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Application of High Density Linkage Map Derived from Genotyping by Sequencing for Detection of QTL Conferring Resistance to Leaf Rust Races Spread in Egypt

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Abstract: Wheat is a major source of carbohydrates in Egypt, leaf rust disease is known to be the most common rust disease affected wheat genotypes. This study aimed to apply a constructed high density linkage map through Genotyping-by-sequencing (GBS) for detection of QTL resistant to important leaf rust races spread in Egypt. The applied map contained 3,641 markers distributed on 21 chromosomes and spanned 1,959 cM with an average distance of 1.8 cM between markers. A mapping population of 204 RILs (F6:8) obtained from the cross between two parents of winter wheat 'Harry' x 'Wesley' through single seed decent method was used to identify QTL region associated with leaf rust resistance genes in wheat. Under the Green house condition in Egypt leaf rust pathotypes i.e NTTJT, PTTGS, PTTTT, TTTBT and TTTTT were used. High-density linkage map based on GBS derived SNPs were applied in this study for QTL mapping. The wheat genotype Harry was resistant to all tested pathotype while, the wheat genotype Wesley was susceptible to all tested pathotype. One major stable QTLs, for race NTTJT was identified on chromosome 6A flanked by markers XSNP3958 and XSNP3957 with a LOD 4.22. The identified SNP marker may be used to screen for resistance to specific races of leaf rust found in Egypt in further spring wheat breeding programs as marker assisted selection.

Keywords: Wheat, Leaf Rust, QTL Mapping, GBS by Sequencing

1. Introduction

Wheat is the most important cereal crop, cultivated in a vast area across the world. Leaf rust disease considered as a one of the main diseases threaten wheat in most of its production regions. Wheat improvement programs are annually intensified to release resistant wheat cultivars [1-3].

In Egypt, leaf rust is the most common and important wheat diseases. It causes severe losses in grain yield which reached 23% on some varieties depending on environmental conditions, level of resistance, dominant physiologic races and the stage of crop development when initial infection occurs [4-7].

The winter wheat population used in this study was established to widen its application for identifying QTLs for various agronomic and to breed these traits into commercial wheat cultivars and breeding lines [8]. Identified QTL linked to important genes may be used as marker assisted selection in wheat breeding programs and to verify the presence of genes of interest. Transfer of genes from winter and spring wheat through hybridization is known to be adopted and gave promising results [9, 10]. It has been estimated that 80% of the advanced spring wheat lines at CIMMYT (Mexico) carry at least some of the genetic material from winter types [11].

Innovations and advances in next generation sequencing tool like genotyping-by-sequencing (GBS) is utilized for SNP

detection and genotyping [11-13].

One of the most important applications of the constructed high-density genetic maps is the study of quantitative trait loci (QTLs) linked to important agronomical traits for different crops. Also benefit can be gained from its ability to concentrate on regions of concern, and reduce data set in more controllable manner [14]. Plant breeding programs are based on variation and polymorphisms of DNA of different crops. This variation can be detected by using DNA markers. The introduction of new technology for genotyping like technology known as next-generation sequencing (NGS) has paved the road of new opportunities for high-throughput genotyping in various plant species to be used to detect and score single nucleotide polymorphisms (SNPs) [15, 16]. After the construction a high-density linkage map QTL analysis can be carried out to detect SNP markers associated with traits like leaf rust resistance [17].

The objective of this study is to detect QTLs resistant to important leaf rust races spread in Egypt using constructed high density linkage map through genotyping-by- sequencing (GBS) under the Egyptian environmental conditions.

2. Materials and Methods

2.1. Plant Materials

A mapping population of 204 RILs (F6:8) obtained from the cross between 'Harry' x 'Wesley' through single seed decent method was used in this study. Seeds were obtained from the seed laboratory of Nebraska University Lincoln, USA and were evaluated at seedling stage under the Egyptians condition for detection of SNP linked to QTL for leaf rust resistance.

