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Phenotypic and Genotypic Characterization of New Kabuli-Type Chickpea Lines in Australia for Resistance to *Ascochyta* Blight

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Abstract: *Ascochyta* blight (AB) is a major threat to Kabuli-type chickpea production worldwide. This study aimed to identify AB-resistant Kabuli-type chickpea lines through combined phenotypic and genotypic screening. Twenty-six Kabuli-type chickpea lines were phenotyped at the seedling stage using spray inoculation with conidial suspension. Genotyping employed marker-aided selection (MAS) with markers linked to quantitative trait loci (QTL) for AB resistance. The allele-specific marker, CaETR, closely linked to QTLAR1, and the sequence-tagged microsatellite (STMS) markers GAA47, TAA146, and TA194 linked to QTLAR1, QTLAR2, and QTLAR3 were used to assess their utility in distinguishing between resistant and susceptible chickpea lines. The study revealed that none of the lines tested were completely resistant (R) phenotypically. However, some lines, such as AVTCPK#6 and AVTCPK#14, were found to be moderately resistant (MR). Of the two MR lines identified phenotypically, only AVTCPK#6 was found to have bands linked to QTLs for adult plant resistance. The other MR line for AB showed the presence of bands in only one or two of the four markers used. These MR lines can be further utilized in chickpea breeding programs for the development of AB-resistant chickpea cultivars. It is recommended that these results be verified through repeat experiments, using more diverse isolates, and including additional chickpea lines as reference checks for resistance and susceptibility. The allele-specific marker, CaETR, closely linked to QTL_{AR1} and sequence-tagged microsatellite (STMS) markers GAA47, TAA146 and TA194 linked to QTL_{AR1}, QTL_{AR2}, and QTL_{AR3} were used to explore these markers' utility in discriminating between resistant and susceptible chickpea lines. The study showed that phenotypically, none of the lines tested are completely resistant (R). However, some lines, namely AVTCPK#6 and AVTCPK#14, were found to be moderately resistant (MR). Of the two MR lines identified phenotypically, only AVTCPK#6 was identified to have bands linked to QTLs for adult plant resistance. The other MR line for AB showed the presence of bands in only one or two markers among the four markers used. These MR lines can be exploited further in chickpea breeding programs for the development of AB-resistant chickpea cultivars. It is recommended that these results are verified by repeat experiments, using more as well as diverse isolates alongside additional chickpea lines for resistant and susceptible reference checks.

Keywords: *Ascochyta rabiei*; marker-aided selection (MAS); phenotype; genotype

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1. Introduction

Chickpea (*Cicer arietinum*) is a high-value winter legume crop after dry beans and peas [1]. Currently, chickpeas are grown in numerous regions around the world, including but not limited to multiple examples on the Indian subcontinent, across the Great Plains region of the United States, in Western Asia, and throughout the Mediterranean. In 2023, countries such as India, Turkey, Pakistan, Mexico, Argentina, the United States, Ethiopia, Australia, Myanmar, and Russia are some of the countries with the highest rates of chickpea production in the world [2]. In 2022, around 18.1 million metric tons of chickpeas were

produced worldwide [3]. The multifarious importance of chickpea crops in the human diet, soil fertility improvement, and animal feed has increased its popularity. However, improvements in productivity are still lagging behind because chickpeas are faced with several abiotic or biotic challenges throughout the crop growth cycle. Vulnerability to fungal diseases such as *Ascochyta* blight (AB) is one of the important biotic factors limiting the production of chickpeas throughout the world [4].

Ascochyta blight (AB), caused by the fungus *Ascochyta rabiei*, is recognized as a destructive disease of chickpeas in Australia [5], with yield loss reported as high as 100% [6]. The severity of this disease is significantly increasing worldwide, with the reported occurrence of AB in more than forty countries, including Australia [7]. Central Queensland through New South Wales, Victoria, and southern Australia are major places for chickpea production, with minor production in northwestern Australia. Despite Australia being one of the world's largest producers of chickpeas at an estimated 876.5 thousand metric tons in 2022 [3], it has always been challenging for farmers to protect chickpea crops from AB to obtain a potential yield [8]. Due to these challenges, the growing market in 2023 within Australia currently sits at under 500,000 tons, as indicated in a recent study reported by ABC Rural [9].

