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Research Application Summary

Genome Wide Association Mapping for Turcicum Leaf Blight (*Exserohilum Turcicum*) Resistance in Sorghum MAGIC Population

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Abstract

Turcicum Leaf Blight (TLB) caused by *Exserohilum turcicum* is among the most destructive diseases in sorghum causing upto 50 % or more yield losses. Different methods can be used to control TLB and among them is the use of resistant varieties coupled with the use of molecular markers. Therefore, the present study was undertaken to identify Single Nucleotide Polymorphism (SNP) markers and candidate genes associated with TLB resistance. Six multi-locus models of genome wide association study were conducted with Best Linear Unbiased Predictors (BLUPs) obtained from 198 sorghum MAGIC lines screened in Uganda in 2019 using 79,728 SNPs. Fifteen SNPs associated with TLB across seven chromosomes explained phenotypic variation ranging from 0.06-23.99% with the exception of chromosomes 2, 4 and 8. Potential candidate genes were predicted at 220kb down and up-stream of the associated SNP positions including NBS-LRR, NB-ARC and a remorin gene involved in disease response in plants. Results from the study give an insight into understanding the genetic architecture of TLB and the SNPs and candidate genes that can be useful for Marker Assisted Selection after validation.

Key words: BLUPS, candidate genes, MAGIC, Tercicum leaf blight, single nucleotide polymorphism, sorghum

Résumé

Le sorgho (*Sorghum bicolor (L.)* Moench) est une céréale consommée dans le monde entier, en particulier dans les zones arides et semi-arides où il constitue un aliment de base. Il est également utilisé comme fourrage pour les animaux et comme source émergente de biocarburant dans les pays développés. L'une des principales contraintes à la consommation de sorgho est la faible digestibilité des protéines. Le but de cette étude était d'élucider les déterminants génétiques qui influencent la digestibilité des protéines en utilisant une population MAGIC diverse de sorgho. La protéine brute totale (CP) a été déterminée en utilisant la méthode micro-Kjeldhal et le test de pepsine. La protéine in-vitro (IVPD) a été obtenue comme la différence entre la protéine brute et ce qui restaitaprès la digestion par la pepsine, exprimée en pourcentage. L'analyse de variance (ANOVA) a révélé des différences significatives de digestibilité parmi les génotypes testés ($p < 0,05$). Les lignées MAGIC M171, M189, M188, M053 et M178 avaient la digestibilité des protéines la plus élevée et la plus stable avec respectivement

87 %, 84 %, 80 %, 80 % et 78 %. Parmi les témoins, SEKEDO avait une digestibilité des protéines de 78 %, comparée aux quatre scores élevés de digestibilité des protéines pour les lignées MAGIC M171, M189, M188 et M053. L'étude d'association génomique à l'échelle du génome (GWAS) utilisant 45 120 SNPs filtrés sur la base des six modèles multi-locus, a identifié des signaux significatifs pour la CP, la protéine in-vitro (IVP) et la digestibilité des protéines (DGTY) sur les chromosomes chr1, chr2, chr3, chr4, chr6, chr8, chr9 et chr10 dont quinze SNPs étaient associés à DGTY, CP et IVP. Ceux-ci incluaient S1_70639927, S1_66629979, S1_55423981 associés à la digestibilité des protéines (DGTY). S1_2878532, S1_1737743, S1_67953960, S1_52591415, S1_58341942, S1_55391000, S1_55375200 étaient en étroite association avec les protéines brutes et S1_49220551, S1_52851942, S1_61819221, S1_55937022 et S1_56962389 étaient en étroite proximité avec les gènes contrôlant les protéines in-vitro. Le SNP S1_66629979 à 66,7 Mb a été détecté près de Sobic.002G287400, une protéine de type NIN responsable de l'activation de la réponse transcriptionnelle du nitrate en se liant aux éléments cis-répondant au nitrate dans une cascade entraînant une réaction catalytique. Un gène de nitrate réductase (NR) 1, l'un des gènes NIN, catalyse la première réaction dans l'assimilation des nitrates en réduisant le nitrate en nitrite jusqu'à l'ammoniac, puis à l'azote, qui est essentiel dans la formation des acides aminés. L'étude met en évidence des loci candidats impliqués dans la digestibilité des protéines, contribuant ainsi aux efforts d'établissement de la sélection assistée par marqueurs pour ce trait chez le sorgho.