2.2. Assessment of Seedling Reaction of Wheat Genotypes Against leaf Rust Races Under Greenhouse Condition

The assessment study was done in the greenhouse of Wheat Diseases Res. Dept., Plant Pathology Res. Inst., ARC, Giza, Egypt during 2017/18 growing season. Seeds of 206 tested wheat genotypes were sown in 6 cm square plastic pots. Five seeds of the different wheat genotypes were sown in the corner of each pot in a clockwise order. Seven days old seedlings of the tested genotypes, when the first leaf full emerged, were inoculated with each of the urediniospores of the five pathotypes i.e. NTTJT, PTTGS, PTTTT, TTTBT and TTTTT which were the most common during 2017/18 growing season [18]. Inoculation was carried out by shaking with urediniospores over seedling leaves of the tested genotypes. The Inoculated seedlings were incubated in moist chamber for 24 h at 18-20ºC and 100% RH, and then moved to greenhouse benches for 12 days at 20± 2°C [19].

2.3. Disease Assessment

Infection type (IT) data for each of the tested wheat genotypes were recorded 12 days after inoculation using standard infection type scoring scale; 0 - 4 [20]. Infection type 0 (no visible signs of infection), 0; (hypersensitive reaction or flecks), 1 (small uredinia surrounded by necrotic area) and 2 (moderate size of uredinia surrounded by necrosis) were considered as low infection type (resistant) and infection types 3 (moderate size of uredinia without necrosis or chlorosis) and 4 (large size of uredinia) were considered as high infection type (susceptible).

2.4. Marker Data Information of the Used High-Density Linkage Map

The plant materials used in this study were genotyped using GBS library construction, genotyping and SNP calling based on the high-density linkage constructed by Hussain *et al.* (2017) [8]

2.5. QTL Mapping

QTL mapping was performed using BIP functionality in QTL ICI Mapping v4.181. Inclusive Composite Interval Mapping of Additive (ICIM-ADD) function in QTL ICI Mapping was selected as mapping method to detect additive QTL. The mapping parameters step for ICIM-ADD was set at 1.0 CM, and a probability of 0.05 in stepwise regression was selected for each mapping method. The LOD threshold for QTL detection was determined by 1000 permutation test analyses using a Type I error set at $P < 0.05$. Disease score values were used to map QTLs using ICI Mapping Software'.

3. Results and Discussion

Evaluation of the tested genotypes against leaf rust pathotypes at seedling stage under greenhouse condition

The reaction of the two parents; Harry and Wesley and 204 (F6:8) genotypes obtained from the cross between two parents through single seed decent method were evaluated at seedling stage under greenhouse condition using five pathotypes i.e. NTTJT, PTTGS, PTTTT, TTTBT and TTTTT (Table 1). The wheat genotype Harry was resistant to all tested pathotypes. While, the wheat genotype Wesley was susceptible to all tested pathotypes. A total of 79 wheat genotypes were resistant and 125 were susceptible against pathotype NTTJT. On the other hand, a total of 77 wheat genotypes were resistant and 127 were susceptible against pathotype TTTTT. A total of 75 wheat genotypes were resistant and 129 were susceptible against pathotype PTTGS. While, a total of 73 wheat genotypes were resistant and 131 were susceptible against the two pathotypes PTTTT and TTTBT (Table 1).

Table 1. *Evaluation of the two parents Harry and Wesley and 204 wheat genotypes from the crosses between the two parents against five leaf rust pathotypes at seedling stage under greenhouse condition during 2017/18 growing season.*

