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RESEARCH PAPER

Efficiency of marker-assisted selection in detection of ascochyta blight resistance in Tunisian chickpea breeding lines

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Summary. Ascochyta blight (AB) resistance reactions were studied in 23 chickpea cultivars, mainly advanced lines and Tunisian varieties from the Tunisian chickpea breeding program, growing both at two locations and under controlled conditions. Two co-dominant markers both associated with AB resistance were also used in this study; the CaETR marker tightly linked to QTL_{AR1} in combination with the SCAR $SCY17_{590}$ marker linked to QTL_{AR2} to explore their usefulness in discriminating between resistant and susceptible chickpea genotypes. These two markers contribute efficiently in the selection of new chickpea varieties with better combinations of alleles to ensure durable resistance to AB. The advanced line V10 presenting the resistance allele for CaETR, but being still heterozygous for the $SCAR17_{590}$ was characterized as resistant to moderately resistant in field studies and under controlled conditions. This line could be very useful for developing a new variety that is fixed for both resistance alleles and expresses good levels of resistance to AB in different chickpea cropping environments. These markers are very useful in assisting chickpea breeding programs, especially thanks to their robustness, their co-dominance and their utility across different genetic backgrounds.

Key words: chickpea**,** Ascochyta blight, resistance, molecular markers, selection.

Introduction

Chickpea (*Cicer arietinum* L.) is widely grown around the world and occupies the third position among food legumes in terms of cultivated areas (11.97 millon ha; FAOSTAT-Agriculture, 2010). Chickpea is considered a vital source of protein in many countries, particularly in South and West Asia, and North and East Africa. The average annual yield worldwide (0.78 t ha^{-1}) is considered to be much lower than its potential yield (Singh *et al.*, 1994). Biotic and abiotic constraints cause around 4.8 and 6.4 million tonnes of global annual yield losses, respectively (Ryan, 1997). Among biotic stresses, Ascochyta blight (AB) caused by *Ascochyta rabiei* (Pass.) Labr. is a destructive foliar disease that can cause complete loss of the crop in many chickpea growing regions around the world (Pande *et al.*, 2005). Total yield losses have been recorded in many regions including Pakistan, India, European countries and the Mediterranean regions, where various AB epidemics have occurred (Hawtin and Singh, 1984; Singh *et al.*, 1984; Kaiser *et al.*, 1998; Pande *et al.*, 2005). In Tunisia, chickpea was grown mainly as a spring crop, but problems with spring drought and low production led the chickpea research breeding program to focus on the development of new high yielding winter chickpea varieties. For the winter cropping, AB is

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considered the most important yield-limiting factor in Tunisia (Halila and Harrabi, 1990) and other Mediterranean countries. Therefore, the introduction of winter sowing in the Mediterranean basin required the development of cultivars with improved resistance to AB, which could ensure a sharp increase in production (Singh and Reddy, 1996).

Host plant resistance is one of the major components of integrated AB management and the most economical approach. Therefore, breeding cultivars with durable resistance to AB is essential for the integrated management of the disease. However, resistant cultivars are difficult to obtain due to the continuous evolution of the fungus and appearance of new pathotypes that overcome the resistance of existing cultivars. In addition, disease resistance is considered to be a quantitative trait and numerous QTLs have been identified on the chickpea genetic map (Millán *et al*., 2010). Breeders are attempting to combine genes in a new cultivar to improve the level and durability of resistance, but this process is further complicated when different QTLs or genes control the same phenotype.

Marker-assisted breeding (MAB) for AB resistance would facilitate the development of new chickpea cultivars. Various AB resistance quantitative trait loci (QTLs) have been reported in the literature (Collard *et al*., 2003; Udupa and Baum, 2003; Iruela *et al*., 2006; Tar'an *et al*., 2007), but none of them have been reported to be used in MAB.

Two quantitative trait loci (QTL_{AR1} and QTL_{AR2}) associated with resistance to AB have been successfully targeted using allele specific markers. A co-dominant SCAR marker ($SCY17_{590}$) tightly linked to QTL_{AR2} has been reported (Iruela *et al*., 2006) and was successfully employed to characterize blight resistance sources in chickpea (Imtiaz *et al*., 2008). A new co-dominant molecular marker (CaETR) was developed by Madrid *et al*. (2012a) based on allelic sequence length polymorphism in an ethylene receptor-like gene located in the genomic region of QTL_{AR1}, which confers AB resistance in chickpea, and which explained 33.8% of phenotypic variation. These markers not only discriminated resistance and susceptible phenotypes of chickpea to AB, but also easily detected heterozygous genotypes (Madrid *et al*., 2012b).