Mots-clés : Digestibilité des protéines, Sorghum bicolor, Ouganda, Population MAGIC, Loci de traits quantitatifs

Introduction

Turcicum leaf blight (TLB) has been known to be a major disease affecting sorghum and maize (Adipala *et al.*, 1993; Ceballos *et al*., 1991). The high evolutionary potential for this pathogen, characterized by the ability to undergo sexual reproduction in the field emphasizes the importance of developing durable resistance. Mating type and race differential study in Uganda suggested a great potential of having more virulent races of *E. turcicum* in the future (Ramathani, 2010), therefore, for disease management, if there are no improvements on the elite released sorghum lines an epidemic is bound to happen. The genetics of turcicum leaf blight resistance in sorghum has been extensively studied using bi-parental populations but are still poorly understood because of several factors, including low marker densities (a pool of mapped restriction fragment length polymorphism (RFLP) and/or simple sequence repeat (SSR) markers) and the small population sizes used in many studies (Beshir, 2016; Zhang *et al*., 2020). However, the new advances in genomic technology and mapping populations like the sorghum MAGIC lines (Ongom, 2016), have greatly increased the larger marker density required for QTL mapping resources thus scientists embrace the Genome-Wide Association studies (GWAS) tool to dissect QTL underlying complex traits and identification of functional genes. Similar studies have been carried out in maize (Kaefer*et al*,.2017) that identified genomic regions on chromosomes 1,2,3,4,6,7,8,9 and 10 that confer resistance to northern leaf blight in maize. Therefore, this study was carried out to identify sources of resistance to TLB in the sorghum MAGIC population in Uganda and to determine the regions that confer resistance to the disease through association mapping studies using MAGIC lines.

Materials and Methods

Plant Materials and Field Trials layout. The plant genetic material used in this study consisted of 198 Multi-Parent Advance Generation InterCross (MAGIC) genotypes and twelve checks. The experiments were conducted in two sites (Makerere University Agricultural Research Institute Kabanyolo (MUARIK) and Ngetta Zonal Agricultural Research and Development Institute (NgettaZARDI) in Lira, northern Uganda. These sites have varied conditions, with MUARIK being humid and wet, and Ngetta ZARDI being drier and with less rainfall. The experiments were laid out in a Randomized Complete Block Design (RCBD) with two replications. Each genotype was planted in two rows 4 metres long plot at a spacing of 75 x 30 cm. The plants were thinned to two plants per stand at two weeks after germination. The plants were protected from stem borers and leaf-feeding insect pests following the recommended plant protection measures. Weeding was done manually at different periods until harvesting.Disease severity assessment was done visually at weekly intervals for five weeks. Disease severity scores were recorded on whole plant basis based on a scale of 1-9. Other agronomic traits that included panicle length and width, days to 50% flowering, stay green, plant height and grain yield were also recorded. Data collected were subjected to analysis of variance (ANOVA) using 'R' statistical software.

Resistance datasets based on AUDPC and weekly scores were used to identify the SNPs associated with resistance to TLB. Genome-Wide Association Study (GWAS) for marker trait association was performed using R software using mrMLM package with 79,728 SNPs markers used to identify genomic regions associated with the disease. Significant SNPs were mapped onto the sorghum reference genome in Phytozome v13 using JBrowse (Goodstein *et al*., 2012) based on physical positions obtained during SNP calling

