Genetic analysis of agronomical traits in recombinant inbred lines of cowpea (Vigna unguiculata(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 QTLs involved in the genetic variation of agronomical traits in cowpea under 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
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 plants1,4. 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 sequencing projects have shown that SNPs single bases are the most abundant type of DNA variation occurring frequently in or near coding sequences and may be the cause of functional differences between alleles6. Since the 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 genomics7,9. 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 cost10. 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 such as cowpea, Vignaunguiculata whose whole genome is limited in its genetic resources1,12.

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 yield12,14.

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 cowpea13,15-17. 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 collaboration17. 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 interest18,20. 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 a density 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 stressed conditions.

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 “Bambev21” and “Mouride” cowpea cultivars developed by Senegalese Agricultural Research Center (CNRA) and commonly used by farmers in Senegal. Mouride is a high yielding cowpea cultivar with resistance to drought while the cowpea cultivar Bambev 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 stress conditions at the experimental station of CNRA in Bambe (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 (Institut Senegalais 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 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/m² 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 Bamby21, Mouride and 96 RILs was isolated following the procedure early reported by some authors. 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®, 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 were tested on Bamby21 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. 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. 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. MapQTL 4.0 software was used for QTL analysis according to Muchero et al., with a slight modification. 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 (MGM) 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. 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 MGM analysis. MapChart 2.2 software 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 × Bamby21) for agronomic traits under the two water regimes are summarized in Table 1. Cowpea cultivars Mouride and Bamby21 exhibited 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. Transgressive segregates 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 traits are 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.
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                    | Water treatment | RILS | Water treatment × RILs interaction |
|                 | Mean | range | Mean | range | Mean | range | NS | *** | NS | *** | *** | *** | *** |
| **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 | *** | *** | NS | *** |
| BY              | 2363.1* | 1327.5 | 1425.9 | 369-7216.1 | 3112.* | 2476.2 | 2848.3 | 2.8-98.1 | NS | *** | *** | 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 | 2.8- | NS | *** | *** |
| SWP             | 1.5*  | 0.7   | 0.7  | 0-6.9 | 1.30  | 1.48 | 1.3  | 9.7- | NS | *   | 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 | 873.4 | 991.8 | 0.3- | NS | *** | NS |
| HI              | 0.24  | 0.2   | 0.2  | 1376.32 | 0.45  | 0.32 | 0.4  | 2.3  | 2.8- | NS | *** | *** | *** |

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 significance level 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 number or alphabetical letter for 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 10 were 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 groups indicated 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 Vignaunguiculatalinkage SNP map

| Linkage group (LG) | Size (cM) | Segregation | Mendelian segregation | Distorted segregation | Average density (cM) | Total |
|-------------------|-----------|-------------|-----------------------|-----------------------|---------------------|-------|
| 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                   |
| Total             | 1281,8    | 174         | 125                   | 6,5                   | 299                 |
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 Bambye 21 under well-water treatment

| Trait s | QTLs | LG | Position (cM) | Marker interval | Kruskall–Wallis | MQM |
|---------|------|----|---------------|----------------|-----------------|-----|
|         |      |    |               |                |                 |     |
| W100_1  | 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  | 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  | 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  | 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  | 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   | 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   | SNP_3 | 3  | 47.9-70       | 1_0296-1_162   | 0.05-0.0001     | 1.62-3.50 | 2.2 | 10.2-31.6 |
| SNP_4   | 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   | 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   | DSF_2 | 3  | 28.1-48.9     | 1_1065-1_0964  | 0.05-0.01       | 2.2-1.7  | 2   | 12.4-16.6 |
| PWP8    | PWPL_1 | 1  | 0-10.8        | 1_0731-1_0811  | 0.05-0.001      | 0.4-1.85  | 1.8 | 3.5-11.5 |
| PWP8    | PWPL_2 | 5  | 4-21.3        | 1_0806-1_0419  | 0.05-0.01       | 1.55-1.93 | 1.6 | 10.8-15.7 |
| SNP     | SNPL_1 | 1  | 0-10.8        | 1_0731-1_0811  | 0.05-0.00001    | 1.94-2.64 | 1.9 | 5.2-16.6 |
| SNP     | 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    | PLPL_2 | 10 | 45.4-57       | 1_0780-1_0628  | 0.05-0.001      | 1.83-2.46 | 1.9 | 13.3-16.6 |
| SWP     | SWPL_1 | 1  | 0-10.8        | 1_0731-1_0811  | 0.05-0.001      | 0.5-2.05  | 2   | 2.9-12.7 |
| SWP     | SWPL_2 | 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 |
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 Significance level | LOD | LOD threshold | R² |
|--------|-----|----|---------------|----------------|-----------------------------------|-----|---------------|----|
| 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   | 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   | 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    | 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   | 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 numerals or alphabetical letter for well-watered or water-stressed. Common QTLs across water treatments were shown as bold-face in table 5
DISCUSSION

It is well established and documented that water deficit during reproductive development in cowpea significantly reduces yield\(^1\text{,}^6,^7\). 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\(^14,^28,^30\). A wide range in variation was observed for all measured traits among water-stressed compared with their well-watered counterparts. Some authors have also reported genetic variability for the stress response that could well be seen upon exposure of 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-anthesis drought 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\(^1\). This map has the distinction of allowing a cross reference to recently published cowpea 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\(^3,^12\). The low number of loci detected for agronomical traits in this study may be attributed to low level of polymorphism (SNP)

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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 * at the end of markers.
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. For example, QTLs affecting maturity in cowpea (Mat_b), R² 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 agromorphological 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 agromorphological traits. Data supporting similar relationships of yield with yield component have been reported on sunflower 3. One of the most important chromosomal 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, 35-37. Earlier studies have also recommended maintaining a high harvest index as the best strategy for improving crop yield under water limiting conditions 38. 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 Vigna unguiculata. 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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