The objectives of this study were (i) to assess the resistance level of chickpea advanced lines, cultivars and Tunisian commercial varieties in different environments and under controlled conditions and (ii) to explore the usefulness of $SCAR17_{590}$ and CaETR markers associated with AB resistance in discriminating between resistant and susceptible chickpea genotypes in the national chickpea breeding program.

Material and methods

Plant material

Twenty three chickpea genotypes were evaluated for their reaction to AB in field, under controlled conditions and through molecular analysis: 12 advanced lines (V1, V2, V3, V4, V5, V6, V7, V8, V9, V10, V12 and Béja 2) from the Tunisian chickpea breeding program, 7 Tunisian commercial varieties ('Béja 1', 'Nour', 'Bouchra', 'Nayer', 'Kasseb', 'Chétoui' and 'Amdoun 1'), a susceptible Spanish local variety ('Blanco Lechoso'), and 3 lines (ILC482, WR315 and JG62) (Table 1). 'Blanco Lechoso' is frequently used as check and spreader in Spanish chickpea breeding program (Juan Gil, Departamento de Genetica, University of Cordoba, Cordoba, Spain, personal communication), ILC482 is a tolerant accession to AB from ICARDA, WR315 is a resistant line to all races of fusarium wilt (FW) and JG62 is a resistant line to race 0.

Evaluation of genotypes for reaction to ascochyta blight

Field trials

Chickpea genotypes were sown during the cropping season 2010–2011 in two different environmental conditions in Tunisia; Oued Béja experimental station localized in the Béja region (36°44'03.53"N; 9°13'37.73"E) and Oued Méliz experimental station in Jendouba region (36°28'44.58"N; 8°29'37.87"E). In each location, the trial was established according to a randomized complete block design with four replications. Each plot was sown in a single row (2 m long) with 0.5 m between rows and a density of ten seeds per linear meter. The susceptible check to AB, 'Amdoun 1', was used as a spreader and sown in a single row repeated every two tested entries. The trial was surrounded by three rows of the spreader in order to provide uniform inoculum. The field inoculation was provided by spreading about 100 g per row of infected stem debris collected on the susceptible check from the previous cropping season at each location. To maintain a favourable environment for

Table 1. Pedigree and origin of the 23 chickpea cultivars and advanced lines studied.

Chickpea cultivar	Pedigree	Origin
Nour	X96TH61-1	INRAT-ICARDA
V4	X96TH61-2	INRAT-ICARDA
Béja 1	(Amdoun 1 x ILC3279) x ILC200	INRAT-ICARDA
V7	X98TH86-1	INRAT-ICARDA
V5	X96TH24	INRAT-ICARDA
V10	X96TH61-3	INRAT-ICARDA
V9	X96TH62-1	INRAT-ICARDA
Béja 2	(Amdoun 1 x ILC482) x ILC191	INRAT-ICARDA
V1	X96TH86-2	INRAT-ICARDA
Bouchra	FLIP84-79C	ICARDA
V2	X96TH62-2	INRAT-ICARDA
V6	X96TH61-4	INRAT-ICARDA
Nayer	FLIP84-92C	INRAT-ICARDA
V8	X96TH86-3	INRAT-ICARDA
Kasseb	FLIP83-46C	ICARDA
Chétoui	ILC3279	Ex. USSR-ICARDA
V3	X97TH85	INRAT-ICARDA
V12	X96TH63	INRAT-ICARDA
Amdoun 1	Amdoun 1	INRAT-TUNISIA
ILC482	ACC.N°267780-68	Turkey-ICARDA
Blanco Lechoso	-	Spain
WR315	WR315	ICRISAT
JG62	JG62	ICRISAT

AB development, fields in the two experimental locations were frequently irrigated. The evaluation of chickpea genotypes for AB reaction was performed by using a rating scale of 1 (highly resistant) to 9 (highly susceptible) based on the severity of infection on leaves, stems and pods as proposed by Singh *et al.* (1981). Data related to the level of infection were scored and collected at two, four, six and eight weeks after inoculation.

