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Genetic analysis of agronomical traits in recombinant inbred lines of cowpea (Vignaunguiculata(L.) Walp.)under two water regimes.

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ABSTRACT

Single nucleotide polymorphism (SNP) markers were used to develop a genetic-linkage map and to identify QTLsinvolved in the genetic variation of agronomical traits in cowpeaunder two water regimes. A total of 1536 SNP GoldenGate assay were used to screen for polymorphism in a cowpea population of recombinant inbred lines. A total of 299 SNP markers amplified polymorphic products of which 228 mapped to the 11 cowpea linkage groups with an average distance of 6.5 cM between markers. The new SNP genetic map with a total length of 1281,8 cM were aligned with the consensus cowpea map allowing filling some gaps, which will increase QTLs analysis. A total of 31 QTLs affecting agronomic traits were identified and mapped to cowpea genomic regions. Among them 45% explaining from 3 to 35% of genetic variation were detected for both water conditions. Co-locations between QTLs were identified on several linkage groups among them QTLs affecting harvest index (HI) and grain yield suggesting their common genetic bases. Because, HI has been shown as the most stable and highly correlated parameter with cowpea yield under stress; our results will enable the efficiency of MAS and enhance genetic progress in cowpea.

Keywords:Cowpea; Water regimes; SNP; Cowpea genetic map;QTLs;genetic variation; HI;MAS

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INTRODUCTION

The development and use of molecular marker technologies have significantly contributed to understanding of genetic basis for many traits of interest of important crop species. Various DNA markers methods such as Restriction Fragment Length polymorphism (RFLP), Random Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP) and Simple Sequence Repeat (SSR) have been used successfully to study the genomic structure and to construct genetic maps of important plants[1-4]. More recently, a new type of molecular marker has gained much interest in the scientific community based on single base substitutions and/or small insertion–deletion polymorphisms (SNPs)[5]. Genome sequencingprojects have shown that SNPs single bases arethe most abundant type of DNA variation occurring frequently in or near coding sequences and may be the cause of functional differencesbetween alleles[6]. Sincethe potential of SNP markers is clearly demonstrated in many genome studies analysis for identification and characterization of genes underlying agronomic importance or quantitative trait variables for a better understanding of the plant functional genomics[7-9]. Currently, a variety of approaches for large-scale SNP identification are available and are increasing in number with the involvement of next-generation sequencing techniques (454, Illumina, SOLID…) that can be used to discover many SNPs in a species at much lower cost[10]. Therefore the occurrence of SNP throughout the genome has become ideal for studies regarding genetic variation, linkage mapping, population structure analysis, association genetics, map-based gene isolation, and plant breeding and will be useful especially in plants suchas cowpea, Vignaunguiculatawhose whole genomeis limited in its genetic resources[11, 12].

Cowpea is a widely consumed food crop and a primary source of protein for many people in developing countries. Cowpea has become increasingly popular in recent years in Africa because of many uses as vegetable, as dry grain for human consumption and as fodder for livestock. Apart from its commercial and nutritional importance, cowpea is also known for its adaptation to drought, its high potential to biologically fix nitrogen in marginal soils. Despite the economical importance of cowpea with regard to food security and income for subsistence farmers in developing countries, cowpea is among ‘‘orphan crops’’[11, 12]. In addition this crop is grown in regions where water deficit frequently occurs and significantly reduces yield [13, 14].

Water stress decreases plant growth and productivity, by slowing the rate of cell division and expansion. Many studies have generated significant amounts of information regarding the agronomical traits related to drought tolerance in cowpea[13, 15-17].Unfortunately these phenotypic relationships have less shown at a molecular level.Understanding of genetic basis of grain yield by studying yield components is valuable in cowpea improvement and can help attain food security in developing countries.

Recently, 183,118 of expressed sequence tags (ESTs) from 17 cDNA libraries have been generated on cowpea within international projects and collaboration[12]. The EST libraries have proven to be excellent resources for gene discovery, molecular marker development, analysis of gene expression at the level of the whole genome, and identification of candidate genes for phenotypes of interest[18-20]. The 183,118 ESTs discovered on cowpea sequenced yielded _10,000 high-confidence SNPs from which an illumina 1,536-SNP GoldenGate genotyping array was developed. These SNP markers are important Molecular tools to enhance genetic progress in cowpea.

In this study, we evaluated SNP GoldenGate assay to develop adensity cowpea genetic-linkage map and to identify QTLs involved in genetic variation of agronomical traits in a population of cowpea recombinant inbred lines (RILs) under well-watered and water stressedconditions.

MATERIALS AND METHODS

Plant materials.The mapping population of 96 RILs (F2:10) used in the present work was developed through single seed descent method from F2 plant derived from a cross between “Bambey21” and “Mouride” cowpea cultivars developed by Senegalese Agricultural Research Center (CNRA) andcommonly used by farmers in Senegal. Mouride is a high yielding cowpea cultivar with resistance to drought while the cowpea cultivarBambey 21 is susceptible.The RILs and their parental varieties were used for agronomical analysis.

Field experiment.

Two experiments were conducted under the fully irrigated (non-stress) and water stressconditions at the experimental station of CNRA in Bambey (Senegal) during 2005 and 2008 dry season from March to June (March - June).The experiment consisted of a split-plot design with three blocks. The main plot consisted of water treatments (well-watered and water-stressed) and subplot consisted of genotypes (RILs and parental lines). The RILs and their two parents were sown in each water regime with three replications. Each replication consisted of two 5 m- long rows, with cowpea planted 25 cm apart with a distance of 50 cm between rows. The farm was managed according to standard procedures recommended by ISRA (InstitutSenegalais de Recherché Agronomique.), with a single starter dose of 150 kg.ha^{-1}complex N-P-K (6-20-10) manually. Weeding in both water regimes was manually conducted. The water deficit treatment was imposed when plant were 20 days of age at anthesis stage by withholding watering for a period of 28 days. Well-watered plants received sufficient water (300 mm) to maintain the normal growth of cowpea plants. Three plants per replicate and per water treatments were randomly chosen for evaluation of the studied traits.

Agronomical traits measurements.

