Article

Application of Genomics Approaches for the Improvement in Ascochyta Blight Resistance in Chickpea

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Abstract: Advancements in high-throughput genotyping and sequencing technologies are enabling the development of a vast range of genomic tools and resources for a new revolution in plant breeding. Several genotyping-by-sequencing (GBS) methods including capture-based, genome complexity reduction and sequencing of cDNA (GBS-t) are available for application in trait dissection, association mapping, and genomic selection (GS) in crop plants. The aims of this study were to identify genomic regions conferring resistance to Ascochyta blight (AB) introgressed from the wild *Cicer echinospernum* into the domesticated *C. arietinum*, through a conventional recombinant inbred population genotyped using a variety of GBS methods. Evaluation of GBS methods revealed that capture-based approaches are robust and reproducible while GBS-t is rapid and flexible. A genetic linkage map consisting of 5886 polymorphic loci spanning 717.26 cM was generated. Using field phenotyping data from two years, a single genomic region on LG4 was identified with quantitative trait loci (QTL) mapping. Both GBS methods reported in this study are well suited for applications in genomics assisted plant breeding. Linked markers for AB resistance, identified in the current study, provide an important resource for the deployment into chickpea breeding programs for marker-assisted selection (MAS).

Keywords: legume; single nucleotide polymorphism; target capture; RNA-Seq; fungal disease resistance; molecular breeding

1. Introduction

Chickpea (*Cicer arietinum* L.) is the third most important grain legume crop after soybean and pea, grown worldwide on c. 17.8 Mha with an annual production of 17.2 million tonnes [1]. Due to its high protein content, chickpea serves as an important protein source in the vegetarian diet of many developing nations. Under optimal growth conditions, chickpea productivity can reach up to 6 t/ha; however, exposure to biotic and abiotic stresses can reduce the average yield to <1 t/ha [2]. Among these stresses, Ascochyta blight (AB), caused by the fungus *Ascochyta rabiei* (Pass.) Labr. [teleomorph: *Didymella rabiei* (Kovacheski) von Arx (=*Mycosphaerella rabiei* Kovacheski)], is one of the major diseases that can cause a yield loss of 10–100%. Different strategies can be applied for the management of AB, such as short-acting seed treatments, strategic foliar fungicide application, and growing resistant varieties [3]. Development of resistant varieties is the most effective and viable option for ongoing sustainability and has been one of the major aims for chickpea breeding programs across the world. Conventional breeding approaches have been successful, to some extent, in improving the levels of AB resistance in breeder's germplasm especially for cultivated chickpea. Previous studies have reported several major resistance genes along with multiple minor genes controlling AB [4–7]. However, major resistance sources being

incorporated and widely used in breeding programs are always at a risk of boom-and-bust cycles due to constant evolution of pathogen structure [6]. Introgression of minor resistance sources along with major sources through genomics assisted breeding is anticipated to provide durable AB resistance. To maintain AB resistance in chickpea varieties, it is necessary to explore new sources of resistance not only within the cultivated gene pool but also from wild gene pools. Wild chickpea relatives adapted to different conditions and geographical locations offer tremendous genetic diversity [8]. Previous studies have identified new sources of AB resistance in *C. bijugum* K. H. Rech., *C. echinospermum* P. H. Davis, *C. pinnatifidum Jaub*. & Sp., and *C. reticulatum* Ladiz. [6,9]. Due to the feasibility of introgression and generation of fertile hybrids, *C. echinospermum* and *C. reticulatum* are known to be the common wild relatives of chickpea used in germplasm enhancement through conventional breeding [10].