No.	Genotype	Leaf rust pathotype / Infection type					No.	Genotype	Leaf rust pathotype / Infection type				
		NTTJT	PTTGS	PTTTT	TTTBT	TTTTT			NTTJT	PTTGS	PTTTT	TTTBT	TTTTT
1	HW_8	3	3	4	4	4	54	HW_92	0	3	4	4	3
2	HW_9	3	3	4	4	4	55	HW_93	3	4	3	4	3
3	HW_11	0	0,	1	0	0	56	HW_97	3	4	3	4	3
4	HW_12	3	3	3	4	3	57	HW_98	0	3	3	3	3
5	HW_13	1	0	0,	2	1	58	HW_100	2	2	1	2	1
6	HW_15	4	3	4	4	3	59	HW_102	4	3	3	4	3
7	HW_16	3	3	4	4	3	60	HW_103	4	3	3	4	3
8	HW_17	3	0	4	4	4	61	HW_104	0	0,	2	4	1
9	HW_18	0,	2	3	2	0,	62	HW_106	3	3	4	4	0
10	HW_20	3	3	0,	3	3	63	HW_109	4	4	4	3	2
11	HW_24	3	3	2	3	3	64	HW_110	0,	0,	1	0,	4
12	HW_25	0,	1	4	2	3	65	HW_113	3	1	4	3	4
13	HW_26	3	3	0,	3	3	66	HW_115	3	1	4	3	3
14	HW_27	0,	0,	1	1	0,	67	HW_117	3	4	4	4	3
15	HW_29	1	0	0,	2	1	68	HW_118	4	4	4	4	4
16	HW_30	0,	1	4	4	0,	69	HW_119	1	3	0,	0	2
17	HW_31	3	3	1	3	4	70	HW_120	3	3	4	4	3
18	HW_37	1	2	2	3	3	71	HW_121	3	0,	0,	0,	1
19	HW_40	3	4	4	3	4	72	HW_122	0,	0,	1	1	1
20	HW_41	4	4	4	3	4	73	HW_123	0,	1	1	2	2
21	HW_43	0	2	1	1	0,	74	HW_124	3	1	1	3	3
22	HW_44	3	3	4	3	3	75	HW_125	4	3	4	3	4
23	HW_46	1	0	0,	0	0	76	HW_127	4	3	4	3	4
24	HW_47	3	0	3	3	4	77	HW_128	3	0,	4	4	3
25	HW_48	0	1	2	0	0	78	HW_130	3	4	4	0	2
26	HW_52	3	4	3	3	3	79	HW_131	3	4	4	4	4
27	HW_53	0,	2	3	0,	0,	80	HW_132	3	4	4	4	4
28	HW_54	3	3	3	3	3	81	HW_133	2	1	0	2	0,
29	HW_56	3	3	0	1	0,	82	HW_135	3	4	4	4	4
30	HW_62	3	3	3	3	3	83	HW_136	3	3	3	3	4
31	HW_64	4	0,	0,	1	0,	84	HW_137	3	1	0	2	2
32	HW_65	4	3	4	4	4	85	HW_138	3	3	3	3	3
33	HW_66	3	3	4	4	4	86	HW_139	3	3	3	3	3
34	HW_67	4	3	4	4	4	87	HW_140	2	3	3	3	2
35	HW_68	3	3	0	4	4	88	HW_141	3	3	4	4	0
36	HW_69	3	0	4	4	4	89	HW_142	3	1	3	1	0
37	HW_70	3	4	0	4	3	90	HW_144	3	0	3	3	3
38	HW_71	3	4	3	4	4	91	HW_147	3	1	0,	0	2
39	HW_72	4	4	4	4	4	92	HW_148	3	3	3	3	3
40	HW_75	4	4	4	4	1	93	HW_149	3	0	0	0	2
41	HW_76	4	4	0	4	4	94	HW_152	3	2	0,	0	1
42	HW_78	1	4	1	1	4	95	HW_153	3	3	3	0	3
43	HW_79	2	0,	1	1	3	96	HW_154	1	3	3	3	3
44	HW_80	3	3	2	4	3	97	HW_155	1	2	2	1	0,
45	HW_82	3	3	3	0,	3	98	HW_157	0,	2	1	2	2
46	HW_83	1	2	0,	1	3	99	HW_159	3	3	3	3	1
47	HW_84	2	1	0,	0	1	100	HW_160	3	3	3	3	3
48	HW_85	3	3	4	4	4	101	HW_164	4	3	4	4	4
49	HW_86	3	3	4	4	3	102	HW_165	4	4	3	3	4
50	HW_87	3	3	4	4	4	103	HW_167	4	0	4	4	4
51	HW_88	0	0,	1	1	0,	104	HW_169	4	3	4	0,	3
52	HW_89	0	2	0,	1	1	105	HW_170	4	3	3	3	3
53	HW_90	0	0,	1	0,	3	106	HW_172	4	2	4	3	3