Days from sowing to flowering (DSF) and days from sowing to maturing (DSM) were recorded when 50% of the plants per plot in the field were at flowering and maturity stage. At physiological maturity a sample of three plants from each plot was

harvested and separated into various parts. All parts were oven-dried at 70° C for 72 h. Thereafter, pod number per plant (PNPL), pod length per plant (PLPL), total pod weight per plant (PWPL), Grain weight per pod (SWP), grain weight per plant (SWP), grain number per pod (SNP), grain number per plant (SNPL), and 100-grain weight (W100) were determined. Grain yield (GY) and total biomass (BY) were collected on a plot basis at g/m^2 and calculated at Kg/ha by harvesting all plants in each plot for RILs and their parental lines in each replication for the 2 water regimes. Then the harvest index (HI) was estimated.

Genomic DNA isolation and SNP genotyping.

Seeds for each RILs and the parental cultivar were sown in plastic pots filled with steam-sterilized soil mix UCMIX-3 (http://agops.ucr.edu/pdfs/soil_mix_recipes.pdf)at the University of California Riverside greenhouse, in August 2008.Two weeks after planting, fresh plants materials were collected for DNA extraction. The genomic DNA of Bambey21, Mourideand 96 RILs was isolated following the procedure early reported by some authors[12]. Briefly, genomic DNA was isolated from 98 parental genotypes and RILs by using Plant DNeasy (Qiagen) starting with 100 mg of young trifoliate leaves. DNA concentration was determined by using a Quant-iTdsDNA Assay Kit Broad Range (Q33130) (Invitrogen) and fluorescence (485 nm / 535 nm, 1.0 s) measured with a microtiter plate reader (PerkinElmer / Life Sciences; Wallac Victor^2, 1420 Multilabel counter). Samples were adjusted to 80 ng/L in Tris-EDTA buffer. A total of 1536 SNP markers mapped onto the cowpea consensus genetic linkage Map[12] were tested on Bambey21 and Mouride using the GoldenGate assay. The Golden Gate genotyping assay was performed at the University of California Los Angeles genotyping facility using 250 ng of DNA for each assay. A Visual FoxPro script was developed to automate phase assignment of RIL genotype calls based on parental genotypes, tally no-calls, heterozygotes, and nonparental alleles for each RIL and calculate allele frequencies.

Statistical analysis,Map construction and QTL analysis.

Agronomical traits data were analyzed by ANOVA with Statistix 8 package[21]. Correlation analysis was performed to examine the relationship between the measured traits in each of the conditions and between lines for the same traits across water treatments.Map Disto1.7.0 was used to construct the genetic linkage map[22]. Chi-square-tests were performed for segregation distortion of each locus. Loci were assembled into groups using likelihood odds (LOD) ratios with a LOD threshold of 4.0 and a maximum recombination frequency threshold of 0.35. Multiple locus order estimates were produced for each linkage group using Map Disto1.7.0. The likelihoods of different locus orders were compared and the locus-order estimate with the highest likelihood was selected for each linkage group. Kosambi mapping function was used to calculate map distances (cM) from recombination frequencies[23]. MapQTL 4.0 software[24] was used for QTL analysis according to Mucheroet al., with a slight modification[11]. First, the non-parametric Kruskall–Wallis package was used to identify markers associated with agronomical traits. Data for each water condition and for each trait were analyzed separately. Markers showing significant association at the more stringent 0.005 significance level or higher were considered putative. Putative QTL were confirmed by the multiple-QTL model mapping (MQM) package using the automatic cofactor selection option. Given the large difference in linkage group sizes, LOD thresholds were determined at the 0.05 significance level for each linkage group and each water treatment using the permutation test [25]. During each analysis, 1000 permutations were performed. The 50th highest LOD was taken as the LOD significance threshold for each linkage group, and then the highest LOD significance threshold for each linkage group was used as the final LOD threshold to identify QTL in all conditions. QTL were considered significant when the significance thresholds were met concurrently for both the Kruskall–Wallis and the MQM analysis. MapChart 2.2 software [26] was used for graphical presentation of linkage groups and map position of the SNP markers and QTL.

RESULTS

Phenotypic Variation among cowpea genotypes and effect of water status on measured traits.Phenotypic performance of RILs and their parents (Mouride×Bambey21)for agronomic traits under the two water regimes are summarized in Table 1.Cowpea cultivars MourideandBambey21exhibited significant differences in DSF, DSM, BY,SNPL, SWPL and GY under both water regimes. But in the stressed water treatments, differences were observed among them only in PLPL, PWPL, SNP, W100, and SWP. These parental phenotypes also segregated in the RIL population. Significant differences were observed between RILs for most agronomic traits studied under the two water conditions ($p < 0.001$). Water stress consistently affected the expression of DSM, SNP, SWP, SWPL, GY and HI ($p < 0.05$) whereas biomass traits, DSF, SNPL, PNPL and W100 showed no significant difference under both water regimes. Significant interaction water regime × RILs at 0.001 probability level was observed for most of the traits indicating a high level of genotype by environment..Transgressivesegregants were observed for all agronomic traits measured with some RILs showing high and others showing low phenotypic data than parental extremes under both water treatments. Correlations between grain yield (GY) and other studied traitsare presented in Table 2. There was high correlation between yield and phenology, biomass and yield related traits particularly under water-stress conditions ($r = 0.37$ $P < 0.001$).GY showed a high positive association with harvest index,pod length, pod weight, biomass yield, seed number, Pod number, seed weight ($P < 0.001$) under stress. In general, GY and phenology were negatively correlated whereas 100-grain weight seems no correlated in GY.

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Table 1. Phenotypic performance for agronomic traits of cowpea RILs and their parents evaluated across well-watered

and water-stressed treatment.