Advances in next-generation sequencing technologies have provided opportunities to enrich genomic and genetic resources for chickpea. Currently, large-scale genomic resources are focused on a collection of reference genomes (kabuli type [11], desi type [12], wild chickpea [13]) as well as collection of SNP markers [3,14] and genetic maps [2]. Discovery of genome wide SNPs using genotyping-by-sequencing (GBS) approaches is a vital tool for marker trait association studies leading to genetic enhancement. High-throughput SNP genotyping approaches include complexity reduction-based methods [15–17], skim genome sequencing, and target capture assays [18]. Recently, high-throughput SNP genotyping methods, including restriction site-associated DNA sequencing (RAD-Seq) as well as skim genome sequencing, were used to genotype chickpea mapping populations [19,20]. In this study, a combination of approaches including capture-based and GBS-transcriptomics (GBS-t) sequencing were utilised to genotype an interspecific RIL mapping population and identify regions associated with AB resistance. Target capture-based systems are more reproducible and specific and they provide enrichment of targeted regions [21]; however, they require prior knowledge of the genome sequence and structure in order to select specific regions for amplification. GBS-t is a type of target capture assay that also serves as complexity reduction method by targeting the whole exome of any given species. This strategy is scalable, economical, and allows for deeper sequencing coverage compared to whole genome approaches. It is also highly suitable for species with limited genomic resources available and has been exemplified across a broad range of species and ploidy levels [22–24].

Molecular breeding offers many advantages over traditional breeding in terms of time and cost savings as well as breeding efficiency and selection accuracy. Genetic linkage map construction for use in trait dissection and marker-assisted breeding has been a part of chickpea breeding programs. As a result, a large number of maps based on different types of markers such as SSRs and SNPs have been generated [3,25,26]. Both intraspecific as well as interspecific maps were published; however, more emphasis has been given to the interspecific maps, particularly those between *C. arietinum* and *C. reticulatum* [27]. The rationale for the use of interspecific mapping populations has been the low levels of genetic diversity that are known to be present within the gene pool of cultivated chickpea [28]. Multiple quantitative trait loci (QTLs) for resistance to ascochyta blight have been identified, from both inter and intra-specific crosses, for resistance at different growth stages (including seedling, flowering, and pod filling) under greenhouse and field conditions [3,5,7,20]. These QTL analyses have historically accelerated the dissection of genetic control of AB, thus allowing marker-assisted selection (MAS) to be implemented in breeding processes [3,29]. A limitation of MAS is that it focuses on the selection of major resistance genes that can be dramatically overcome by rapid adaptation of *A. rabiei* pathogen. Moreover, there is limited research on genes underlaying these QTLs and the resistance mechanism against AB infection.

As genetic diversity in chickpea suffers from a domestication bottleneck, it is imperative to utilize wild relatives to introgress agronomically important traits and expand the gene pool [30]. Introgression from wild relatives will likely deliver a single genomic region

that would provide enhanced major gene or gene set resistance. Major gene resistance, when used in combination with other sources to improve the quantitative resistance level within the germplasm, is likely to be the most effective mean of control of AB pathogens. In this study, we used a recombinant inbred mapping population derived from a cross between *C. arietinum* (genotype Sonali) and *C. echinospermum* (genotype 04067-81-2-1-1) that was generated to introgress AB resistance into commonly cultivated genetics. This study evaluated capture-based and GBS-t sequencing approaches utilising Illumina sequencing. SNPs identified using both approaches were used for genetic linkage map construction and QTL analysis for identification of genomic region for AB resistance.

2. Materials and Methods

2.1. Plant Materials and Nucleic Acid Extraction

Seeds from a total of seven chickpea varieties/advanced breeding lines (Genesis114, Howzat, ICC3996, Lasseter, Sonali, Yorker, 04067-81-2-1-1) were obtained from NSW-DPI, Tamworth, Australia. These seven lines were extensively used in chickpea breeding programs and were the parents of RIL mapping populations segregating for key traits of interest. Multiple seeds of each genotype were germinated and maintained for 2–3 weeks in glasshouse at $22 \pm 2\,^{\circ}\mathrm{C}$ under a 16/8 h (light/dark) photoperiod, in individual pots filled with potting mix (AGH Mix, Bio Gro, South Australia, Australia) at the premises of Agriculture Victoria, Bundoora, Victoria, and Australia.