Table 1. *Continued.*

No.	Genotype	Leaf rust pathotype / Infection type					No.	Genotype	Leaf rust pathotype / Infection type				
		NTTJT	PTTGS	PTTTT	TTTBT	TTTTT			NTTJT	PTTGS	PTTTT	TTTBT	TTTTT
107	HW_173	3	3	4	3	0	157	HW_235	0,	0,	0,	1	0
108	HW_174	1	2	0	0,	1	158	HW_236	3	4	4	3	3
109	HW_175	4	3	3	3	4	159	HW_237	2	4	1	2	0,
110	HW_176	1	3	1	2	0	160	HW_238	3	4	3	3	3
111	HW_177	1	4	4	4	4	161	HW_239	3	4	3	2	3
112	HW_178	1	4	0,	4	4	162	HW_240	3	4	3	4	3
113	HW_179	2	4	3	0	3	163	HW_241	4	4	4	4	4
114	HW_181	2	4	3	4	3	164	HW_242	4	4	4	3	4
115	HW_182	3	3	0	1	2	165	HW_243	3	4	4	4	4
116	HW_184	0	3	3	0	3	166	HW_244	4	4	4	3	4
117	HW_186	3	4	0	0	3	167	HW_245	4	2	3	0,	1
118	HW_187	0	4	0	0	1	168	HW_246	2	2	1	3	3
119	HW_191	0,	2	0	0	1	169	HW_247	1	3	4	3	0,
120	HW_192	3	3	4	3	4	170	HW_248	3	3	3	4	4
121	HW_193	3	2	3	0	4	171	HW_249	3	0	3	3	4
122	HW_194	0,	3	1	0,	2	172	HW_250	3	1	4	3	4
123	HW_195	0	3	0,	0,	3	173	HW_251	0,	2	3	0,	0,
124	HW_197	0,	1	1	0	0	174	HW_252	1	1	0,	2	1
125	HW_198	0,	0,	2	1	0,	175	HW_253	0	0	4	4	3
126	HW_199	0,	1	2	1	1	176	HW_254	0	3	4	4	3
127	HW_200	0	3	4	3	4	177	HW_255	0	3	4	4	4
128	HW_201	0	1	0	2	2	178	HW_256	0	3	4	4	2
129	HW_203	3	4	3	4	1	179	HW_257	4	3	4	4	4
130	HW_206	1	0,	0	2	1	180	HW_258	4	3	3	3	0,
131	HW_207	3	4	4	4	4	181	HW_259	0,	1	1	0,	0,
132	HW_208	1	3	2	2	1	182	HW_260	3	3	3	3	3
133	HW_209	2	0,	1	2	1	183	HW_261	0,	3	3	4	3
134	HW_210	3	3	3	3	3	184	HW_262	4	3	3	4	4
135	HW_211	1	3	1	0,	0,	185	HW_263	4	3	3	4	4
136	HW_212	4	3	3	4	3	186	HW_264	2	3	1	4	1
137	HW_213	3	4	3	4	3	187	HW_265	4	4	3	0	3
138	HW_214	3	3	3	4	3	188	HW_266	3	4	3	3	3
139	HW_215	3	4	4	3	3	189	HW_267	2	4	0,	4	3
140	HW_216	3	4	4	3	3	190	HW_268	3	4	3	0,	2
141	HW_217	0,	0,	0,	3	0,	191	HW_269	1	0	3	0,	0,
142	HW_218	0,	2	1	0,	1	192	HW_270	3	4	3	4	3
143	HW_219	4	3	3	3	3	193	HW_271	3	1	1	4	3
144	HW_221	2	3	2	2	4	194	HW_272	0,	0	3	4	2
145	HW_222	3	3	4	4	4	195	HW_273	3	3	4	2	1
146	HW_224	1	1	0,	1	0,	196	HW_274	3	3	4	3	3
147	HW_225	0,	3	4	3	0,	197	HW_275	3	3	4	3	3
148	HW_226	0,	0,	1	2	0,	198	HW_276	1	0,	4	3	2
149	HW_227	1	3	2	2	1	199	HW_277	0	0	4	4	4
150	HW_228	3	4	3	4	3	200	HW_278	3	2	4	4	0
151	HW_229	4	1	4	4	3	201	HW_279	3	3	3	4	4
152	HW_230	4	2	4	4	3	202	HW_280	3	3	3	4	4
153	HW_231	1	3	0,	0,	0,	203	HW_281	3	3	2	1	2
154	HW_232	4	3	4	4	1	204	HW_282	0,	0	1	2	1
155	HW_233	4	2	3	3	3	205	Wesley	3	4	3	3	3
156	HW_234	4	4	4	3	3	206	Harry	0	0,	0,	1	1

The five leaf rust pathotypes i.e. NTTJT, PTTGS, PTTTT, TTTBT and TTTTT used in this study were the most common and frequent in Egypt during 2017/18 growing season [18]. The 20 differential monogenic used for identification and designation of these leaf rust resistance genes in Egypt were *Lr* 1, 2a, 2b, 2c, 3a, 3ka, 9, 10, 11, 14b,

15, 16, 17, 18, 21, 24, 26, 30, 36 and 42 [21]. These races were virulence to most of the 20 differential monogenic lines. The leaf rust resistance genes *Lr* 2a, *Lr* 9, *Lr* 10 and *Lr* 14b are the most effective genes at adult plant stage under Egyptian field conditions [5, 22].

