**
SW (g)	WW	F4	2.7	4.19	4	3.29	4181663, 4175954	23.5	−2.04	0	13.19	****
	WW	F3	2.9	2.18	6A	5.99	4178051	11.3	−1.89	2.5E−05	8.55	**
	DS	F3	2.8	1.99	7A	35.38	4175814	10.4	−1.37	5E−06	8.19	**
PW (g)	WW	F4	2.8	3.50	4	3.29	4181663, 4175954	19.9	−2.54	0	10.87	****
				3.03	5	30.51	42010841	17.6	−2.61	0	16.57	*******
	WW	F3	2.9	2.10	2	85.95	4181165	11.0	2.43	5E−05	8.24	**
	DS	F3	2.8	1.93	7A	35.38	4175814	10.1	−1.63	1.5E−05	8.09	**
100SW (g)	DS	F4	2.8	2.45	2	71.63	4179802	13.8	−3.40	−5E−05	8.93	**
HI	WW	F3	2.8	3.33	5	13.99	4183046, 4181791	16.8	0.04	5E−07	10.68	****
	WW	F4	2.7	1.77	4	3.29	4181663	11.3	−0.05	0	5.76	*
SP	DS	F3	2.8	1.93	9	30.66	37313543	11.5	0.06	5E−07	8.15	**
	WW	F4	2.9	2.64	3	17.13	4184228	17.8	−0.06	0	9.87	***
DTF	WW	F4	2.9	2.34	6B	5.51	4181907	12.2	−1.00	0	9.51	***
	DS	F4	2.8	2.52	9	26.20	4182850	12.2	−1.12	0	8.96	**
NL	DS	F3	2.8	1.88	10	32.66	4178651	9.9	−4.88	5E−05	8.23	**
	DS	F4	2.8	2.43	7A	75.43	4180470	11.2	6.03	5E−05	10.19	***

(Continues)

TABLE 4 (Continued)

Traits	Treatment	Generation	GW LOD	IM LOD	Linkage group	Position (cM)	Nearest marker	PVE%	Additive effect	Dominance effect	KW value	Significant levels
PH (cm)	DS	F3	2.8	1.99	10	37.09	4181438-1	10.4	-1.53	5E-05	9.67	***
	DS	F4	2.9	2.41	3	87.10	4183509	12.9	-1.25	-5E-05	10.76	****
PL (cm)	WW	F3	2.7	2.26	6A	10.36	4181745	11.8	-0.93	-5E-05	9.35	***
	DS	F3	2.8	1.85	3	156.88	4181117	9.7	-0.94	-5E-05	9.67	***
IL (cm)	WW	F3	2.9	2.18	11	1.01	4181329, 24385209	11.4	-0.22	5E-06	12.15	****
	DS	F3	2.9	2.11	2	87.45	27636104	11.0	-0.22	-5E-06	8.26	**
	DS	F4	2.7	2.38	8	0	4178576	12.8	-0.22	-5E-06	12.83	****
P/I	WW	F3	2.7	1.81	10	2.53	24384187, 37713638	9.6	0.58	0	10.39	***
	DS	F3	2.9	2.17	2	100.03	4182352	11.6	0.95	-5E-06	9.50	**
	DS	F4	2.8	1.94	2	100.03	4182352	10.7	0.92	5E-06	8.66	**

Note: Yield-related traits: SDW, shoot dry weight; NS, number of seeds per plant; NP, number of pods per plant; NDP, number of double-seeded pods per plant; SW, seed weight per plant; PW, pod weight per plant; 100SW, 100-seed weight; HI, harvest index; SP, shelling percentage; morphological traits: DTF, days to flowering; NL, number of leaves per plant; PH, plant height; PL, petiole length; IL, internode length; P/I, petiole internode ratio; DS, Drought-stressed; WW, Well-watered; GW LOD, Genome-Wide logarithm of odds; IM LOD, Interval mapping logarithm of odds; PVE, phenotypic variation explanation; KW, Non-parametric Kruskal–Wallis test, Significant level *$p < 0.1$, **$p < 0.05$, ***$p < 0.01$, ****$p < 0.005$, *****$p < 0.001$, ******$p < 0.0005$, *******$p < 0.0001$, *ns* not significant.

the F_3 and F_4 generations (F_3: $r_{DS} = 0.51$, $p < 0.01$, $r_{WW} = 0.54$, $p < 0.01$; F_4: $r_{DS} = 0.70$, $p < 0.01$, $r_{WW} = 0.63$, $p < 0.05$) (Tables S2 and S3), and these two traits were located in the same genomic regions (87.10 cM, nearest marker: 4183509) with 12.90% of the PVE on LG3 under drought-stressed conditions in the F_4 generation. Traits including PH, SDW, SW, NS, NP, PW, 100SW, IL and P/I are useful for selection of individuals in response to drought stress (Varshney et al., 2014). The significant differences observed among individual lines ($p < 0.05$) and the interaction between treatment and individual lines for yield-related and physiological traits would suggest that individual lines in the segregating populations could be selected for superior performance under multiple environmental conditions (Zhao et al., 2016).

The genetic linkage map obtained in the present study could be used for the identification of molecular markers linked to important agronomic traits and syntenic regions in other closely related species such as cowpea. Integrating genetic linkage maps from different crosses or using a larger mapping population size will facilitate the development of fine and high marker density maps. Together with a fully assembled and annotated genome of bambara groundnut, the task of identifying markers associated with target traits and the function of candidate genes associated with specific traits will become a reality. The identified markers associated with target traits will be useful in breeding selection to accelerate bambara groundnut improvement through MAS breeding. The development of DArT sequencing technology and the emergence of powerful genome editing techniques will further contribute to molecular breeding progress in bambara groundnut.

5 | CONCLUSION

The present genetic linkage map covered 1,040.92 cM across 11 linkage groups with an average interval distance of 5.23 cM among 234 DArTseq-based SNP markers in the F_2 segregating population from S19-3 × DodR. Significant and putative QTLs for yield-related and morphological traits under drought-stressed and well-watered conditions in the F_3 and F_4 segregating generations were identified. QTLs associated with NS, NP and PW under well-watered conditions and IL under drought-stressed conditions in the F_3 generation were co-located on LG2 with overlapping confidence intervals, while NS, NP, NDP, SW, PW and HI under well-watered conditions in the F_4 generation were co-located on LG4 with overlapping confidence intervals. QTLs identified under well-watered conditions would reflect the intrinsic genetic mechanisms underlying yield-related and morphological traits. Multiple significant QTLs for NDP and PW were observed, suggesting inheritance of

double-seeded pods and pod yield was controlled by many genes. The significant ($p < 0.05$) reduction observed in yield-related and morphological traits and a decrease in PVE under drought-stressed conditions compared to well-watered conditions, suggesting the traits identified under well-watered conditions were unable to fully express their potential trait values under drought conditions. Several QTLs with ≥20% of the PVE were identified as major QTLs to control these traits, including NS, NDP and SW. The major QTLs identified in this study are essential to support the development of improved varieties of bambara groundnut in molecular-enabled breeding programmes.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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