Ascochyta rabiei is a necrotrophic fungus that affects the above-ground part of the plant, i.e., stem, leaf, pods, and seeds, causing necrotic lesions. Cool, cloudy, and humid seasons encourage disease development, infection, and spread [10]. A number of control strategies for the management of this disease have been employed. Fungicide application is mostly preferred, but frequent applications have led to fungicide resistance development [11,12] and are also costly; therefore, the return from chickpea production is reduced for growers.

Several studies on the successful development of resistant varieties have been reported, attempting to improve the level of disease resistance by conventional breeding approaches [13], but the constant evolution of pathogens challenges breeders to explore new sources of gene diversity. A few of the cultivars reported as resistant and moderately resistant, i.e., PBA HatTrick and Genesis 090, are now causing high levels of disease symptoms because of the outbreaks of aggressive strains of these pathogens in Australia [14]. Furthermore, chickpea is a crop with limited genetic diversity [15], so utilizing the available cultivars to address existing and emerging diseases using modern technologies such as marker-aided selection (MAS) could ensure effective, efficient, and reliable screening [16]. Castro et al. [16] describe the importance of MAS as a useful tool to identify the genetic background of tested lines and select desirable alleles at the molecular level, which can be used for the introgression of multiple useful alleles.

For the successful use of the MAS method, markers tightly linked to traits of interest should be selected and employed. Quantitative trait loci (QTL) analysis and association mapping have enabled the identification of molecular markers associated with specific traits [17]. Several of the QTLs with a low-to-moderate effect, located in all the linkage groups working against AB, have been identified by genetic mapping [18]. Markers have been reported for use to screen AB resistance in recombinant breeding lines derived from an interspecific and intraspecific cross in chickpeas to determine the presence of QTL [16,19–22].

To improve resistance to AB in chickpeas, the screening of existing germplasm is required. Therefore, in the present study, both the phenotypic and genotypic screening of Kabuli-type chickpea lines were employed to determine their resistance level against AB, which can provide useful information in the identification and development of lines/cultivars for incorporation in the chickpea AB-integrated disease management program.

2. Materials and Methods

2.1. Plant Material and Trial Location

A total of twenty-six Kabuli-type chickpea lines sourced from AgriVentis Technologies Pty Ltd. (North Sydney, Australia) were used in this study (Table 1).

Table 1. List of Kabuli-type chickpea lines used in the experiment.

Line ID	Line ID
AVTCPK#1	AVTCPK#15
AVTCPK#2	AVTCPK#16
AVTCPK#3	AVTCPK#17
AVTCPK#4	AVTCPK#18
AVTCPK#5	AVTCPK#19
AVTCPK#6	AVTCPK#20
AVTCPK#8	AVTCPK#21
AVTCPK#9	AVTCPK#22
AVTCPK#10	AVTCPK#24
AVTCPK#11	AVTCPK#25
AVTCPK#12	AVTCPK#27
AVTCPK#13	AVTCPK#28
AVTCPK#14	AVTCPK#29

The experiment was carried out in climate-controlled glasshouse conditions in the laboratory of the Institute of Life Sciences and the Environment Research Facilities at the University of Southern Queensland, Toowoomba, Queensland, Australia. Commercially available chickpea cultivars developed and released by Chickpea Breeding Australia (CBA) were used as reference checks (controls).

2.2. Growing Environment and Experimental Design

Phenotyping for AB resistance was performed following the procedure by Newman et al. [23] with some modifications. The experiment was laid out in a Completely Randomized Design (CRD) with six replications.

Seeds of the Kabuli-type chickpea lines were surfaced and sterilized in 1.2% sodium hypochlorite (NaOCl) (Glitz Bleach, Pascoe's, Welshpool, WA, Australia) solution for five minutes, followed by three which were rinsed in sterile distilled water (SDW). One to two seeds per line were sown in each sterilized square pot (50 mm width × 50 mm length × 122 mm height) filled with a pasteurized vermiculite/sand mix (4:1 ratio) and maintained in the glasshouse with a 25 °C/20 °C day/night temperature. Seven commercially available chickpea cultivars were used as checks: Almaz (MR/MS), Flipper (MR/MS), Genesis 090 (R), Jimbour (S), PBA Pistol (VS), PBA Seamer (R) and Yorker (MR/MS) [5] were sown to serve as checks. For uninoculated negative checks, a set of each line was grown in separate trays.