Traits	Water-stressed				Well-watered				Effect		
	Mouride	B21	RILs		Mouride	B21	RILs				
			Mean	range			Mean	range	Water treatment	RILS	Water treatment ×RILs interaction
Phenology											
DSF	53.8*	41.3	45.7	37- 54	50.*	42.3	45.4	37-56	NS	***	NS
DSM	75.3***	57.3	67.1	56 -90	71.*	60.3	64.9	55-83	*	***	***
Biomass											
PLPL	11.4	13.*	9.8	1 -18	13.5	11.8	12.4	5.5-	NS	*	NS
PWPL	16.9*	7.7	6.7	0.1-53.1	23.5	21.4	22.4	17.3	NS	***	***
BY	2363.1*	1327.5	1425.9	369-7216.1	3112.*	2476.2	2848.3	2.8-98.1 406-8243	NS	***	***
Yield components											
SNP	8.3	5.8*	5.02	1-11	10.17	7.50	7.4	2-12	*	***	***
SNPL	74.0**	36.	33.9	1-204	145.90*	88.67	101.7	1.2-	**NS**	***	***
PNPL	7.0	8.3	7.2	1-41	20	16.0	18.8	465	**NS**	***	***
W100	15.8	17.6*	14.7	2-26.6	16.19	18.33	16.9	2-81	**NS**	***	NS
SWP	1.5*	0.7	0.7	0-6.9	1.30	1.48	1.3	9.7-	*	***	**NS**
SWPL	14.3**	4.8	5.1	0.1-61	72.71*	17.02	17.1	36.3	*	***	***
GY	583.3**	270.7	169.9	0.2-1376.32	1244.2*	873.4	991.8	0.3-	*	***	
HI	0.24	0.2	0.2	0.18-0.25	0.45	0.32	0.4	2.3 2.8-72 38-2971 0.08-1.2	*	***	* *

The significant differences between parental lines, RILs, water treatment and effect of water treatment are shown by *,***,** and *: significant at 0.001, 0.01 and 0.05 probability level. NS: non-significant.

Table 2. Correlation coefficients between yield and agronomical traits under well-watered and water-stressed conditions

traits	Well-watered	water-stressed
	Grain yield	
Phenology		
DSF	-0,1596	-0,3831***
DSM	-0,1803	-0,3724***
Biomass		
PLPL	0,0962	0,3290**
PWPL	0,5255***	0,5008***
BY	0.506***	0.678***
Yield components		
SNP	0,2472*	0,3379**
SNPL	0,4079**	0,6832***
PNPL	0,3128**	0,6986***
W100	0,1022	0,2027

***,** and *: Significant at 0.001, 0.01 and 0.05 probability level.

NS: non-significant

Foot note for tables 1 and 2. The agronomical traits are : Days from sowing to flowering (DSF), days from sowing to maturing (DSM), pod number per plant (PNPL), pod length per plant (PLPL), total pod weight per plant (PWPL), Grain weight per pod (SWP), grain weight per plant (SWP), grain number per pod (SNP), grain number per plant (SNPL), and 100-grain weight (W100), Grain yield (GY), total biomass (BY) and harvest index (HI).

Linkage map and QTL analysis for agronomic traits.

A total of 299 (19%) of the 1536 SNPs screened with the GoldenGate assay were polymorphic in the parents and the RILs population.Of the 299 markers, 125 markers exhibited significant segregation distortion at the 0.05 significancelevel as calculated by the JoinMap program. The marker distribution among the linkage groups in our map is presented in Table 3. A total of 71 markers showed high segregation distortion and/or could not be placed definitively (LOD >3) were removed in linkage map.The resulting map consisted of 228 markers SNPs placed in 11 linkage groups and covered a total length of 1281, 8 cM (Figure 1). Linkage groups and SNP annotation in this study were aligned with previous cowpea consensus maps so that allowed a cross reference.A total of 13 agronomic traits were analyzed for QTLs identification. QTLs were designated as the abbreviation of the trait followed by numerals numberor alphabetical letterfor well-watered or water-stressed. Map positions and effect of these QTLs are summarized in Tables 4 and 5. Only 21 significant associations were detected between SNP markers and phenotypic data under well water condition and 10 QTL for 9 agronomic traits were identified under stress water treatment by both Kruskall–Wallis and MQM analyses. Among these 31 QTLs detected, 14 were common across water treatments.

Under well-watered condition (table 4), 1 up to 5 QTLs for the same traits has been detected on 6 of the 11 linkage groups. 100-grain weight was significantly linked to 5 QTLs (W100_1, W100_2, W100_3, W100_4, W100_5) located on 4 cowpea linkage group (2, 4, 5, 10) with R^2 estimates from 6% to 25%. Also 5 chromosomal regions explaining together from 7% to 31% of the total variance were associated with grain number per pod (SNP). Concerning traits as day from sowing to flowering (DSF), pod weight by plant (PWPL), seed number per plant (SNP) and pod length by plant (PLPL), 2 QTLs respectively located or co-located on linkage group 1, 2, 3, 5 and 10were identified with phenotypic variances explained from 3.5% to 34.6% suggested the importance of these chromosomal regions in the variation of these traits. For

trait such pod weight by plant, Day from sowing to physiological maturity and seed weight per pod, only 1 QTL were identified. Co-location between QTL for SNPL, SNP, SWPL, PWPL, DSF were founded on linkage group 1 under well water treatment (Figure 1). There was overlap between some QTLs on linkage groupsindicated the existence of a common genetic base for these traits.

Under water-stress condition 10 QTL for 6 morph-agronomic traits were identified (table 5). The percentage of phenotypic variance explained by these QTLs (R^2) ranged from 5.2% to 35.3%. In 100-grain weight, 3 QTLs involved (W100_a, W100_b W100_c) were detected on linkage group 2, 4 and 10 respectively. The locus W100_a had a negative additive effect while W100_b and W100_c had additive positive effect. Under this-stressed condition two QTLs controlling Day from sowing to physiological maturity (Mat_a and Mat_b) were found on linkage group 1. This region also hosted QTL for Day from sowing to flowering (DSF_a) at high significance level. QTLs controlling grain yield (GY_a) and harvest index (HI_a) were co-located on linkage group 6 (figure 1) and indicate the existence of a common genetic base for these traits under drought. This result is in accordance with the correlation coefficient between GY and HI under water-stressed condition. Two chromosomal regions linked to pod length by plant (PLPL_a and PLPL_b) were identified on linkage group 3 and 10 respectively.