An interspecific RIL mapping population was developed by crossing a susceptible cultivar, Sonali (*C. arietinum*), and a highly resistant line, 04067-81-2-1-1 (*C. echinospermum*). The RIL mapping population consisted of 134 individuals based on single seed descent from F_2 to F_6 progeny in the glasshouse (NSW-DPI, Tamworth, Australia).

For nucleic acid extractions, 3–4 young leaflets were harvested, snap frozen in liquid nitrogen and stored at $-80\,^{\circ}\mathrm{C}$ until required. Genomic DNA extraction was performed using DNeasy® 96 Plant Kit (QIAGEN, Hilden, Germany) and RNA extractions using RNeasy® 96 Kit (QIAGEN) following manufacturer's instructions, with the exception that Dithiothreitol (DTT) (Sigma-Aldrich, St. Louis, MO, USA) was used instead of β-*mercaptoethanol*. Tissue disruption was performed in a 96-well plate (QIAGEN Microtubes) using a Mixer Mill 300 (Retsch®, Haan, Germany) and was eluted in 40 μL of elution buffer and stored at $-80\,^{\circ}\mathrm{C}$. Concentration and quality being confirmed by a Thermo Scientific™ NanoDropTM UV-Visible spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA) and integrity evaluated using a TapeStation 2200 platform, using either gDNA ScreenTape or RNA ScreenTape (Agilent Technologies, Santa Clara, CA, USA).

2.2. GBS-t Sequencing Library Preparation, SNP Identification and Target Enrichment Array Design

Following RNA extraction from above mentioned seven chickpea varieties/advanced breeding lines, paired-end sequencing libraries (SureSelect Strand Specific RNA Library Preparation Kit [Agilent Technologies]) were constructed with insert sizes of 350 bp. The resulting libraries were evaluated using the TapeStation 2200 platform (Agilent Technologies). Each library was generated with a unique barcode and pooled in equal mass. Pooled libraries were quantified using the KAPA library quantification kit (KAPA Biosystems, Wilmington, MA, USA). Sequencing was performed using an Illumina HiSeq 2000 Sequencing platform producing 2×150 bp reads, and each sample was sequenced to a depth of 5–6 million paired reads per sample (Illumina Inc., San Diego, CA, USA).

Following fastq data generation, the raw sequence reads were filtered using a custom perl script to remove adaptor sequences along with reads and bases of low quality ($Q \leq 30$). Reads with 3 consecutive unassigned nucleotides (N) were also trimmed and, finally, any reads shorter than 50 bp in length were removed from the final set. The remaining high-quality trimmed sequence reads were aligned to the chickpea reference genome sequence from cultivar CDC Frontier [11] using TopHat2 (version 2.1.1) [31]. Variant calling was performed using SAMtools (version-1.5) [32] and BCFtools [33]. The final VCF output was

then filtered based on various parameters: depth (DP → 10), minor allelic frequency (MAF 0.05), maximum missing data (20%), and base quality (Q30) using vcftools [33].

For target enrichment array design, a final set of SNPs was selected based on uniform distribution across the genome and flanking sequences 100 bp both upstream and downstream from each target SNP were sent to Agilent Technologies for target capture assay design (Table S1).

2.3. Genotyping the RIL Mapping Population

GBS-t sequencing libraries (SureSelect Strand Specific RNA Library Preparation Kit [Agilent Technologies]) were used, and SNP identification of RIL mapping population was performed, as described above, with slight modifications on the number of paired-end reads generated (around 2–3 million paired-end reads for each RILs).

