Symposium Paper* **ورقة قدمت في حلقة علمية***

Molecular Tools Developed for Disease Resistant Genes in Wheat, Barley, Lentil and Chickpea: A Review

Aladdin Hamwieh[1], Fida Alo[1] and Seid Ahmed[2]
(1) International Center for Agricultural Research in the Dry Areas (ICARDA), Cairo, Egypt, Email: a.hamwieh@cgiar.org; (2) ICARDA, Rabat, Morocco.

Abstract

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The major objective of using molecular tools by the plant pathologists is to identify markers linked to the resistant genes. The best markers are those located within the genes or tightly linlked to the resistant genes. This paper will review the most recent markers/QTL linked to resistant genes reported in wheat (strip rust, leaf rust, and stem rust), barley (stripe rust, leaf rust, and stem rust), lentil (Fusarium wilt, Ascochyta, Anthracnose and Stemphylium blight) and chickpea (Ascochyta blight and Fusarium wilt). The markers tightly linked to the resistant genes could be used as markers assisted selection in the breeding program to improve effectiveness and efficiency of variety development.

Introduction

Many cultivars of wheat, barley, lentil, faba bean and chickpea cultivars are released in CWANA through screening of the materials under field condition in addition to have high yield. However, field phenotyping is affected by variation in pathogen population and weather conditions as well as disease measurements. The molecular markers linked to the gene of resistance could be fast and reliable way for selection. However, to find these kind of markers are not easy because many disease resistant mechanisms are quantitative traits as they are controlled by many genes across the genome. Furthermore, multiple pathogens have races indicating multiple resistant genes, therefore, plant breeder requires many markers linked to these genes in order to develop germplasm with combined resistance.

Such markers used for selection are called markers assisted selection (MAS). Unfortunately, there is no enough MAS are available for the breeders and there is a need of more research to identify better markers linked/associated with resistant genes. In cereals and mainly in wheat, maize and rice, more genomic DNA sequences have been developed and published in the public domain. This huge database enabled the scientists to identify more MAS to be applied widely to these crops (http://maswheat.ucdavis.edu/). Unfortunately, the cool season food legumes (mainly in lentil, chickpea and faba bean) have less research and less data sequence in the public domain, and therefore they need more researches to identify markers linked to resistant genes in the future.

In this review, we present the latest marker/QTL reported for resistant genes and MAS in the breeding program in wheat, barley, lentil and chickpea.

Wheat

In recent years the most important limiting factors of wheat production are Leaf rust (*Puccinia triticina* Eriks.), stripe rust (*Puccinia striiformis* f. *tritici* Eriks.) and stem rust (*Puccinia graminis* f. sp. *tritici*)

Wheat strip rust (*Yr*) (*P. s. tritici*) - The 90K single nucleotide polymorphisms (SNP) chips have been used to map numerous QTL associated to stripe rust resistance in various panels of wheat germplasms using the genome-wide association study (GWAS) approach (8, 43, 51, 63, 74). The genotyping by sequencing (GBS) technique uses new sequence technologies have been applied also to explore SNP markers. Li *et al.*, (41) constructed a consensus map containing 28,644 GBS sequence tags at 3757 loci with the average marker distance 0.88 cM, and three rust resistance genes (Yr18/Sr57/Lr34, Yr29/Sr58/Lr46, and Yr30/Sr2/Lr27) and 15 QTL were validated with high resolutions. The large number of diversity array technology "DArT" and SNP markers provided a powerful tool to identify new resistant and virulent genes (8, 38, 45, 71). For example, several SNP markers were determined and validated for *Yr62, YrSP* and *Yr76* (22, 47, 72), *Yr51* (55), *Yr56* (5) and *Yr58* (13).

Several simple sequence repeats markers (SSR) such as *Xgwm533* (120 bp) have been identified and used in MAS to incorporating resistance genes against yellow, leaf and stem rust resistance genes Yr30/Lr27/Sr2 into wheat cultivars, (62), and *cssfr1- cssfr7* marker for the genes *Yr18/Lr34/Sr57Pm38* (40). A cleaved amplified polymorphic sequences (CAPS) marker *XLr57/Yr40-MAS-CAPS16* was an effective marker for gene *Yr40/Lr57* (39).

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SSR markers used for *Yr* genes only were: *Xbarc8* for *Yr15* (54), *Xcfa2149* for *Yr48* (44); and *Xwmc776* for *Yr60* (31), *Xpsp3000 and Gli-B1* for gene Yr10 (6), *Xsun533* and *Xsun476* for *Yr58* (13), *STS7/8* for *Yr5* (12), and three markers *Xbarc8*, *Xgwm413* and *Xgwm273* for *Yr15* (50, 73).