Tableau 3. Description of the 11 linkage groups of the *Vignaunguiculata*linkage SNP map

Linkage group (LG)	Size (cM)	Segregation		Average density (cM)	Total
		Mendelian segregation	Distorted segregation		
1	171,1	18	4	10,1	22
2	168,4	27	13	5,4	40
3	203,2	22	14	6,6	36
4	81,3	9	0	11,6	9
5	96,5	12	4	7,4	16
6	88,8	15	5	4,9	20
7	115,4	13	4	7,6	17
8	106,4	17	2	9.6	19
9	100,1	14	8	5,9	22
10	77,7	17	0	5,9	17
11	72,9	5	5	10,41	10
Unlinked		5	66		71
Total	1281,8	**174**	**125**	**6,5**	**299**

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Table 4. Quantitative trait loci (QTL) for agronomical traits revealed by Kruskall–Wallis and multiple-QTL model mapping (MQM) analysis in a cowpea recombinant inbred population derived from a cross between cowpea cultivars Mouride and Bambey 21 under well-water treatment

Traits	QTLs	LG	Position (cM)	Marker interval	Kruskall–Wallis	MQM		
					Significancelevel	LOD	LOD threshold	*R*2
W100	W100_1	2	62,4-84,9	1_1406 - 1_1431	0.05-0.005	1.23-3.85	2	11-25.1
	W100_2	4	0,0-8,7	1_1445-1_0153	0.01-0.001	2.81-1.57	1.7	11.1-20.8
	W100_3	5	0,0-5,8	1_0806 - 1_0292	0.005-0.0005	1.51-1.93	1.9	9.5-12.9
	W100_4	10	20,2-28,5	1_1118-1_1081	0.05-0.005	0.81-2.03	1.8	12.3-15.5
	W100_5	10	72,6-77,7	1_0598-1_0065	0.01-0.001	0.95-2.4	1.8	6-14.7
SNP	SNP_1	1	0-10,8	1_0731-1_0811	0.01-0.00001	1.14-2.72	2	7.3-20.3
	SNP_2	1	149,6-170,2	1_0357-1_1526	0.05-0.01	1.41-3.09	2	9.4-27.1
	SNP_3	3	47,9-70	1_0296-1_1162	0.05-0.0001	1.62-3.50	2.2	10.2-31.6
	SNP_4	10	12,2-36,2	1_1118-1_0111	0.05-0.001	1.72-3.65	1.8	13.2-27.1
	SNP_5	10	38,4-42,1	1_0111-1_0077	0.01-0.001	1.42-2.02	1.8	8.9-12.4
DSF	DSF_1	1	134,3-171,1	1_0640-1_1526	0.05-0.00005	2.03-6.34	2	12.8-34.6
	DSF_2	3	28,1-48,9	1_1065-1_0964	0.05-0.01	2-2.17	2	12.4-16 .6
PWPL	PWPL_1	1	0-10,8	1_0731-1_0811	0.05-0.001	0.4-1.85	1.8	3.5-11.5
	PWPL_2	5	4-21,3	1_0806-1_0419	0.05-0.01	1.55-1.93	1.6	10.8-15.7
SNPL	SNPL_1	1	0-10,8	1_0731-1_0811	0.05-0.00001	1.94-2.64	1.9	5.2-16
	SNPL_2	5	55,4-58,1	1_1359-1_1095	0.05- 0.001	1.57-1.97	1.7	3.6-12.2
PLPL	PLPL_1	10	38,4-42,1	1_0111-1_1098	0.01-0.001	1.32-2.33	1.9	8.4-14.3
	PLPL_2	10	45,4-57	1_0780-1_0628	0.05-0.001	1.83-2.46	1.9	13.3-16.6
SWPL	SWPL_1	1	0-10,8	1_0731-1_0811	0.05-0.001	0.5-2.05	2	2.9-12.7
SWP	SWP_1	2	62,4-71,5	1_1406-1_1096	0.01-0.005	1.68-2.59	2.1	9-15.9
DSM	DSM_1	1	137,6-171,1	1_0357-1_1526	0.001-0.0005	2.46-4.37	2	13.7-28

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Tableau 5: Quantitative trait loci (QTL) for agronomical traits revealed by Kruskall–Wallis and multiple-QTL model

mapping (MQM) analysis in a cowpea recombinant inbred population derived from a cross between cowpea genotypes

Mouride and Bambey 21 under stressed-water treatment

Traits	QTL	LG	Position (cM)	Marker interval	Kruskall–Wallis	MQM		
					Significancel evel	LOD	LOD threshol d	R^2
W100	**W100_a**	2	62.4-71.5	1_1406-1_1096	0.005-0.001	1.66-2.42	2.1	10.3-16.6
	W100_b	4	0.0-3.8	1_1445-1_0153	0.05-0.001	0.82-1.95	1.7	5.2-12 .7
	W100_c	10	52-77.7	1_1049-1_0065	0.05-0.0001	1.25-3.05	1.8	7.9-18.5
DSM	**DSM_a**	1	109-148.6	1_0256-1_0775	0.05-0.001	1.95-2.81	1.9	14.8-33.1
	DSM_b	1	167.2-171.1	1_0775-1_1526	0.05-0.001	1.89-2.16	1.9	13.4-14.4
PLPL	PLPL_a	3	21.1-49.9	1_1065-1_0964	0.05-0.001	1.98-3.33	2.1	13.5-22.8
	PLPL_b	10	45.4-58	1_0780-1_0628	0.01-0.01	1.74-2.19	1.8	10.8-14.8
HI	HI_a	6	51.9-68.8	1_0943-1_0010	0.05-0.01	1.01-2.04	1.8	6.5-17.5
GY	GY_a	6	55.4-58.1	1_0706-1_0639	0.005-0 .0001	1.53-2.15	1.8	9.1-13.2
DSF	DSF_a	1	101-171.1	1_0256-1_1526	0.01 -0.001	1.98-5.46	2	12.2-35.3

Foot note for tables 4 and 5. The traits are: 100-grain weight (W100), grain number per pod (SNP), days from sowing to flowering (DSF), total pod weight per plant (PWPL), grain number per plant (SNPL), pod length per plant (PLPL),grain weight per plant (SWPL), Grain weight per pod (SWP), days from sowing to maturing (DSM), harvest index (HI) and Grain yield (GY)