Target enrichment was performed according to the manufacturer's instructions (Sure-Select Target Enrichment [Agilent Technologies]), except for a reduction in reaction volume (1/4 of the reaction). High-quality DNA (200 ng) was randomly fragmented using MspJI restriction enzyme (New England Biolabs, Ipswich, MA, USA), as described previously [34]. All reads were pair-end sequenced using the MiSeq platform (Illumina Inc.). The generated sequence data were trimmed as before and then aligned to the chickpea reference genome sequence, i.e., the CDC Frontier [11] using BWA MEM [35]. SNP calling was performed using SAMtools (version-1.5) [32] and BCFtools [33] with a defined SNP list from target enrichment assay. VCF output was processed with the same parameters used for GBS-t sequencing data filtering using vcftools [33].

Additionally, 12 markers (2 SSRs and 10 SNP markers) flanking the AB resistance QTLs from a previous study [3] were screened on Sonali X 04067-81-2-1-1 RIL population. SSR genotyping was performed, as described previously [3], as well as SNP genotyping using KASP^{TM} genotyping chemistry (LGC Genomics, Middlesex, UK) with some modifications [36].

2.4. Genetic Linkage Mapping

SNP genotyping data showing contrasting alleles between the parental genotypes were selected for linkage mapping. A chi-squared test was performed on the filtered SNPs and any markers showing significant deviation from 1:1 ratio ($p < 0.01$) were excluded from further analysis. Linkage maps were constructed from the final set of SNPs using the statistical software QTL IciMapping V4.1 [37] with a logarithm of odds (LOD) score of 14. To finalize the linkage map, marker ordering and rippling were performed using RECORD and COUNT algorithms, respectively. The Mapchart 2.2 software [38] was used to visualise the final genetic map. Visual comparisons of the genetic linkage maps with the physical map was performed using the Strudel software package [39].

2.5. AB Inoculation, Disease Rating and Statistical Analysis of Phenotyping Data

The phenotypic evaluation of AB resistance in Sonali X 04067-81-2-1-1 RIL population was performed, as described previously [3]. Briefly, the RIL population and parents were sown in a 0.6 m single row plot (25 seeds/plot) in field in Tamworth, NSW, Australia in 2014 and 2015 in a randomized complete block design with three replicates. Once plants were established, inoculation was performed with the spore suspension (500,000 conidia/mL at a rate of 100 L per hectare). Disease symptoms were scored using a scale of 1–9 based on whole plot severity.

The means of the phenotypic scores were estimated after the adjustment for any spatial patterning within the trial. Models were fitted using residual maximum likelihood (REML), as implemented in GenStat (GenStat Committee, 2002) [40]. Phenotypic scores from both trials (2014 and 2015) were plotted and the score distributions were compared in R [41]. Best Linear Unbiased Estimates (BLUEs) analysis was used to calculate the broad sense heritability (H2) using ASReml v4.1 [42].

2.6. Identification of Genomic Regions for AB Resistance

To identify genomic regions associated with AB resistance, QTL mapping was performed. Both simple interval mapping (SIM) and composite interval mapping (CIM) were performed in Windows QTL Cartographer version 2.5 [43]. An arbitrary LOD threshold of 2.5 was used to determine significance for SIM. For CIM, significance levels for LOD thresholds were determined using 1000 permutations. Based on the sequences of SNP loci flanking QTL intervals, genomic regions containing putative candidate genes were retrieved (based on annotation).

3. Results

3.1. Target Enrichment Array Design from Advanced Varieties/Advanced Breeding Lines

A total of 789,525,381 high-quality trimmed paired end transcriptome reads obtained from seven chickpea varieties/advanced breeding lines were aligned to the reference genome of chickpea (v1.0). Table S2 provides details of paired end reads that were mapped to the reference from each genotype. A total of 368,449 base variants were identified that were filtered based on depth, quality score, missing data, and minor allele frequency to generate a final set of 26,563 SNPs. Finally, 11,222 SNPs were selected based on uniform distribution across the chickpea genome and used for target capture design. Details of these selected SNPs, genomic location, and probe sequences are provided in Table S1.