2.3. Isolate and Inoculum Preparation

An isolate of *A. rabiei* (AR0357) obtained from Griffith University, Queensland, Australia, classified as high pathogenicity in Group V, and as reported in *A. rabiei* Dashboard (https://shinotate.pp18000.cloud.edu.au/shiny/Asco_dashboard/, accessed on 15 October 2023), was used. The isolate was grown and maintained on V-8 juice (Campbell Australia Pty Ltd., North Strathfield, NSW, Australia) agar at 25 °C, then kept at 4 °C until used. For inoculum preparation, the *Ascochyta* isolate was grown on V-8 juice agar plates at 21 °C. After 10 days, pycnidiospores were harvested by scraping the fungal growth from culture plates and blended with SDW using a handheld mixer. The spore suspension was then filtered through four layers of sterile gauze cloth, and the spore concentration was adjusted using a haemocytometer with 5 × 104 conidia/mL. Tween 20 (Sigma-Aldrich, Darmstadt, Germany), at a rate of 0.05%, was added to the inoculum suspension before spraying to the test lines.

2.4. Inoculation and Disease Assessment

The chickpea test lines were transferred in a Holman misting tent (Holman Industries, Osborne Park, WA, Australia), then spray-inoculated with the spore suspension for 20 s until runoff, and dried for 30 min, following the procedure as described by Isenegger et al. [24]. Afterwards, humidity was applied using a 505 Condair Humidifier (Condair Pty Ltd., Hornsby, Australia) and maintained at 20 ± 1 °C/26 ± 1 °C night/day temperature and 100% relative humidity for 72 h. Seedlings uninoculated were used as a negative control. At 10 days post-incubation, the intensity of disease symptoms on the whole plant for each entry was evaluated and rated based on a 1–9 rating scale by Pande et al. [25] (Table 2). An ANOVA of the ratings and a post hoc mean comparison by Tukey's HSD test was performed using the GenStat Twenty-third Edition Software (Version 23.1.0.651) [26].

Table 2. Rating scale used for *Ascochyta* blight (1–9) disease severity assessment.

Symptoms	Infected Area (%)	Rating Scale	Disease Reaction
No symptoms, immune	0	1	Asymptomatic
Minute lesions/spots on the apical stem	1–5	2	Resistant (R)
Apical stem slightly dropping and lesions up to 5 mm in size.	6–9	3	Resistant (R)
Apical steam-clear dropping and obvious lesions on all the plant parts.	10–15	4	Moderately Resistant (MR)
Obvious lesions on all plant parts, defoliation, and broken branches	16–20	5	Moderately Resistant (MR)
Obvious lesions on all plant parts, defoliation, and broken branches with some plants killed.	20–40	6	Moderately Susceptible (MR)
Same symptoms as 6: up to 25% of the plants killed.	41–75	7	Susceptible (S)
Same symptoms as 6: up to 50% of the plants killed.	76–100	8	Very Susceptible (VS)
Same symptoms as 6: up to 100% of the plants killed.	100	9	Highly Susceptible (VS)

2.5. MAS for AB Resistance

Four previously published markers, CaETR, GAA47, TA146, and TA194, linked to QTLs for AB resistance were used (Table 3) [16,19,21,22] for genotyping the 26 Kabuli- type lines and 7 chickpea cultivar checks.

Table 3. Details of the molecular markers used for *Ascochyta* blight resistance marker-assisted selection.

Marker Type		Primer Sequence (5′-3′)	Linkage Group-QTLs	Reference
Allele-specific	CaETR	Fw: CAGGAAGTTCAATGGCCCTA Rev1:TAAGTTGTGACAAAAGACTCAATCG Rev2:TAAGTTGTGACAAAAGACTCAATCG	*LG4 QTL*$_{AR1}$	[27]
Sequence-tagged microsatellite (STMS) markers	GAA47	Fw: CTAAGTTTAATATGTTAGTCCTTAAATTATRev: ACGAACGCAACATTAATTTTATATT	*LG4 QTL*$_{AR1}$	[28]
	TA146	Fw: TTTTTGGCTTATTAGACTGACTT Rev: TTGCCATAAAATACAAAATCC	*LG4 QTL*$_{AR2}$	
	TA194	Fw: TTTTTGGCTTATTAGACTGACTT Rev: TTGCCATAAAATACAAAATCC	*LG4 QTL*$_{AR3}$	

About 100 mg of leaves from three-week-old seedlings from each line were collected for DNA extraction. The DNA extraction kit Qiagen DNeasy® Plant Pro Kit (Qiagen Pty Ltd., Clayton, Australia) was employed for the extraction of total cellular DNA, as described by the manufacturer. The quantification of extracted genomic DNA was completed using a DS-11+Spectrophotometer (DeNovix Inc., Wilmington, DE, USA). The resulting DNA was dissolved with sterile ultrapure water and adjusted to 50 ng/µL for Polymerase Chain Reaction (PCR) assays.