The QTLs were designated as the abbreviation of the trait followed by Arabic numeralsor alphabetical letterfor well-watered or water-stressed. Common QTLs across water treatments were shown as bold-face in table 5

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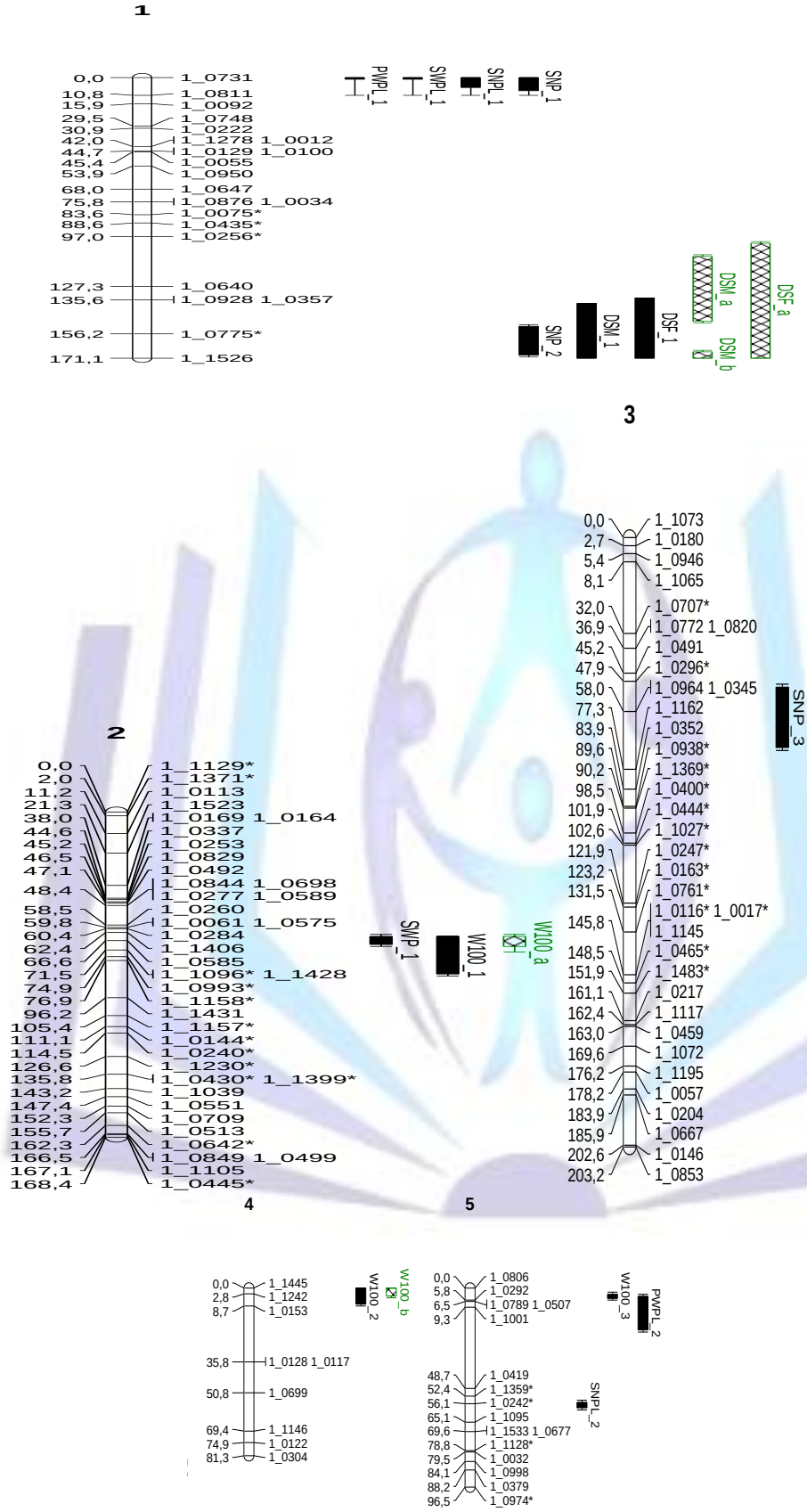

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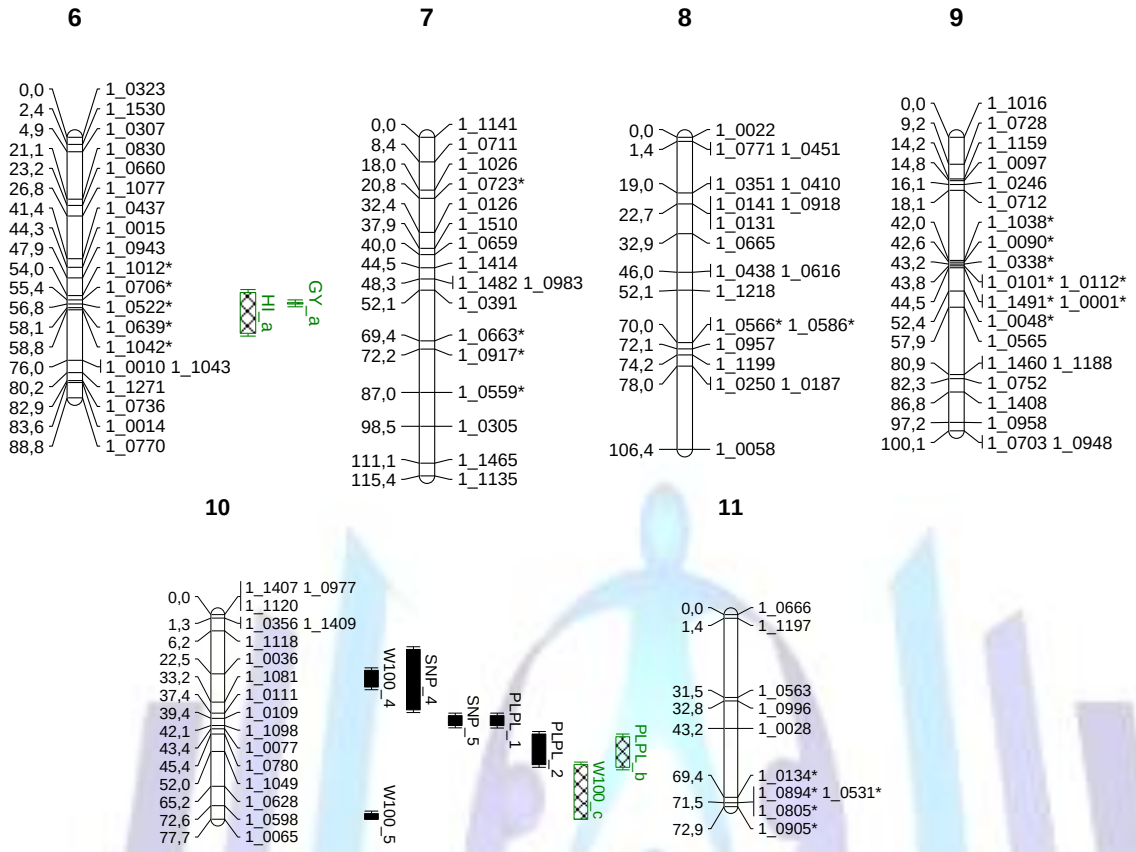