3.2. Genotyping the RIL Mapping Population and Genetic Linkage Map Construction

To identify SNPs in the RIL mapping population, capture-based and GBS-t sequencing approaches were performed. In the capture-based approach, sequencing generated a total of 48,503,448 paired end reads with an average of 350,000 reads per line. Most of the probes (10,732; 95%) were identified as being on-target sequences with reads distributed evenly across all targeted regions. Among them, 55% of the probes (5854) were polymorphic between the two RIL parents. A final set of 2479 SNP markers were selected for linkage mapping after additional filtering based on missing data (<20%), heterozygosity (<10%), and χ^2 analysis ($p < 0.05$) (Table S3).

From GBS-t sequencing of the RIL mapping population, a total of 329 million high-quality reads were used for analysis. After the initial filtering based on read quality, read depth and missing data, 3856 polymorphic SNP markers were identified. After performing a χ^2 analysis ($p < 0.05$), a final set containing 3591 segregating SNP markers was used for linkage mapping (Table S3). From previously published AB resistance QTL flanking markers, two SSR and three SNP markers were also included in linkage mapping (Table S3).

Polymorphic marker data from all these genotyping approaches were combined (6075); only 5919 unique markers (based on the genomic position) were used for linkage map construction. Mapping resulted in final set of 5886 markers that were assigned to eight major linkage groups (that equaled to the eight chromosome) and three satellites (Figure 1, Table 1 and Table S4). The proportion of loci assigned to LGs was 99%, with a small proportion marker unlinked. Total map length was calculated to be 717 cM with an average inter-marker distance of 0.12 cM. The marker distribution across the LG's were not uniform; LG4 had the highest number of markers (1934) and LG5 with the lowest (105). Marker order on LG's were compared to the chickpea genome, which identified significant commonality (Figure S1). However, inconsistencies were observed for a handful of markers which were assigned to different LG's and some marker order was not co-linear (especially on LG7).
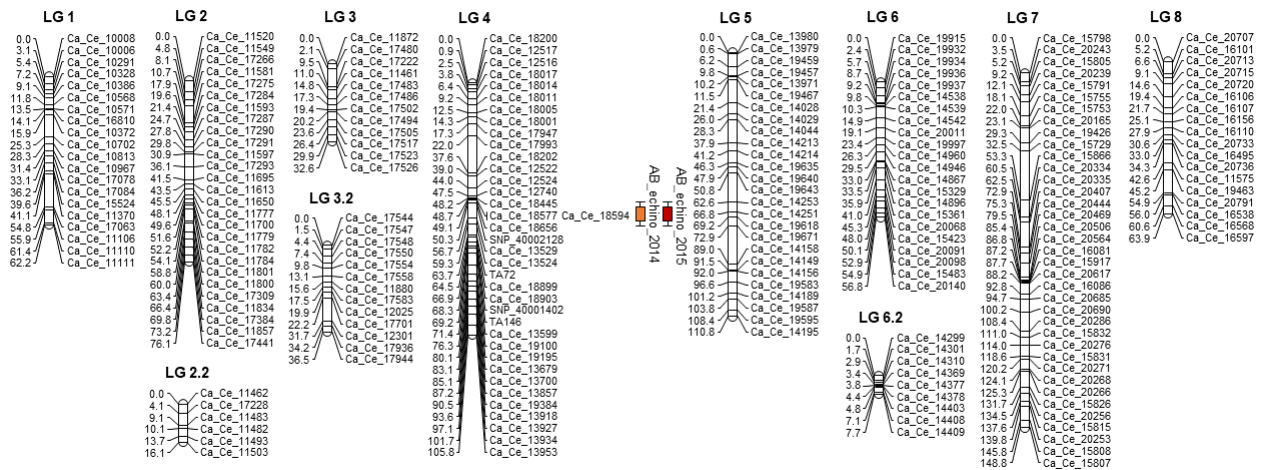
Figure 1. Genetic linkage map of the Sonali X 04067-81-2-1-1 cross, showing the location of genomic region for AB resistance. The name is provided at the top of each LG. Distances of the loci (cM) are shown to the left and names of loci are shown to the right side of LGs. For presentation purposes, only selected markers are shown on the map.