Greenhouse trial and inoculation

Seeds from all genotypes used in field conditions were disinfected and pregerminated then transferred to peat pots having a volume of 0.5 L at a rate of one seed per pot. The trial was conducted using a Completely Randomized Design with eight replications. Plants were grown under glasshouse conditions with 16 hours photoperiod and a temperature of 20°C and 10°C during the day and at night, respectively. Infected chickpea debris, collected from chickpea plots in the experimental station of Kef (36°07'31.26"N; 8°43'19.61"E) during the cropping season 2010–2011, were cut out at the level of lesions, disinfected and placed on Petri dishes containing malt agar at 20°C for fungal development. A monospore culture of *A. rabiei* was used to prepare the inoculum. The inoc-

ulum concentration was adjusted at 2×10^5 spores mL^{-1} and plants were inoculated 15 days after sowing by spraying the suspension. In order to maintain humidity and to promote fungal infection, plants were misted frequently with water. The AB disease score was recorded, using the 1–9 scale (Singh *et al*., 1981), when the susceptible check 'Amdoun 1' presented the first symptoms of AB. Plants were scored seven times at a rate of once per week.

Statistical analysis

The disease scores in the field and under controlled conditions were used to calculate the area under the disease progress curves (AUDPC) of each genotype (Campbell and Madden, 1990). Analysis of variance was applied to the AUDPC data from the two locations and from controlled conditions according to the following model:

$$x_{ij} = \mu + g_i + \beta_j + \varepsilon_{ij}$$

Where x_{ij} is the individual observation, μ the general mean, g_i the effect of ith genotype, β_j the effect of jth block and ε_{ij} is the residual error.

Considering the data of two locations, combined analysis was applied following the model:

$$x_{ijk} = \mu + g_i + l_k + \beta_{j(k)} + gl_{jk} + \varepsilon_{ijk}$$

Where l_k is the effect of kth location and gl_{jk} is the effect resulting from interaction between ith genotype and kth location.

Correlation analysis between results at each location was performed by Spearman Rank Correlation (r).

The statistical analyses were carried out using the SAS software (version 8.0). LSD all-pairwise Comparison test was used to compare genotypes.

Molecular marker analysis

Markers previously developed by Iruela *et al*. (2006) ($SCY17_{590}$) and Madrid *et al*. (2012b) (CaETR), and linked to QTL_{AR2} and QTL_{AR1} respectively, were used in this study. Genomic DNA was isolated from leaves of the 23 genotypes previously described using DNAzol reagent (Invitrogen Carlsbad, CA, USA) according to the manufacturers' instructions. PCR reactions and cycling conditions were carried out as described previously by Iruela *et al*. (2006) for $SCY17_{590}$ and Madrid *et al*. (2012b) for CaETR. Amplification products were analyzed on 6% polyacrylamide gels in 1 × TBE buffer and visualized by ethidium bromide staining.

Results

Field evaluation for AB resistance

The analysis of variance of AUDPC revealed significant differences among genotypes in terms of resistance to AB (P<0.001) at both locations, Oued Béja and Oued Méliz (Table 2). At the Oued Béja site, the 23 tested genotypes were classified into different groups based on AUDPC values (LSD test, Table 3). Thus, 'Nour', V4, 'Béja 1', V7, V5, V10, V9, Béja 2, V1, 'Bouchra', V2, V6, 'Nayer', V8, were the most resistant genotypes that did not show significant differences among themselves. The genotypes WR315 and JG62 were the most susceptible cultivars followed by 'Blanco Lechoso', ILC482, 'Amdoun 1' and V12. At Oued Méliz, based on AUDPC values following the same criteria and comparison LSD test (Table 3), 'Nayer', 'Chétoui', V8, V9, 'Nour', 'Bouchra', 'Kasseb', V7, Béja 2, V4, V10, V3 and V5 were considered as the most resistant ones, whereas WR315 and JG62 were considered the most susceptible. The Spearman Rank Correlation coefficient (r = 0.5) expressed a positive correlation for genotype reactions between the two locations. In fact, we observed that 'Nour', 'Nayer', 'Bouchra', 'Béja 2', V4, V5, V7, V8, V9 and V10 were the most resistant genotypes at both locations (Table 6). In contrast, the two genotypes WR315 and JG62

Table 2. Analysis of variance of ascochyta blight severity (measured as Area Under the Disease Progress Curve) of 23 chickpea genotypes grown at two different locations and under controlled conditions.