Figure 1: Location of putative QTL associated with agronomical traits on a cowpea genetic linkage map constructed using SNP markers.*QTL detected under well water treatment are represented in black solid bars and under stressed-water are represented by green bars. Distorted markers are indicated with a * at the end of markers.*

DISCUSSION

It is well established and documented that water deficit during reproductive development in cowpea significantly reduces yield [16, 17]. In this study the water deficit treatment imposed at anthesis stage showed significant variability for agronomical traits in recombinant inbred lines of cowpea. A large variability concerning agronomical traits has been previously reported in cowpea and is recognized as important criterions for improving drought resistance in cowpea [14, 27, 28]. A wide range in variation was observed for all measured traits among water-stressed compared with their well-watered counterparts. Some authorshave also reported genetic variability for the stress response that couldwell be seen upon exposure plant to an induction stress [3, 27]. Among the agronomical traits evaluated 100-grain weight is not correlated with yield under the two water regimes. However the other traits measured were significantly associated with yield, mainly under stress condition and indicate their contribution to yield maintenance of cowpea under pre-anthesisdrought condition. Similar relationships between cowpea yield and phenology, biomass under water stress conditions have been reported [17, 28, 30]. Although phenology is reported as a key component in cowpea breeding [14, 31], maturity and flowering were negatively correlated with yield in this study that suggest cowpea plant responds to water stress by shortening the flowering and the seed filling period.

To determine the genetic determinism of all studied traits known as quantitative traits, cowpea linkage-saturated maps have to be developed, which requires a large number of polymorphic markers. In our study only 19% of 1,536 SNP loci were successful genotyped among parental cowpea varieties and the RILs. The SNP map constructed in this study consisted of 229 markers placed in 11 linkage group and presumably corresponds of the 11 chromosomes in the cowpea genome (x = 11).This maphasthe distinction of allowing a cross reference to recently publishedcowpea consensus SNPs map [12]. Common markers were found on the same linkage groups between the two cowpea maps. Consequently, linkage groups in this study were aligned with the consensus SNP cowpea map allowing filling some gaps, which will increase the quantitative and qualitative trait analysis for *Vignaunguiculata.*

The base of QTL detection is to associate variation of the measured phenotype with markers genotypes in segregating population. QTLs for agronomical trait identified in this study underlined that several putative genomic regions are involved in the response of cowpea under water regimes variation. The number of loci detected per agronomical trait was larger in non-stressed conditions. These results do not support the high variability of measured traits observed in the present study, and stand in contrast to other studies which revealed more QTL in stressed conditions than in non-stressed ones [3, 32]. The low number of loci detected for agronomical traits in this study may be attributed to low level of polymorphism (SNP)

detected on the cowpea parental varieties, Mouride and Bambey 21. Only 19 % of the SNPs screened were polymorphic in the parents and the RILs population.Other studies that utilized SSR (Simple Sequence Repeat) technologies have shown fewer polymorphisms on the same mapping population [30]. Using AFLP, SSR and SNP, we demonstrated that SNP was the most efficient in detecting polymorphism between Mouride and Bambey 21(data not shown). The lack of significant molecular differences lowered the evaluation of genetics effect in the expression of most traits under drought in cowpea RIL population. Genetic variability in the stress response has been suggested to be mainly due to the differential expression of stress-responsive genes [33, 34.]

Most of the QTLs identified were specific to each water regime but a limited number of QTLs were common under both water treatments. Among these, QTLs affecting flowering and maturity on linkage group 1 were common to both water treatments. Also QTLs affecting 100-grain weight in cowpea on linkage group 2, 4, 10 and QTL affecting pod length on linkage group 10 were common under both well-watered and water-stressed conditions. However, the phenotypic variance explained by some of common QTLs was different under the two water treatments. Example, For QTLs affecting maturity in cowpea (Mat_b), R^2 range to 13.4% - 14.4% under water-stressed conditions whereas it range from 13.7 - 28 % for the same QTL under Well-watered conditions (DSM_1). The differential effect of QTL over water regime can explain the genetic component of the control of the expression of statistically significant "RIL _water treatment" interaction component [3]. This explain why Days from sowing to flowering in cowpea which did not show significant "RIL _water treatment" interaction has common QTL with almost the same effect of 12,2 % - 35,3 % and 12,8 % - 34,6 % under well water and water deficit conditions respectively.