In this study A mapping population of 204 RILs (F6:8) obtained from the cross between two parents of winter wheat 'Harry' x 'Wesley' through single seed decent method was used to identify QTL region associated with leaf rust resistant genes in wheat. The QTL analysis detected single QTL associated with leaf rust resistance evaluated under the Egyptian environment in 2017/18 (Table 2). NTTJT was found to be located on chromosome 6 A flanked by markers XSNP3958 and XSNP3957 with a LOD 4.22 (LOD score of 3 declared significant QTL). Additive effect QTL was detected on chromosomes 6A with phenotypic variance explained by individual QTL 9.7% in the RIL population. The additive effects (9.7) of detected QTL was negative suggesting increasing alleles for leaf rust resistance was contributed by the parent 'Harry' susceptibility from 'Wesley'.

Studies for identification leaf rust genes on chromosome 6A were reported by many researchers [12, 23, 24]. Spielmeyer et al. (2007) [25] identified a QTL on chromosome 6A that contribute for 8% of the variation for coleoptile length, 14% of seedling leaf width and was associated with increased plant height. Yang et al. (2012) [26] identified SNP and allelic-specific PCR markers development for TaGW2-6A, a gene linked to wheat kernel weight. Su et al. (2011) [27] Identified and developed a functional marker of TaGW2 associated with grain weight in bread wheat (Triticum aestivum L.). Yang et al. (2012) [26] identified SNP and allelic-specific PCR markers for TaGW2, a gene linked to wheat kernel weight. A splice acceptor site mutation in TaGW2-A1 increases thousand grain weight in tetraploid and hexaploid wheat through wider and longer grains [28].

Inheritance of resistance to leaf rust Puccinia triticina Erik studies on common wheat *Triticum vulgare* were started from approximately one hundred years ago [29, 30]. Recently advances in DNA tools and molecular markers tools were able to detect a significant number of rust genes distributed across wheat cultivars around the world and their wild relatives. Currently, there are 80 genes detected for leaf rust, 49 genes were identified for yellow rust and 58 genes for stem rust [30-33].

Many studies that have been carried out on the genetic characterization of the genes responsible for resistance against leaf rust in wheat have shown that the genes responsible for the resistance at the seedling stage is monogenic and race specific [34].

Table 2. *Significant QTL detected for leaf rust resistance using high-density linkage map.*

Trait ID	Trait Name	Chr. 1	Pos2	Left Marker	Right Marker	LOD	PVE (%)3	Add4
1	NTTJT	6A	14	XSNP3958	XSNP3957	4.22	9.698	-0.44

1 Chromosome where the QTL was located.

2 Chromosome position (cM) of the QTL.

3 Phenotypic variance explained.

4 Additive effect (positive values of the additive effect indicated that alleles from parent 'Harry' were in the direction of increasing trait score and negative indicated that alleles from parent 'Wesley' were in the direction of increasing trait score).

Both winter or spring are very related and are belong to *Triticeae*, they have two different growing seasons. It has been assessed that eighty percent of the nontraditional modern spring wheat lines at CIMMYT (Mexico) carry at least some of the genetic material from winter types. Based on the pattern of expansion of coverage using winter X spring (WxS) derivatives, an increase of 8% yield was attributed to the use of winter wheat in W x S crosses [25, 36, 37]. Mapping of the vernalization genes in wheat was followed by cloning and sequencing of the genes in a less complex diploid relative of common wheat, *T. monococcum* [38]. These findings encourage breeding programs involving both spring and winter wheat to invest the identified gene in winter and spring wheat genetic improving programs.

4. Conclusions

This Study identified one major stable QTLs, for race NTTJT on chromosome 6A flanked by markers XSNP3958 and XSNP3957 with a LOD 4.22. To date, no GBS analysis to locate QTLs for leaf rust races spread in Egyptian environment in wheat has been conducted, thus the recording of these GBS sequence tags and associated QTLs will assist wheat breeders in developing wheat varieties with promising alleles through the use of marker assisted selection.

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