Variation source	Oued Béja		Oued Méliz	
	df	Mean squares	df	Mean squares
Block	3	557575ns	3	65975.4ns
Genotype	22	416451***	22	41658.5***
Error	66	31961	66	8996.6
	CV=25.99 %		CV=15.02%	

*** Significant at level $P \leq 0.001$; ns: not significant.

Table 3. Area Under the Disease Progress Curve (AUDPC) mean values and classification of the 23 genotypes studied at two field locations and under controlled conditions.

Genotypes	Oued Béja[a]		Oued Méliz[a]		Controlled conditions[a]	
	N[1]	*AUDPC*	N[2]	*AUDPC*	N[3]	*AUDPC*
Nour	1	365 a	5	550 abc	13	1478 cdefgh
V4	2	410 ab	10	585 abcde	11	1358 cdef
Béja 1	3	435 ab	17	690 def	19	1830 i
V7	4	477 abc	8	575 abcd	17	1695 ghi
V5	5	481 abc	13	615 abcdef	7	1264 cd
V10	6	506 abc	11	600 abcdef	8	1317 cde
V9	7	509 abc	4	545 abc	6	1229 c
Béja 2	8	509 abc	9	585 abcde	15	1641 efghi
V1	9	520 abc	14	630 bcdef	-	-
Bouchra	10	526 abc	6	550 abc	12	1371 cdefg
V2	11	537 abc	15	635 bcdef	4	1223 c
V6	12	543 abc	16	660 cdef	2	883 ab
Nayer	13	562 abc	1	490 ab	3	1195 bc
V8	14	578 abcd	3	525 ab	20	1836 i
Kasseb	15	656 bcd	7	560 abcd	5	1228 c
Chétoui	16	661 bcd	2	510 ab	9	1341 cdef
V3	17	714 cd	12	605 abcdef	1	851 a
V12	18	814 de	20	720 fg	10	1351 cdef
Amdoun1	19	829 de	19	720 fg	18	1799 hi
ILC482	20	1020 e	21	725 fg	21	1846 i
Blanco Lechoso	21	1036 e	18	715 ef	-	-
WR315	22	1537 f	23	885 h	14	1572 defgh
JG62	23	1538 f	22	850 h	16	1668 fghi

*, Mean followed by the same letter do not differ according LSD test ($P<0.05$).
N^1, N^2 and N^3 are rank of the genotypes in term of AUDPC mean values in Oued Béja, Oued Méliz and under controlled conditions, respectively.

showed the highest levels of susceptibility to infection in the two field environments (Table 6).

It is important to note that ILC482 was reported in many countries in the 1980s and 1990s to be tolerant to AB, while in Tunisia it was classified with the variety 'Amdoun 1' as susceptible to AB. In addition, the Tunisian variety 'Béja 1', selected and registered by the Tunisian chickpea breeding program for its tolerance to AB, showed susceptibility at Oued Méliz with an AUDPC value of 690 compared to 435 at Oued Béja (Table 3).

The combined analysis of variance for AUDPC showed significant genotype and genotype-location interaction effects (Table 4). It is important to note that the genotype 'Béja 1' was classified 3rd in term of resistance to AB at Oued Béja, while it occupied the 17th position at Oued Méliz (Table 3). 'Chétoui' and 'Nayer' were classified 16th and 13th at Oued

Table 4. Combined analysis variance of ascochyta blight severity (measured as Area Under the Disease Progress Curve) of 23 chickpea genotypes growing at two Tunisian locations (Oued Béja and Oued Méliz).

Variation source	df	Mean square
Genotype	22	338608**
Location	1	146110ns
Block (Location)	6	311775ns
Genotype*Location	22	119501***
Error	132	20479

** and *** are significant at level $P \leq 0.01$and $P \leq 0.001$ respectively; ns: not significant.

Table 5. Analysis of variance of ascochyta blight severity (measured as Area Under the Disease Progress Curve) of 21 chickpea genotypes growing under controlled conditions.

Variation source	df	Mean squares
Genotype	20	664603.03***
Error	145	112365.02
	CV = 23.4%	

*** Significant at level $P \leq 0.001$.

Béja, whereas these two varieties occupied the second and the first position, respectively, at Oued Méliz in term of AB resistance.