Results and Discussion

In the current study, there were significant differences among genotypes for all the traits (Table 1), suggesting that there was significant genetic variability in this population and providing an opportunity to select genotypes with TLB resistance and other agronomic traits. Additional new sources of resistance were identified from the MAGIC population as well as the local checks that included genotype 2003-PP34FG-S7-429, 2003-PP34FG-S7-44, 2003-PP34FG-S7-331, 2003-PP34FG-S7-8 and Alenga. Turcicum leaf blight was observed in the two locations albeit at different severity levels and this could be attributed to the variability in the amount and distribution of rainfall, humidity and temperature received in the growing season. Low TLB severity at all dates and AUDPC was observed in MUARIK than in NgettaZARDI indicating low disease pressure in MUARIK. This perhaps could also be due to the farming systems along the Lake Victoria basin where MUARIK is located where sorghum is grown as a secondary crop thus availability of host tissue for infection and maintaining high inoculum levels is low whereas in Lira sorghum is one of the key crops in the farming system (Ramathani, 2010).

Table 1. Combined analysis of variance for Turcicum leaf blight and agronomic traits evaluated across two locations and two seasons (2019 A and 2019 B)

Source of variation	Df	54 DAP	61 DAP	68 DAP	75 DAP	82 DAP	AUDPC
Rep	1	0.1	0.3	0.5	0.0	0.2	1235.0
Location	1	2.3***	44.2***	253.4***	504.6***	945.2***	470725.0***
Season	1	34.4***	84.2***	172.4***	42.0***	64.8***	148046.0***
Genotype	209	0.5***	1.4***	3.0***	3.9***	4.5***	2752.1***
L×G	209	0.2***	0.3***	0.9***	1.3***	1.4***	868.0***
S×G	209	0.2***	0.3***	0.6***	0.8***	1.0***	519.8***
L×S	1	13.2***	06.8***	4.4***	52.9***	251.5***	7842.0***
L×S× G	209	0.2***	0.2***	0.6***	0.9***	1.0***	554.7***
Residuals	839	0.1	0.1	0.4	0.6	0.6	315.7
Mean		1.3	1.9	2.4	2.7	3.1	78.7
CV (%)		43.2	40.3	52.7	54.6	50.1	45.0

***=p<0.001; **=p<0.01; *=p<0.05; SOV=Source of variation ; df==degrees of freedom ; DAP=Days after planting; AUDPC=Area under disease progress curve

Genome-wide association analysis based on the six multi-locus models identified a total of fifteen SNPs distributed across the 10 chromosomes of sorghum with the exception of chromosomes 2, 4, 7 and 8 (Figure 1). Interestingly, the most significant SNPs were harbored on chromosome five and six that include S1_302160520, S1_302527383, S1_302517403, S1_302529375, S1_302517420, S1_302561410 and S1_302318685. The SNP markers linked to TLB disease resistance guided in identification of several candidate genes identified through GWAS that could potentially play a role in countering pathogen attacks in sorghum. Several positional candidate genes among them several nucleotide-binding-site, leucine-rich repeat (NBS-LRR),NB-ARC and remorin gene REM39 involved in plant defence responses (Mayada *et al.*, 2016; Zhang *et al*. 2020).

Conclusions

Identified new disease-resistant accessions like genotype 2003-PP34FG-S7-429, 2003-PP34FG-S7-44,2003-PP34FG-S7-331 and 2003-PP34FG-S7-8 among others from the sorghum MAGIC population can be intergrated in sorghum breeding programs whereby the identified resistance sources may be potential parents in a hybrid development program. Significant SNPs mostly on chromosome 5 identified accounted for 19%–25% of the genetic variance, suggesting that these genomic regions have relatively large major gene effects on resistance to TLB. The SNPs can be converted into robust markers and validated in different populations and genetic backgrounds and used for marker-assisted selection programme or used in genomic selection scheme for TLB resistance in sorghum.