The PCR reaction was carried out in a total volume of 10 µL containing 50 ng of genomic DNA, 5 µL of 2X Taq Master Mix New England Biolabs (NEB, Notting Hill, VIC, Australia), 0.5 µL each of the primer (10 µM), and 3 µL sterile ultrapure water. For PCR amplification using primers CaETR-Fw and CaETR-Rev1, initial denaturation was carried out for 1 min at 95 °C and then subjected to 30 cycles at 95 °C for 30 s, 60 °C for 30 s, 72 °C for 1 min, followed by a final extension at 72 °C for 7 min, as described by [28]. In contrast, for multiplex PCR using CaETR-Fw and two reverse primers, CaETR-Rev1 and CaETR-Rev-2, the reaction mixture was prepared as mentioned above except for the primer concentration (0.25 µL each of reverse primer, 10 µM). For initial denaturation, the reaction mixture was carried out for 5 min at 95 °C, then subjected to 30 cycles at 95 °C for 30 s, 62 °C for 30 s, 72 °C for 50 s, followed by a final extension at 72 °C for 7 min [27].

For sequence-tagged microsatellite (STMS) primers, GAA47, TA146 and TA194, the reaction mixture was carried out for 2 min at 96 °C denaturation, then subjected to 35 cycles of 96 °C for 20 s, and an annealing step at 55 °C for 50 s (GAA47, TA146), while for TA194, the reaction mixture was conducted at 59 °C for 50 s with an extension step of 60 °C for 50 s, followed by the final extension at 60 °C [19].

Amplification products were electrophoresed on 3% agarose (Agarose MB, Astral Scientific, Taren Point, NSW, Australia) gels in a 1XTBE buffer with 4 µL/100 mL of GelRed® Nucleic Acid Gel Stain (Biotium, Gene Target Solutions Pty Ltd., Dural, NSW, Australia). The Quick-Load® 100 bp DNA Ladder (New England Biolabs, Notting Hill, VIC, Australia) was used as a size standard. The DNA banding patterns were visualized and documented using the Biovision software (Version 4.2) in a Quantum Gel Doc System (Vilber Smart Imaging, Seoul, Republic of Korea).

3. Results

3.1. Phenotypic Evaluation for Ascochyta Blight

Each plant was assessed for the intensity of disease symptoms on the whole plant and assigned a rating between 1 and 9. Water-soaked lesions on the stem and leaf were the initial symptoms observed, which later turned into brown lesions, followed by the drying and then breaking of terminal parts; these were the most common symptoms observed (Figure 1). No data were obtained from lines AVTCKP#7, AVTCKP#23, and AVTCKP#26 due to poor germination.

An analysis of variance (ANOVA) for rating data yielded significant variation among the Kabuli-type chickpea lines ($p < 0.001$) in their reaction to AB. A mean comparison based on Turkey's post hoc test revealed that most of the tested lines (29 lines), including the checks, were either moderately susceptible (MS, susceptible (S), or very susceptible (VS) to AB at $p < 0.05$ (Table 4). No line was found to be highly resistant (HR) or resistant (R) to the disease. Only two lines, AVTCPK#6 and AVTCPK#14, and the check varieties PBA Seamer and Genesis 090 were found to be moderately resistant (MR) with an average rating of five.

Table 4. Mean disease severity ratings for Ascochyta blight on artificially inoculated Kabuli-type chickpea lines and check cultivars of chickpeas.