Evaluation of AB resistance under controlled conditions

The AUDPC analysis of variance revealed significant differences among genotypes ($P<0.001$) (Table 5). The advanced lines V3 and V6 showed high resistance to AB under controlled conditions (Table 3). In comparison with the disease reaction in the field, 'Béja 1' and 'Béja 2', selected as tolerant to AB in our breeding program, were classified in the same group of the susceptible genotypes 'Amdoun 1', JG62 and ILC482 (Table 3). On the other hand, the highly susceptible genotype WR315 showed a similar reaction under controlled conditions to the resistant varieties 'Nour' and 'Bouchra'. The relatively high coefficient of variation (CV=23.4%) of ANOVA in this experiment under controlled condition suggests that infection was not very homogenous.

Molecular screening for AB resistance

Results obtained using the CaETR and $SCY17_{590}$ markers, which are linked to QTL_{AR1} and QTL_{AR2},

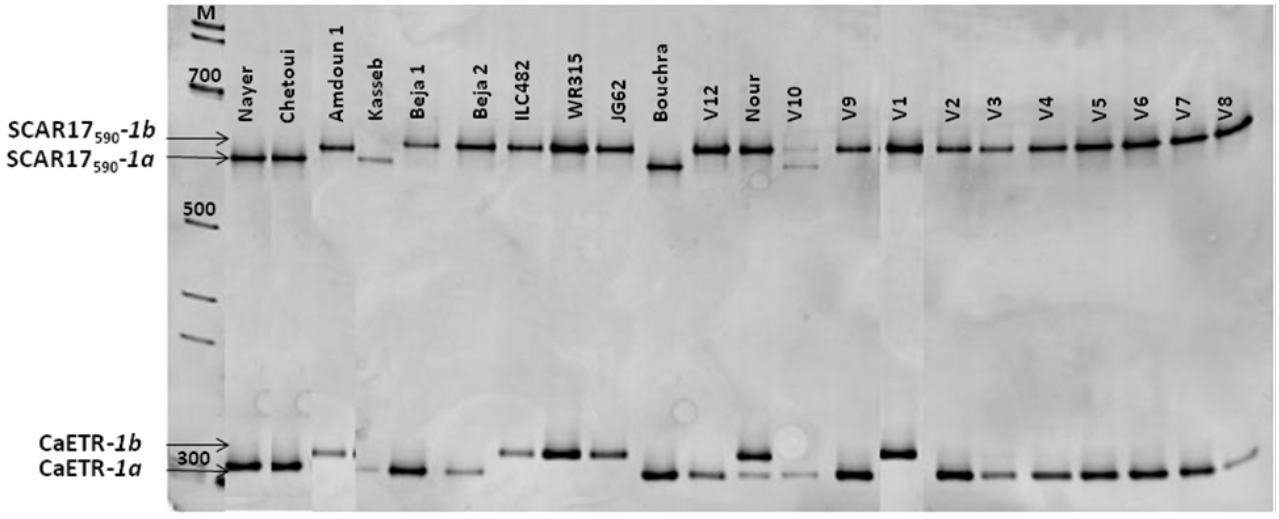

Figure 1. Polyacrilamide gel showing the amplification products using $SCY17_{590}$ and CaETR markers in the 23 chickpea cultivars studied. M, molecular size marker (pb) (Hyperladder IV of Bioline). $SCY17_{590}$-*1a* and $SCY17_{590}$-*1b* correspond to resistant and susceptible alleles to AB (targeting QTL_{AR2}). CaETR-*1a* and CaETR-*1b* correspond to resistant and susceptible alleles to AB (targeting QTL_{AR1}).

Table 6. Association of resistance and susceptible alleles of two DNA markers, $SCY17_{590}$ and CaETR, with resistant and susceptible phenotypes of chickpea cultivars evaluated under field conditions.

Cultivars	Field evaluation[a]		Molecular screening[b]	
	Oued Béja	Oued Méliz	$SCAR17_{590}$	CaETR
Béja 2	R	R	-	+
Bouchra	R	R	+	+
Nayer	R	R	+	+
Nour	R	R	-	H
V4	R	R	-	+
V5	R	R	-	+
V6	R	MR	-	+
V7	R	R	-	+
V8	R	R	-	+
V9	R	R	-	+
V10	R	R	H	+
Béja 1	R	MR	-	+
Chétoui	MR	R	+	+
Kasseb	MR	R	+	+
V1	R	MR	-	-
V2	R	MR	-	+
V3	MR	R	-	+
V6	R	MR	-	+
V12	S	S	-	+
Amdoun 1	S	S	-	-
Blanco lechoso	S	MR	-	-
ILC482	S	S	-	-
JG62	HS	HS	-	-
WR315	HS	HS	-	-