Wheat leaf rust (*Lr*) *(P. triticina)* - To date, 41 genes for leaf rust have been transferred from wild relatives to cultivated genotypes with minor effects (14, 49). Many molecular markers linked to *Lr* resistance genes have been developed. For example: A sequence-tagged site (STS) marker *RGA567-5* were reported for wheat leaf rust resistance genes *Lr1* (15), random amplified polymorphic DNA (RAPD) markers (*S1302609*, *S1326615* and *OPAB-1388*) were successfully converted to sequence characterized amplified region (SCAR) markers co-segregate with *Lr24* (26), SSR marker *cssfr* were reported as marker linked to *Lr34* (40), PCR marker derived from the restriction fragment length polymorphism (RFLP) named as *ABC465* was reported as marker linked to *Lr47* gene (30), *cfd1* and *gwm*508 are two SSR markers located on the short arm of chromosome 6B were used to map the gene *Lr53* (16). SSR markers *cfd71* and *cfd23* were reported as linked to *Lr67* resistant gene (34). The SSR merker *barc130* was linked to *Lr70* (33). Zhou *et al*. (76) developed the STS marker *Hbsf-1* to detect *LrZH84* and *Lr26 resistant genes*. Similarly, Ayala-Navarrete *et al.* (4) developed STS markers linked genes *Lr19* and *Sr25* on chromosome 7DL. In addition, *Xgdm35* marker was reported to be closely linked to Lr41 gene (61). *Lr50* is linked to the microsatellite markers *Xgwm382* and *Xgdm87* on 2BS chromosome (7). More recently, Lr18 using Xgpw7425 and Xwmc75 SSR markers on chromosome 5BL can be used for gene postulation and MAS in wheat (58).

Wheat Stem rust (*Sr*) *(P. g.* f. sp. *Tritici)* - Several markers linked to *Sr* resistant genes have been identified. In brief, *gwm533*, *gwm389* and Stm559n SSR markers were tightly linked to *Sr2* (29, 62), *Xwmc453* and *Xcfd43* markers were linked to *Sr6* (66), SSR marker *Xgwm47* link to *Sr9a* (67), *barc71* and *wmc633* markers were linked to *Sr24* (47, 53), *BF145935* marker linked to *Sr25* (42), BE518879 marker linked to *Sr26* (42, 47), SSR markers *Xbarc51* (codominant), *Xcfa2076* (dominant) and *Xwmc169* (codominant) were used to verify the presence of S*r35* (75), SSR *marker Xwmc477* link to *Sr36* (68), CAPS marker *VENTRIUP-LN2* used for detecting the presence of genes *Sr38*, *Lr37*, *Yr17* and *Sr38* (30), Sr39F2/R2 marker link to *Sr39 (Sr39/Lr35)* (24), FSD-RSA marker reported as the closest marker to *SrCad* resistant gene (35).

Barley

The most important limiting factors of barley production are Barley stripe rust (*P. striiformis* f. sp. *hordei*), Barley leaf rust (*P. hordei*), and Barley stem rust (*P. graminis* f.sp. *tritici*).

***Barley stripe rust (P. striiformis* f. sp. *hordei)* -** Loci contributing to stripe rust resistance observed at the seedling stage have been reported in barley (65). These loci were mapped on chromosome 1H and 6H, using 99 polymorphic markers (11) mapped *rpsGZ* to the long arm of chromosome 4H. Niks *et al*. (52) conjectured that the major gene on chromosome 4H conferring resistance to *P. striiformis* f. sp. *hordei* (*Rpsh*) in the spring barley line 'L94' (*Clho 11797*) was the same as *rpsGZ*. Marker-assisted selection (MAS) has been used to pyramid stripe rust resistance genes derived from different sources into a single line (9, 10, 56). SNPs markers were used to identify three genes on chromosome 4H, 6Hand 7H (20). More recently, eight genes for stripe rust were mapped using Infinium iSelect 9K chip consisting of 7,864 SNPs (70).

***Barley leaf rust* (*P. hordei*) -** *Barley leaf rust* is one of the most serious and damaging foliar pathogens of barley worldwide. Up to day, 25 major genes (*Rph1-Rph24*) conferring resistance to *Ph* have been reported (77). Mammadov *et al.* (48) evaluated diagnostic molecular markers for *Rph5* and *Rph7* resistant genes. They recommended STS markers (*TC2863-12.4* and *ABG70*) as well as *SSR* marker (*AY642926- CA11)* as the most reliable markers for use in MAS for *Rph5* and *Rph7*. Hickey *et al.*, (32) reported another MAS (*bPb-0837*) on chromosomes 5HS closely linked to APR gene *Rph20*. More recently, Singh *et al.* (61) developed a PCR-based marker (*Ebmac 0603*) closely linked to APR gene Rph23. More recently, two loci for leaf rust resistance were mapped using Infinium iSelect 9K chip consisting of 7,864 SNPs (70).

***Barley stem rust* (*P. graminis*) -** Three SSR markers linked to (*Rpg1*) resistant gene have been reported (*RPG1-R-RPG1-N AND RPG1-S*), and two markers (Rpg5-LRK and Rpg5-LRK/PP2C) were reported to (*Rpg5*) gene (18).