Kabuli-Type Chickpea Line ID	Severity Rating [2]	Rank [3]	Disease Reaction [4]
AVTCPK#6	4	a	MR
PBA Seamer (R) [1]	4	a	MR
AVTCPK#14	5	ab	MR
Genesis 090 (R) [1]	5	ab	MR
Yorker (MS/MR) [1]	5.3	b	MS
Jimbour (S) [1]	5.5	b	MS
Almaz (MR/MS) [1]	6	bc	MS
AVTCPK#13	6	bc	MS
AVTCPK#28	7	cd	S
AVTCPK#5	7	cd	S
AVTCPK#1	7.4	de	VS
AVTCPK#24	7.4	de	VS
AVTCPK#16	7.5	de	VS
AVTCPK#21	7.5	de	VS
AVTCPK#25	7.5	de	VS
AVTCPK#22	7.6	de	VS
AVTCPK#12	7.7	de	VS
AVTCPK#20	7.7	de	VS
AVTCPK#17	7.8	de	VS
AVTCPK#8	7.8	de	VS
AVTCPK#10	8	de	VS
AVTCPK#11	8	de	VS
AVTCPK#15	8	de	VS
AVTCPK#18	8	de	VS
AVTCPK#3	8	de	VS
Flipper (MR/MS) [1]	8	de	VS
PBA Pistol (VS) [1]	8	de	VS
AVTCPK#19	8.2	de	VS
AVTCPK#4	8.2	de	VS
AVTCPK#9	8.2	de	VS
AVTCPK#2	8.4	e	VS
AVTCPK#29	8.5	e	VS
AVTCPK#27	8.7	e	VS

[1] As per Moore et al. [5], the resistance ratings for the reference checks are for low-moderate disease pressure situations. [2] The average rating for disease severity from six replications. [3] Means followed by a common letter are not significantly different based on Turkey's post hoc test. [4] Disease reaction based on mean disease ratings, where 1 = highly eesistant (HR), 2,3 = resistant (R), 4,5 = moderately resistant, 6 = moderately susceptible (MS), 7 = susceptible (S), and 8,9 = very susceptible.

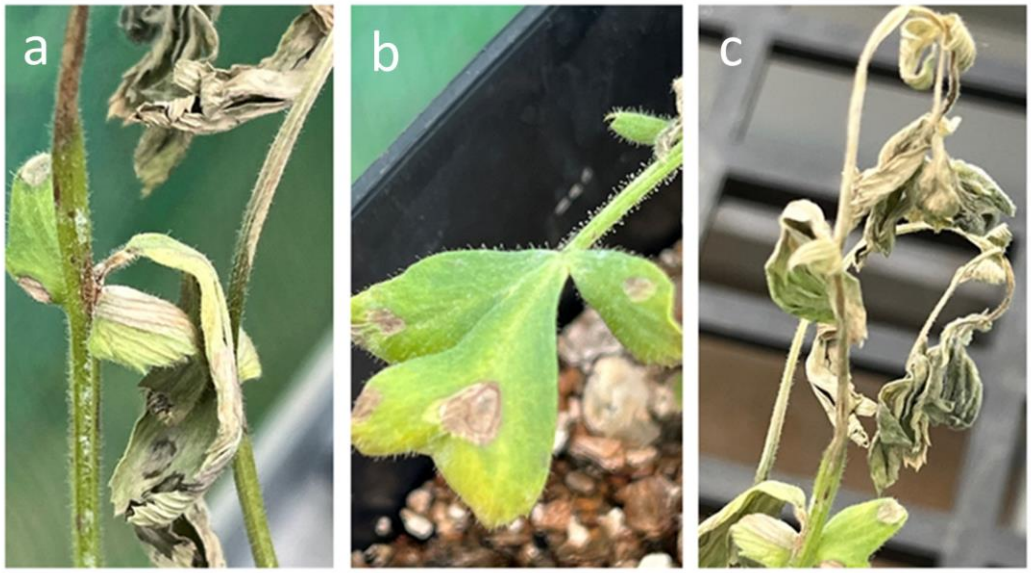

Figure 1. Whole-plant symptoms of Ascochyta blight observed in Kabuli-type chickpea lines: (**a**) necrotic spots on leaves with stem and leaf blight, (**b**) leaf spots on leaves, and (**c**) whole plant blighting.