Table 1. Marker distribution over the LGs of Sonali X 04067-81-2-1-1 genetic linkage map.

Linkage Group	Number of Mapped Markers	Cumulative Map Length (cM)	Average Marker Density
LG1	936	62.23	0.07
LG2	206	76.05	0.37
LG2.2	21	16.08	0.77
LG3	31	32.56	1.05
LG3.2	501	36.54	0.07
LG4	1934	105.76	0.05
LG5	105	110.83	1.06
LG6	1031	56.81	0.06
LG6.2	33	7.68	0.23
LG7	676	148.82	0.22
LG8	412	63.90	0.16
Total	5886	717.26	0.12

3.3. Phenotypic Analysis of RIL Populations and the Identification of Genomic Regions for AB Resistance

The AB disease score showed greater variation in the 2014 trial (range 1.8–5.2, mean 2.74 ± 0.79) than in 2015 (range 3–5, mean 3.68 ± 0.57), depicted in histograms provided in Figure S2. Correlation (Pearson) between the mean AB disease score from the two trials was 0.52 (Figure S3). The estimated heritability of the combined data set was 0.53.

SIM and CIM analyses detected a single genomic region on LG4 associated with resistance to AB (Figure 1). The amount of phenotypic variance (V_p) explained was 34% and 41% from 2014 and 2015 phenotyping trials, respectively (Table 2). Resistance determinant was derived from the *C. echinospermum* parent (04067-81-2-1-1). AB_echino QTL interval was found to be located on the Ca4 between 14–16 Mbp (Figure S3). From the physical interval, potential candidate genes were identified based on annotation, and they include serine/threonine protein phosphatase, receptor protein kinase-like protein, LRR receptor-like serine/threonine-protein kinase, and cysteine proteinase, which have been demonstrated to be involved in disease resistance (Table S5).

Table 2. Identification of QTLs for AB resistance on Sonali X 04067-81-2-1-1 RIL population genetic maps based on CIM.

QTL Name	Chromosome	Flanking Markers	Position (cM)	LOD Threshold	Maximum LOD Threshold	% Phenotypic Variance	Additive Effect
AB_echino_2014	LG4	Ca_Ce_18445	48.15	4.5	13.7	34	0.45
		Ca_Ce_18594, a_Ce_18577	48.68				
		Ca_Ce_18656	49.14				
AB_echino_2015	LG4	Ca_Ce_18445	48.15	4	16.0	41	0.40
		Ca_Ce_18594, a_Ce_18577	48.68				
		Ca_Ce_18656	49.14				