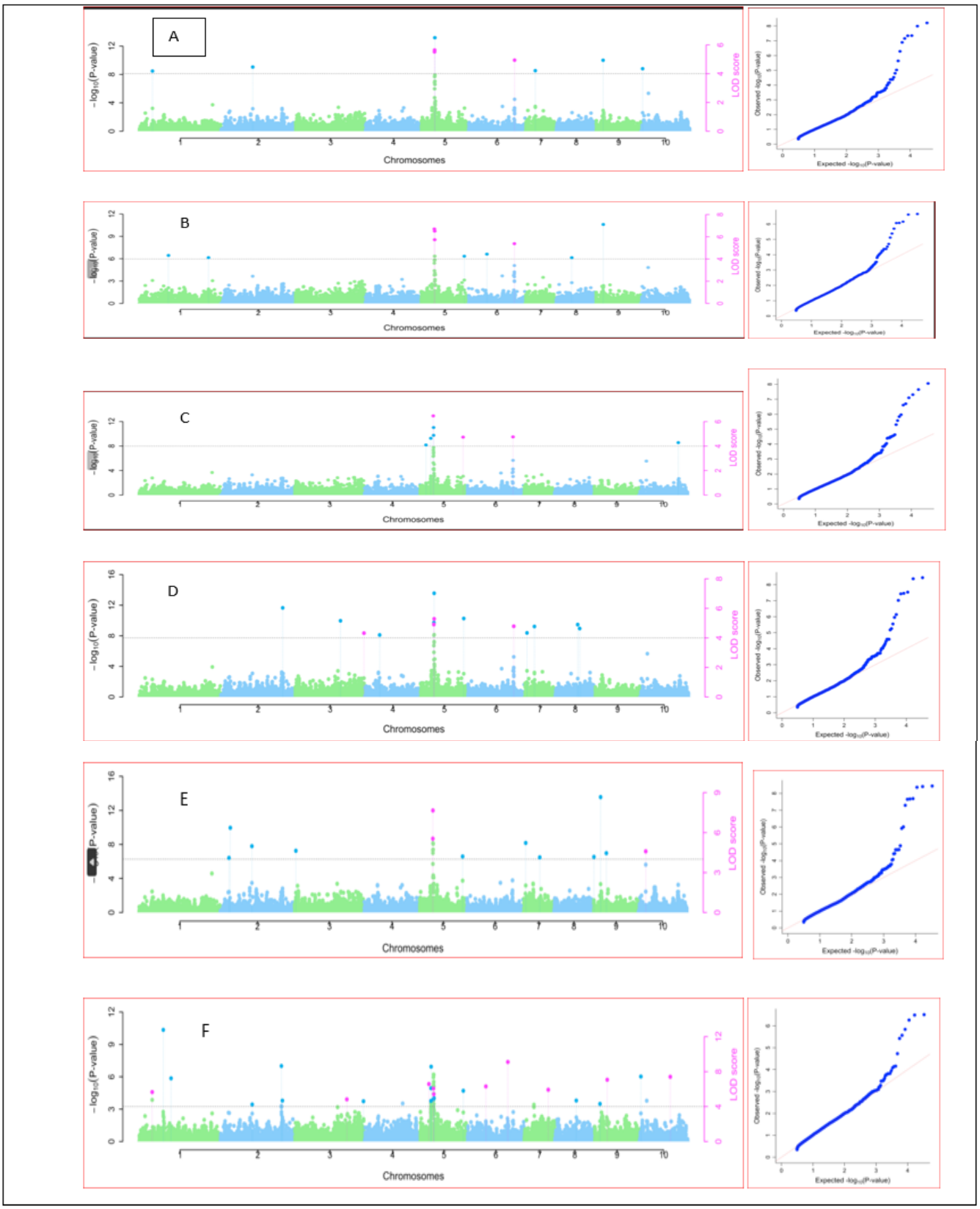

Figure 1. Manhattan plots for combined across environments analysis and respective quantile–quantile (QQ) plots for sorghum MAGIC population. The x-axis shows the 10 sorghum chromosomes with physical positions, the y-axis displays the −log10 (p)-values. The threshold with a critical logarithm odd of 4. A= Area under disease progress curve=severity at 82 DAP; C= severity at 75 DAP; D= severity at 68 DAP; E= severity at 61 DAP; F=severity at 54 DAP

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