3.2. MAS for AB Resistance

The markers CaETR, GAA47, TA146, and TA194, linked to three different QTLs, were employed for *Ascochyta* blight resistance screening. An allele-specific codominant marker, CaETR-linked to QTLAR1, was amplified with CaETR-Fw and CaETR Rev1 primers (Figure 2). For amplification products, the expected sizes for resistance and susceptibility were 1034 bp and 304 bp, respectively, as reported by Madrid et al. [27]. Among the evaluated lines, the amplicon size of 304 bp was detected in most of the lines except for six lines, namely AVTCPK#6, AVTCPK#10, AVTCPK#12, AVTCPK#15, AVTCPK#16, and AVTCPK#21. A very weak band of size 1034 bp or absence of bands observed in some lines may be due to low PCR efficiency in the lines and amplicon associated with the resistance allele [27,29]. Furthermore, the CaETR-Rev2 primer was used in the multiplex PCR together with CaETR-Fw and CaETR Rev-1 for the efficient discrimination of resistant and susceptible genotypes. The band of the 289 bp fragment detected in the same six lines were associated with resistance, whereas other lines displaying a band size 304 bp fragment was associated with susceptibility to the disease.

The STMS markers GAA47 and TA194 were employed to characterize chickpeas for the existence of QTLs, which are the genetic regions that influence phenotypic variation in a complex trait, such as plant reactions to the disease [30]. The marker detected QTL_{AR1} in only one line, AVTCPK#6, while both the QTL_{AR1} AND QTL_{AR2} were detected in AVTCPK#14. In contrast, the other STMS markers, TA146 and TA194, were able to detect QTL_{AR2} and QTL_{AR3}, respectively. Five out of twenty-six tested Kabuli-type chickpea lines and five out of seven check genotypes used were found to have a resistance band size of 120 bp, while the remaining lines had a susceptible band size of 160 bp.

Table 5 summarizes the results obtained from phenotyping and genotyping using four different markers: CaETR, GAA47, TA 146, and TA194. The results show that although some lines were rated susceptible in phenotyping, the genotyping results showed evidence of the presence of resistance genes, such as in AVTCPK#13, AVTCPK#15, AVTCPK#16, and AVTCPK#21. The same was true for the reference checks of Jimbour and PBA Pistol.

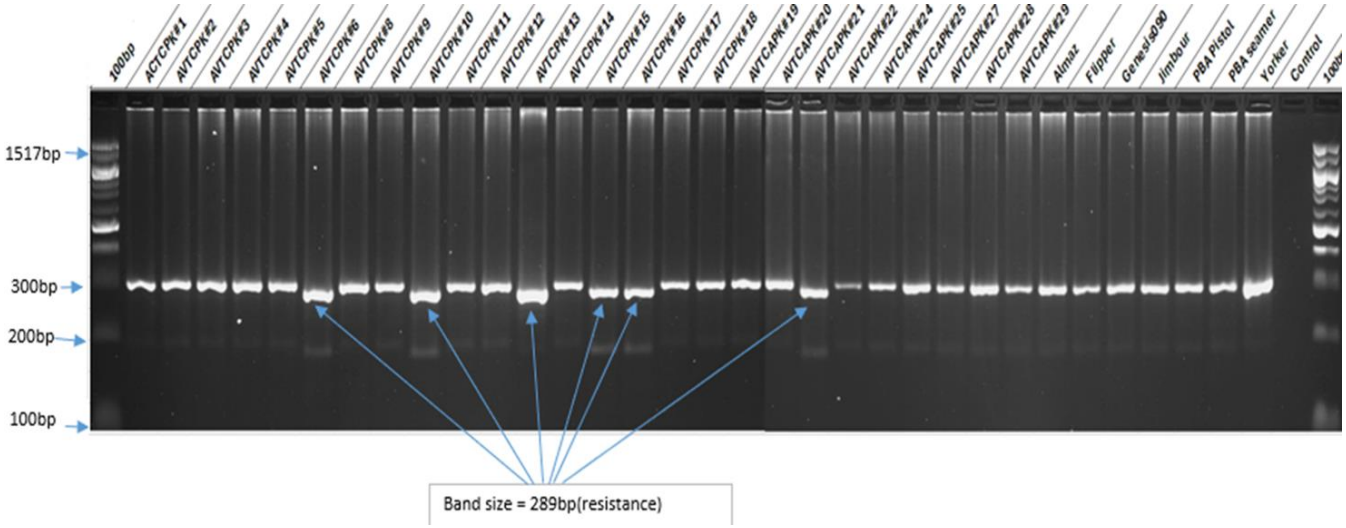

Figure 2. Agarose gel showing the amplicons for resistance and susceptibility of Kabuli-type chickpea lines to AB disease based on CaETR multiplex PCR. CaETR is an allele-specific codominant marker linked to $QTLA_{R1}$ and amplified with CaETR-Fw and CaETR Rev1 primers.