4. Discussion

High-throughput and low-cost sequencing technologies have extensive applications in marker-assisted breeding through linkage mapping, QTL analysis, and MAS [44]. Different GBS approaches vary greatly in the types/amount of data generated, their costs per marker data point, and errors/potential biases. Hence, selecting the best suited method depends on the genome size of the crop, population structure, availability of prior genomic information, reproducibility, ease of application, and the relative scale of the industry. In this study, two high-throughput approaches i.e., capture-based and GBS-t sequencing, were evaluated on a RIL mapping population. Genotyping by capture-based approach had a high proportion (95%) of the targeted SNPs recovered, with ~50% (5854 markers) being polymorphic in the RIL population, whilst the GBS-t approach identified 3856 polymorphic SNP markers, 3591 of which were used for linkage mapping. These results indicate that capture-based approach is more robust, reproducible, and scalable as compared to GBS-t. However, the main caveat/drawback of this method is the lack of flexibility of SNP panels, especially to apply on different germplasm collections such as exotic lines and wild relatives. To address this limitation, custom SNP panels need to be designed and integrated into existing assays, which will incur inevitable additional costs. The alternative is to apply a more flexible approach, such as GBS-t. Even though GBS-t is more flexible as compared to capture-based approach and can deliver functionally associated gene-based markers for the identifcaiton of expression QTLs [45], it still has its own disadvantages including the requirement of high-quality plant material for extraction of RNA and comparatively higher proportion of missing data compared to capture-based approaches. Due to relatively small genome and simple ploidy in chickpea, skim genome sequencing is another suitable approach for detection of genome wide polymorphisms. As the cost of sequencing is declining rapidly with the advent of new experimental protocols, equipment, and applications of sequencing, the limitation of missing data can be alleviated by higher coverage/depth [46]. In brief, two high-throughput genotyping approaches performed in this study have their own pros and cons. GBS-t is an effective genotyping approach for species with limited genomic resources to implement high-throughput genotyping quickly or to evaluate crosses with wild relatives. The development of capture-based assays will then be an obvious extension that incorporates the necessary variants for application in a breeding scenario or germplasm enhancement. Generic costs for both approaches are similar with obvious cost savings for operating at scale; this can potentially deliver greater savings to capture assays. With the availability of efficient computational methods, low-coverage sequencing data can be efficiently imputed to obtain dense marker coverage. There are several computational packages available for imputation, such as Minimac [47], Beagle [48], and LinkImpute [49]. Performance of these packages depends on several factors including the size and structure of population, effective population size, SNP density and sequencing coverage [50].

In this study, SNP markers from GBS data have successfully been used to build a high-density genetic map (5886 markers). The majority of the markers displayed conserved assignments with physical positions on the genome. Uneven marker distribution on certain regions of LG's was observed, which could be due to lack of polymorphism in genes (suppressed for meiotic crossovers hence reduced recombination rates), simple

repeats, retroelements, as well as gene-poor or heterochromatic regions interspersed in the genome [51]. The high-density Sonali x 04067-81-2-1-1 linkage map provided the basis for identification of a single genomic region on LG4 conferring AB resistance. The slight differences observed in the Vp proportions (34% and 41%) in 2014 and 2015 could be due to variability between field conditions of the two screening experiments. Currently, in Australia, chickpea cultivars only possess partial resistance to *A. rabiei* and this level of resistance can easily breakdown as the pathogen is highly variable. Hence, there is an urgent need to introduce novel sources of disease resistance from global germplasm collections, including landraces and wild relatives, into chickpea breeding programs to develop superior AB resistant varieties. A previous study has successfully incorporated high levels of AB and botrytis grey mould (BGM) resistance in cultivated chickpea from wild relatives including *C. judicium* and *C. pinnatifidum.* Populations developed from these crosses were later evaluated, and a proportion of derivatives were resistant to both AB and BGM [52]. Multiple studies have previously been conducted to identify superior sources of AB resistance, identifying both single gene (either dominant or recessive) and multiple genes controlling AB resistance [3,5,20,53]. A recent study which performed the physical mapping of previously reported AB resistance QTLs using flanking SSR and SNP marker sequences the placed majority of QTLs in clusters on Ca2 and Ca4, and only few QTLs on Ca3, Ca5, and Ca6. Previously identified QTLs on Ca4, qAB4.1, and qAB4.2 were mapped at 6–8 Mbp and 21–26 Mbp, respectively [20]. The genomic region identified from this study revealed a physical position at 14–16 Mbp. The fact that the resistance source comes from *C. echinospermum* (genotype 04067-81-2-1-1) suggests it is highly likely to be a novel allele (or set of alleles) that can add value and diversity to the overall resistance breeding strategy. An efficient combination of genotyping approaches along with high-throughput phenotyping can help to determine the precise location of the causal gene responsible for resistant/tolerant phenotypes. GBS approaches for mapping/fine-mapping and gene analysis has been exploited in different species, including pigeonpea for Fusarium wilt and sterility mosaic disease resistance [54], in groundnut for bacterial wilt [55], and in cotton for drought tolerance [56], allowing the identification of associated pathways and candidate genes/SNPs useful for genomics-assisted breeding. Examining the QTL interval from this study helped to identify potential candidate genes, serine/threonine-protein phosphatase, receptor protein kinase-like protein, LRR receptor-like serine/threonine-protein kinase, and cysteine proteinase, all of which play key roles in disease resistance in plants [57]. These genes could be plausible candidates, although further work should be performed for validation. As the resistance source identified in this study originates from a wild relative, there could be additional novel genes present within the QTL region that couldn't be identified using the cultivated genome reference. In the future, long-read sequencing platforms can be used for the comprehensive fine-mapping of this region thereby absolutely defining the gene contents within QTL interval.