Lentil

Lentil is affected by many foliar and soil bore pathogens in many countries. Resistance breeding is the major strategy in reducing the impacts of diseases.

Lentil Fusarium wilt (*Fusarium oxysporum* f. sp. *lentis*)- Several mapping studies indicated different linkage map lengths varied from 751 cM to 4060.6 cM (1, 2, 3, 17, 19, 21, 25, 27, 28, 57, 60, 64). The number of markers were also varied from 283 (28) to 9,793 (2). Hamwieh *et al*. (28) identified Fusarium wilt resistant gene localized on linkage group 6, and this resistance gene was flanked by microsatellite marker SSR59-2B.

Lentil Anthracnose *(Collethotricum truncatum)* - Resistance gene (Lct-2) was mapped by Tullu *et al*. (69). Ascochyta blight resistance is governed by minor genes and three QTLs accounting for 47% (QTL -1 and QTL -2) and 10% (QTL -3) are identified using an F-2 population derived from ILL7537 × ILL6002. Moreover, three QTLs each were detected for resistance at seedling and pod/maturity stages (27). Together these accounted for 34 and 61 % of the total estimated phenotypic variation and demonstrated that resistance at different growth stages is potentially

conditioned by different genomic regions. Recently, QTLs conferring resistance to Stemphylium blight (Stemphylium botryosom) and rust (*Uromyces fabae*) were identified in lentil (59). However, still none of these markers have been applied yet as MAS in lentil breeding program.

Chickpea

In chickpea (*Cicer arietinum* L.), Fusarium wilt (FW) caused by *Fusarium oxysporum* f.sp. *ciceris* and Ascochyta blight (AB) caused by *Ascochyta rabiei* are the two major biotic stresses that cause significant yield losses.

Chickpea Fusarium wilt (*F. o.* f. sp. *ciceris*) - Garg *et al.* (23) used 188 recombinant inbred lines derived from a cross JG 62 × ICCV 05530 to identify the QTL conferring AB and FW resistance in chickpea. Their results indicated significant variation between the lines for FW and AB resistance. Five QTLs were detected for resistance to FW explaining maximum of 31.55% of the total variations. Of these QTLs, three of them on chromosome IV and chromosome VI were identified for resistance to race 1, and for race 3, the QTL were located on chromosome II and IV.

Chickpea Ascochyta blight *(A. rabiei)* - Concerning the current MAS for AB resistance, SCY17 and SCAE19 markers were reported as the best markers linked to AB resistant genes. These two markers were validated on different populations by Iruela *et al*. (37), Imtiaz *et al.* (36) and Madrid *et al*. (46).

Recently, three major conserved quantitative trait loci (QTLs) that confer AB resistance have been reported, two on chromosome II and one on chromosome IV. These QTL explained a maximum of 18.5%, and 25% of the total variation. In total, 27 predicted genes were located in chromosome IV close to these QTL (Hamwieh et al, Unpublished data).

الملخص

حموية، علاء الدين، فدا علو وسعيد أحمد. 2018. تطوير أدوات جزيئية لمورثات مقاومة الأمراض في القمح والشعير والعدس والحمص: مراجعة علمية. مجلة وقاية النبات العربية، 36(1): 50-56.

إن الهدف الرئيسي من استخدام الأدوات الجزيئية من قبل المختصين في الأمراض النباتية هو تحديد المؤشرات الجزيئية (Molecular markers) المرتبطة بمورثات مسؤولة عن المقاومة. أفضل تلك المؤشرات الجزيئية هي الموجودة داخل أو تقع على مسافات قريبة جدا من مورثات المقاومة. هذه الورقة هي بمثابة مراجعة لآخر المؤشرات الجزيئية التي تم نشرها وتوثيقها على أنها مرتبطة بمورثات المقاومة في القمح (ضد صدأ المخطط ، صدأ الأوراق ، صدأ الساق) ، الشعير (الصدأ المخطط ، صفيحة الأوراق ، صدأ الساق)، العدس (الذبول الوعائي، لفحة الأسكوكيتا، لفحة الأنثراكنوز ، ولفحة الستمفيليوم Stemphylium)، والحمص (لفحة الأسكوكيتا، والذبول الوعائي). يجدر الإشارة إلى أن أهمية هذه المؤشرات الجزيئية المرتبطة بشكل وثيق بمورثات المقاومة يكمن في إمكانية استخدامها في مختبرات التقانات الحيوية لتساعد مربي النبات على الانتخاب المبكر بشكل يزيد في دقة الانتخاب وسرعته ويرفع من كفاءة برامج التربية النباتية لتحقيق غاية المربين في دمج مورثات مقاومة عديدة في الأصناف المحسنة.

عنوان المراسلة: علاء الدين حموية، المركز الدولي للبحوث الزراعية في المناطق الجافة (ايكاردا)، القاهرة، مصر، البريد الإلكتروني: a.hamwieh@cgiar.org

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