Table 5. Summary of phenotyping and genotyping results upon screening 26 Kabuli-type chickpea lines and reference check cultivars in their reaction to *Ascochyta* blight.

Line ID	Phenotype [2]	Genotype [3]			
		CaETR	GAA47	TA146	TA194
AVTCPK#1	S	–	–	–	–
AVTCPK#2	VS	–	–	–	–
AVTCPK#3	VS	–	–	–	–
AVTCPK#4	VS	–	–	–	–
AVTCPK#5	S	–	–	–	–
AVTCPK#6	MR	+	+	+	+
AVTCPK#8	S	–	–	–	–
AVTCPK#9	VS	–	–	–	–
AVTCPK#10	MS	+	–	+	–
AVTCPK#11	VS	–	–	–	–
AVTCPK#12	S	–	–	–	–
AVTCPK#13	MS	+	–	–	–
AVTCPK#14	MR	–	–	+	+
AVTCPK#15	VS	+	–	+	–
AVTCPK#16	VS	+	–	–	–
AVTCPK#17	S	–	–	–	–
AVTCPK#18	VS	–	–	–	–
AVTCPK#19	VS	–	–	–	–
AVTCPK#20	S	–	–	–	–
AVTCPK#21	MS	+	–	+	–
AVTCPK#22	S	–	–	–	–

Table 5. *Cont.*

Line ID	Phenotype [2]	Genotype [3]			
		CaETR	GAA47	TA146	TA194
AVTCPK#24	S	–	–	–	–
AVTCPK#25	S	–	–	–	–
AVTCPK#27	VS	–	–	–	–
AVTCPK#28	S	–	–	–	–
AVTCPK#29	VS	–	–	–	–
Almaz (MS/MR) [1]	MS	–	–	+	+
Flipper (MR) [1]	S	–	–	+	+
Genesis 090 (R) [1]	MR	–	–	+	+
Jimbour (S) [1]	S	–	–	+	+
PBA Pistol (VS) [1]	VS	–	–	–	+
PBA Seamer (R) [1]	MR	–	–	–	+
Yorker (MS/MR) [1]	MR	–	–	+	+

[1] As per Moore et al. [5], the resistance ratings of the reference checks are for low-moderate disease pressure situations. [2] S = susceptible, R = resistant, MS/MR = moderately susceptible/moderately resistance, VS = very susceptible. [3] Reactions based on the presence or absence of band sizes specific to being R or S to AB. (+) = resistant, (−) = susceptible.

4. Discussion

Kabuli-type chickpeas are distributed in West Asia and the Mediterranean region. Their large seed size, equal to or greater than nine millimeters in diameter, attracts popularity with a heavy premium not only for human consumption but also as a ruminant feed [31]. Despite common types, Desi- and Kabuli-type chickpeas are genetically variable, with Kabuli-type chickpeas reported to be more genetically diverse than Desi [32,33]. In the present study, we reported the resistance level of Kabuli-type chickpea lines against *Ascochyta* blight (AB).

A number of studies conducted to understand the resistance of AB in chickpeas have been reported. Some of the authors characterize it as monogenic inheritance [34], while others suggest AB resistance in chickpeas to be a result of polygenic inheritance with several QTLs already identified [16,19–21]. Since multiple genes are responsible for influencing the resistance of AB, different molecular markers were used in this research to identify the resistance of Kabuli-type chickpea lines to AB.

In this study, the recommended specific markers CaETR and GAA47 by Gil et al. [35] and Iruela et al. [19] were successfully employed. The CaETR marker, as suggested by Castro et al. [16], was found to be an effective marker for genotyping AB in chickpeas. In the study conducted by Madrid et al. [27] for allele-specific amplification to detect AB resistance in chickpeas, a very weak or absent band was observed in some tested lines while using primers CaETR-Fw and CaETR-Rev-1, which was later addressed by multiplex PCR together with the primers CaETR-Fw, CaETR- Rev-1 and CaETR-Rev-2. Among the markers used, GAA47 was present at a lower frequency than the resistance alleles detected using the other marker. Both of the markers CaETR and GAA47 are related to blight resistance AB Pathotype I [16,36].