QTL mapping has been the method of choice over decades to identify trait-linked markers that can be deployed in breeding for MAS. Genomic regions and markers identified in this study will be highly useful for immediate deployment into chickpea breeding using MAS. However, QTL mapping poses some limitations for traits controlled by multiple genes such as AB [58]. Previous studies on AB in chickpea have focused on the identification of QTLs of moderate to major effects that tend to be highly effective but vulnerable to breakdown with rapid changes in pathogen races. Furthermore, small population sizes as well as the usage of biparental mapping populations in individual studies had limited statistical power to detect quantitative genes conferring partial resistance, which is known to also be an important component for achieving durable resistance to AB. By collating pre-existing datasets and performing meta-analysis, it should be possible to achieve a population size that provides sufficient statistical power to identify novel quantitative genes conferring partial resistance to AB. Several studies have shown that GS outperforms MAS for complex trait in terms of gain per unit time and cost [59,60]. It also enables the simultaneous introgression of multiple loci from cultivated, as well as wild, backgrounds

into elite breeding germplasm for durable resistance. Studies have been published comparing different GS prediction models, the effect of marker density, population structure, and population size on prediction accuracy [61,62]. The effects of prediction accuracy varied for each trait under investigation, especially as accuracy decreases with the increase in the trait complexity [2]. Regardless of population type or trait, the pre-requisite for GS is to develop a training population comprising diverse lines with complete phenotypic and genotypic data. Advances in high-throughput phenotyping and genotyping platforms will play a major role in the application of GS for rapid advancement of genetic gain in breeding programs. A foreground selection based on genotyping/diagnostic markers, followed by GS, could be the way forward for chickpea breeding programs.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/agronomy11101937/s1. Figure S1: Visual representation of the comparison of linkage groups of Sonali X 04067-81-2-1-1 RIL population map with chickpea chromosomes. Corresponding lines indicate the common region. Figure S2: Frequency distribution histograms generated from AB phenotyping using disease scores for 2014 and 2015 trials in Sonali X 04067-81-2-1-1 RIL population. Figure S3: Comparison of the AB disease scores for 2014 and 2015 trials in Sonali X 04067-81-2-1-1 RIL population. Figure S4: Comparison of AB_echino QTL interval from Sonali X 04067-81-2-1-1 RIL population with previously published QTLs on Ca4. Table S1: Name and sequence information of the SNP markers from the target enrichment assay. Table S2: Details on the reads aligned to the chickpea genome from advanced varieties/advanced breeding lines. Table S3: Details on the type and number of markers used for linkage mapping. Table S4. Linkage map statistics from Sonali X 04067-81-2-1-1 RIL population. Table S5: Details of plausible candidate genes identified in the QTL interval and their annotation.

Author Contributions: S.S. performed transcriptome sequencing, SNP genotyping, map construction, QTL analysis, candidate gene identification and contributed to drafting the manuscript. H.V.K. phenotypic data analysis and assisted in drafting the manuscript. K.H. developed the mapping population, performed the phenotyping experiments and contributed to data interpretation. S.B. performed DNA extraction and assisted in genotyping. N.D. assisted in the phenotyping experiments. N.O.I.C. co-conceptualised project and assisted in drafting the manuscript. S.K. co-conceptualised and coordinated the project and assisted in drafting the manuscript. All authors have read and agreed to the published version of the manuscript.

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