Some QTLs controlling agronomical traits as seed number was detected without co-location with any other QTL suggesting these regions contain genes specific implied in the variation of their traits [35]. However, in most of cases, one genome region was found to be associated with more than one trait. Intervals 1_0731- 1_0811 and 1_0357-1_1526 on linkage group 1 were significantly associated with several agro-morphological traits under the two water conditions. In these two intervals, the QTLs controlling SNP, SNPL, PWPL and SWPL under well water condition and DSF and DSM under the two growth conditions were overlapped. Similarly several other overlapping QTLs were also observed for agro-morphological traits on linkage group 2, 3, 4, 5, 6 and 10 which could indirectly affect yield. This result is accordance with the high correlation between yield component, between yield and yield components as reported by this study and by several authors on cowpea [28, 30]. These co-locations indicate the existence of a common genetic base for agro-morphological traits. Data supporting similar relationships of yield with yield component have been reported on sunflower [3]. One of the most important chromosomic regions for yield is located on linkage group 6 where the QTL for grains yield (GY_a) is co-located with QTLs for harvest index (HI_a) in water stressed treatments. Harvest index has been shown as the most stable and highly significant correlation component with yield under stress in cultivated plant and especially in cowpea [30, 36-38]. Earlier studies have also recommended maintaining a high harvest index as the best strategy for improving crop yield under water limiting conditions [39]. In this study HI was positively highly associated with yield in water deficit condition. Therefore, QTLs affecting HI and GY tended to cluster in the same genomic regions suggesting their common genetic bases that will be useful for marker-based approaches to improve drought tolerance in cowpea. One of the principal benefits of the QTL analysis was the identification of relevant genomic regions to be included in breeding program for responsiveness under drought [40]. Identification of QTL influencing several traits in this study could increase the efficiency of marker-assisted selection in cowpea breeding and enhance genetic progress in cowpea.

CONCLUSION

The map constructed in this study allowing a cross reference to the cowpea consensus public map and therefore represents an important genetic tool for quantitative and qualitative trait analysis for *Vignaunguiculata*. Our map enabled us to investigate the genetic basis of cowpea agronomical traits under well water and stressed-water treatment. Such studies permit the identification of constitutive QTLs (common to both water environment) from adaptive ones (specific for a given water treatment) affecting yield, yield component to cowpea. The results showed a common genetic basis between the traits measured, especially between harvest index and grain yield in water stressed treatment and confirm the positive correlation between both traits under water deficit regime. Results obtained in this work open interesting perspectives in the development of cowpea genomics, as the QTLs here were identified could be beneficial for marker-based approaches to improve drought tolerance in cowpea. However these QTLs should be validated in other genetic backgrounds before to be used for improving drought tolerance in cowpea by marker-assisted selection.

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REFERENCES

[1]Menèndez. C. M, Hall. A. E and Gepts, P. 1997. A genetic linkage map of cowpea (Vignaunguiculata) developped from a cross between two inbred, domesticated lines.Theor. Appl. Genet. 95: 1210-1217.

[2] Flandez-Galvez. H, Ford. R, Pang. E. C and Taylor, P. W. 2003. An intraspecific linkage map of the chickpea (Cicerarietinum L.) genome based on sequence tagged microsatellite site and resistance gene analog markers. TheorAppl Genet 106: 1447-56

[3]PoormohammadKiani. S, Talia. P, Maury. P, Grieu. P, Heinz. R, Perrault. A, Nishinakamasu. V, Hopp. E, Gentzbittel. L, Paniego. N and Sarrafi, A. 2007.Genetic analysis of plant water status andosmotic adjustment in recombinant inbred lines of sunflower under two water treatments. Plant Science 172: 773-787

[4] Milczarski. P, Banek-Tabor.A, Lebiecka. K, Stojałowski. S, Myśków. B andMasojć, P. 2007. New genetic map of rye composed of PCR-based molecular markers and its alignment with the reference map of the DS2 X RXL10 intercross. J Appl Genet 48: 11–24

[5]Rafalski, A. 2002. Applications of single nucleotide polymorphisms in crop genetics.CurrOpin Plant Biol**5**: 94–100.

[6] Cho. R. J, Mindrinos. M, Richards. D. R, Sapolsky. R. J, Anderson. M, Drenkard. E, Dewdney. J, Reuber. T. L, Stammers. M andFederspiel, N. 1999.Genome-wide mapping with biallelic markers in Arabidopsis thaliana. Nat Genet. 23: 203–207

[7]Coles. N. D, Coleman. C. E, Christensen S. A, Jellen. E. N, Stevens. M. R, Bonifacio. A, Rojas-Beltran. J. A, Fairbanks. D. J andMaughan, P. J. 2005. Development and use of an expressed sequenced tag library in quinoa (Chenopodium quinoaWilld.) for the discovery of single nucleotide polymorphisms.Plant Science, 168: 439-447

[8] Lai. Z, Livingstone. K, Zou. Y, Church. S. A, Knapp. S. J, Andrews. J andRieseberg, L. H. 2005.Identification and mapping of SNPs from ESTs in sunflower.TheorAppl Genet. 111: 1532–1544.

[9] Balfourier. F, Ravel. C, Bochard. A, Exbrayat-Vinson. F, Boutet. G, Sourdille. P, Dufour. P and Charmet, G. 2006. Développement, utilisation et comparaison de différents types de marqueurs pour étudier la diversité parmi une collection de blé tendre. Les Actes du BRG. 6: 129-14

[10]Mardis, E. R. 2007. The impact of next-generation sequencing technology on genetics.Trends Genet.24**:** 133–141.

[11]Muchero .W, Jeffrey. D. E, Timothy. J. C and Philip, A. R. 2009a. Mapping QTL for drought stress-induced premature senescence and maturity in cowpea [Vignaunguiculata(L.)Walp.TheorAppl Genet. 118: 849–863.

[12] Muchero. W, Ndeye. N. D, Prasanna. R. B, Raymond. D. F, Steve. W, Marti. P, Sarah. H, Cisse. N, Fatokun. C, Jeffrey. D. Ehlers et al. 2009b. A consensus genetic map of cowpea [Vignaunguiculata (L) Walp.] and synteny based on EST-derived SNPs. PNAS Early Edition. September 4, 2009.

[13] Cruz de Carvalho. M. H, Laffray. D and Louguet, P. 1998. Comparison of the physiological responses of Phaseolus vulgaris and Vignaunguiculatacultivars when submitted to drought conditions.Env.and Exp. Botany. 40: 197-207.