A small number of STMS markers (GAA47, TA146, and TA194) are the suggested markers for identifying resistance in chickpeas to AB [16,19–22]. Of the markers used in the present study, the marker TA146 linked with QTL_{AR2} is most commonly detected from the lines evaluated and is reported to be strongly correlated with resistance to *A. rabiei* Pathotype II [16,19,36]. However, in some lines, including the checks, QTL_{AR3} using marker TA194 was noted, indicating a linkage to blight resistance against Pathotype III. Hence, this is an indication of whether the Kabuli-type chickpea lines detected with all the tested

markers have good levels of resistance to different Pathotypes (I,II,III) of AB, which is an occurrence in chickpeas that has been demonstrated by Newman et al. [23].

In the results obtained, two lines were identified that showed moderate resistance by phenotyping. However, only one of these lines showed resistance markers indicating the presence of major QTLs for AB resistance. This suggests that there is some inconsistency between the results obtained from the phenotyping and genotyping of the Kabuli-type chickpea lines at the seedling stage. Similar results were reported by Bouhadida et al. [37] in their study of twenty-three chickpea genotypes and their reaction to AB under controlled conditions. They used molecular analysis with the CaETR marker and found similar discrepancies between phenotypic and genotypic results. The reason behind this inconsistency may be the presence of different QTLs conferring resistance or due to environmental effects, as explained by Bouhadida et al. [37], Castro et al. [16], Mehmood [14], and Iruela et al. [19].

Research on the effect of the chickpea growth stage on AB infection revealed that the incubation period for the pathogen is shorter in the seedling stage, leading to more disease symptoms. Sharma et al. [18] explained that chickpeas can be attacked by AB at any growth stage in cool and humid weather, depending on the availability of the pathogen. However, disease epidemics are most prominent during the flowering and podding growth stages. They suggested that an evaluation for resistance to AB can be conducted at GS 1 (seedling stage) and/or at GS 4 (flowering stage) to GS 5 (podding stage) in chickpeas.

This study supports the idea that the evaluation for AB resistance should be performed on both 10-day-old seedlings and adult plants in locations with high levels of inoculum. This has led to the suggestion of seedling and adult plant resistance screening, as proposed by Basandrai et al. [38], Kaur et al. [39], Kimber et al. [40], Chongo and Gossen [41], Riaz Malik et al. [42], and Trapero-Casas and Kaiser [43], along with genetic analysis using marker-assisted selection (MAS). Additionally, it indicates that a moderately resistant line could be used in breeding as it possesses useful genes for target traits. Although conventional approaches, such as seedling stage resistance phenotyping, have contributed to reducing diseases, the use of modern technologies, such as the MAS method, is expected to enhance selection efficiency when it is performed on seedling material to further reduce losses against this biotic stress.

5. Conclusions

The expansion of the chickpea industry has encountered challenges due to major diseases, one of which is *Ascochyta* blight. Because of the continuous exposure to aggressive pathogens, completely resistant genotypes against AB have not been reported. Considering the importance of chickpeas and the production gap faced by farmers, this research investigated the disease resistance capacity of new Kabuli-type chickpea lines. By phenotyping, there is visual evidence to clearly observe the diversity of genetic material and their behavior in diverse environmental conditions, while in genotyping, using MAS is an effective and rapid method to identify the presence of desired traits via markers in new lines. The findings of this research demonstrate that there is genetic diversity in response to AB among the tested lines. Through the constant screening of breeding lines, the genetic variability among genotypes and pathogens can be determined, leading to improved breeding programs for host resistance. The markers used in this study are useful in selecting resistance against AB. However, to obtain effective results in MAS, the different markers linked to different identified QTLs should be employed because of the complex genetic nature of AB in chickpeas. Further, the identified moderately resistant lines and/or cultivars can be exploited further in chickpea breeding programs for the development of AB-resistant/tolerant chickpea commercial cultivars. As this research is based only on the seedling stage and was based on a single replicated experiment, the test lines can be further validated under field conditions. It is also recommended to screen chickpeas for resistance to AB at different growth stages, going back to the polygenic nature of Kabuli-type chickpea lines and their resistance to AB. If possible, phenotyping and genotyping for disease resistance should be performed simultaneously for more reliable identification of resistance

sources, using diverse AB isolates that can better represent field conditions and potential pathogen variability.

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