[14] Hall. A. E, Cissé. N, Thiaw. S, Elawadc. H. O. A, Ehlersa. J. D, Ismail. A. M, Ferye. R. L, Robertsf. P. A, Kitchg. L. W and Murdockh, L. L.2003. Development of cowpea cultivars and germplasm by the Bean/Cowpea CRSP.Field CropResearch. 82, 2-3 : 103-134.

[15] Roy-Macauley, H. 1993. Activités endoprotéolitiques : mise en évidence et variation en réponse au déficit hydrique chez Phaseolus et Vigna. Etude préliminaire de la réponse du système ubiquinique à la contrainte hydrique. ThèseDoct., Univ. Paris VII, 140p.

[16]Anyia. A. O and Herzog. H. 2004. Water-use efficiency, leaf area and leaf gas exchanges of cowpea under mid-season drought. J. Agr. Crop Sci. 20: 327-339.

[17]Falalou. H., Zombré. G, Diouf. O, Diop. N. N, Guinko. S and Braconnier. S. 2007. Physiological, biochemical and agromorphological responses of five cowpea genotypes **(**Vignaunguiculata (L.)Walp.)to water deficit under glasshouse conditions. Biotechnol.Agron. Soc. Environ. 11 (3): 225-234

[18] Andersen. J. R and Lubberstedt, T. 2003. Functional markers in plants. Trends Plant Sci 8: 554–560

[19] Clarke. B, Lambrecht. M and Rhee, S. Y. 2003. Arabidopsis genomic information for interpreting wheat EST sequences. FunctIntegr Genomics. 3: 33–38

[20] Gupta. P. K, Rustgi. S, Sharma. S, Singh. R, Kumar. N and Balyan, H. S. 2003.Transferable EST-SSR markers for the study of polymorphism and genetic diversity in bread wheat.Theor.Appl Genet. 270: 315-323.

[21] Analytical Software. 2003. Statistix 8 user's manual.Analytical Software, Tallahassee, FL.

[22] Lorieux, M. 2007. MapDisto, a free user-friendly program for computing genetic maps.Computer demonstration (P958). In: Plant and animal genome XV conference. San Diego, 13–17 Jan 2007

[23] Kosambi, D. D. 1944. The estimation of a map distance from recombination values. Ann. Eugen, 12: 172–175.

[24] Van Ooijen. J. W, Boer. M. P, Jansen. R. C and Maliepaard, C. 2002. MapQTL®4.0 software for the calculation of QTL positions on genetic maps. Plant Research International, Wageningen

[25] Doerge. R.W and Rebai, A. 1996.Significance thresholds for QTL interval mapping tests. Heredity 76: 459–464

[26] Voorrips, R. E. 2002. MapChart: software for the graphical presentation of linkage maps and QTLs. J Hered 93: 77–78

[27] Ogbonnaya. C. I, Sarr. B, Brou. C, Diouf. O, Diop. N. N and Roy-Macauley, H. 2003. Selection of cowpea genotypes in hydroponics, pots and field for drought tolerance.Crops Sci. 43: 1114-1120.

[28]Sarr. B, Diouf. O, Diouf. M and Roy-Macauley, H. 2001.Utilisation des paramètres agromorphologiques comme critères de résistance à la sécheresse chez trois variétés de niébé cultivées au Sénégal et au Niger. Sécheresse. 12: 253-266.

[29] Srikanthbabu. V, Ganesh. K, Krishnaprasad. B. T, Gopalakrishna. R, Savitha. M and Udaya Kumar, M. 2002. Identifcation of pea genotypes with enhanced thermotolerance using temperature induction response (TIR) technique.J Plant Physiol. 159: 535-545

[30]Guèye, M. C. 2006. Amélioration de l'adaptation à la sécheresse du niébé : recherche des critères de sélections et approche cartographique génétique. Thèse Doctorat de l'Université. Cheik AntaDiop.

[31] Hall, A. E, Thiaw. S, Ismail. A. M and Ehlers, I. D.1997. Water-use efficiency and drought adaptation of cowpea. In: B. B. Singh; D. L. Mohan Raj, K. E. Dashiell and L. E. N. Jackai (Eds.), Advances in Cowpea Research, IITA, Ibadan, Nigeria, pp. 87-98.

[32]Pelleschi. S, Guy. S, Kim. J. Y, Pointe. C, Mahe. A, Barthes. L, Leonardi. A and Prioul, J. L. 1999. Ivr2, a candidate gene for a QTL of vacuolarinverstase activity in maize leaves. Gene-specific expression under water stress. Plant Molecular Biology 39: 373-380.

[33] Krishnan. M, Nguyen. H. T and Burke, J. J. 1989. Heat shock protein synthesis and thermotolerance in wheat. Plant Physiol 90: 140–145

[34] Joshi. C. P, Kluveva. N. Y, Morrow. K. J and Nguyen, H. T. 1997. Expression of a unique plastid localized heat shock protein is genetically linked to acquired thermotolerance in wheat. TheorAppl Genet 95: 834-841.

[35] Hervé. D, Fabre. F, Flores Berrios. E, Leroux. N, Al charani. G. H, Planchon. C, Sarrafi. A and Gentzbittel, L .2001.QTL analysis of photosynthesis and water status traits in sunflower (Helianthus annuus L) under green housecondition. J Exp Bot. 52: 1857-1864

[36] Passioura, J. B .1977. Grain yield, harvest index, and water use of wheat. J AustInstAgric Sci. 43: 117–120.

[37] Lawn, R. J .1989. Agronomic and physiological constraints to the productivity of tropical grain legumes and prospects for improvement.Expl Agric. 25: 509528

[38]Egli. D. B, Guffy. R. D and Legget, J. E. 1985. Partitioning of assimilate between vegetative and reproductive growth in soybean. Agronomy journal. 77: 917-922.

[39] Ludlow. M. M and Muchow, R. C. 1990. A critical evaluation of traits for improving crop yields in water-limited environments. Adv. Agron 43: 107-153.

[40] Tuberosa. R, Salvi. S, Sanguineti. M. C, Landi. P, Maccaferri. M and Conti, S. 2002. Mapping morpho-physiololgical traits and yield: Case studies, shortcomming and perspectives in drought-stressed maize. Ann. Bot. 89: 941–963