[a] R, MR, S and HS indicated resistant, moderate resistance, susceptible and highly susceptible phenotypes, respectively under field conditions.
[b] + and - indicate resistant and susceptible alleles, respectively; H for heterozygous.

respectively, to screen 23 chickpea genotypes for resistance to AB are shown in Table 4. According to studies by Iruela *et al.* (2006) and Imtiaz *et al.* (2008), the amplified band of 590 bp using $SCY17_{590}$ marker is associated with resistance and a band of 605 bp is associated with susceptibility (Figure 1). Among the 23 studied genotypes, the resistance allele was detected in only five genotypes. The susceptible allele was detected in 19 genotypes with one heterozygous cultivar, V10. For CaETR marker, we detected a band of 289 bp associated with resistance in 17 of 23 genotypes. The band of 304 bp detected in seven cultivars is associated with susceptibility (Madrid *et al.*, 2012b). The variety 'Nour' was revealed to be

heterozygous for the CaETR marker (Figure 1).

In general, all genotypes classified as resistant or moderately resistant in both field locations showed at least one or both resistant alleles associated with QTL_{AR1} and QTL_{AR2}, except the genotype V1, which did not have either resistance alleles (Table 6). The frequency of QTL_{AR1} in our material was higher than QTL_{AR2}. The susceptible genotypes tended to posess susceptible alleles, except for genotype V12, which had the resistance allele of the CaETR marker.

Discussion

Development of AB resistant cultivars is one of the major objectives in the Tunisian chickpea breeding program conducted by the Field Crops Laboratory. The results of this study showed genetic variation for AB disease reaction within the group of genotypes evaluated. The significant genotype-location interaction could be explained in part by the existence of different pathotypes in the two locations. As an example, the resistance of 'Béja 1' decreased at the Oued Méliz location compared to Oued Béja, whereas the resistance of 'Chetoui' and 'Nayer' decreased at Oued Béja comparing to Oued Méliz. In addition, the experimental error was higher in Oued Béja, suggesting that a higher heterogeneity in disease development could also be responsible for the observed results. This heterogeneity was probably caused by the inoculation method using infected debris, although other factors like climatic conditions or inoculum density may also have been sources of variance. It is important to point out that the new variety 'Nour', registered in 2011, showed good performances at both field experimental locations.

The results obtained under controlled conditions could probably be explained by the development of a new, more virulent pathotype in the Kef region. However, the results obtained in this experiment should be confirmed due to relatively high experimental error. It may explain why highly susceptible lines in the field (JG62 and WR315) showed similarly resistant reaction to other resistant genotypes (Béja 2, 'Nour', V7, and V8).

With respect to the characterization of AB resistant chickpea genotypes at the molecular levels, the results revealed that the resistance allele of CaETR was present at higher frequency than the resistance allele detected using the marker $SCY17_{590}$. The CaETR marker used in this study is tagged to QTL_{AR1}, which is the QTL that has been most commonly detected in most of our studied genotypes. These results highlight the usefulness of the presence of the two resistance alleles tagged to QTL_{AR1} and QTL_{AR2} towards good resistance levels to AB in different environments and even with the probable existence of different pathotypes. Both QTLs seem to be associated with pathotype II of AB (Udupa and Baum, 2003; Cho *et al*., 2004; Iruela *et al*., 2006). The recently released variety 'Nour' was heterozygous for the CaETR marker and showed one susceptible allele for $SCAR17_{590}$. This result is probably due to a possible varietal mix in seeds because the selection scheme for several generations applied in the breeding program should lead, normally, to fixed characters and to a homogenous genotype for all loci. The molecular screening of several seed lots of variey 'Nour' using single plant DNA extraction and the two markers ($SCAR17_{590}$ and CaETR) is necessary for two reasons: first, to verify the homogenous state of this variety and discard the heterozygous seed lots, and second to correctly attribute susceptibility or resistance alleles to the markers used in this study.

It is important to notice that genotype V1 was classified as resistant to moderately resistant in field conditions at both locations, but the molecular analysis showed the absence of $SCY17_{590}$ and CaETR resistance alleles. Therefore, considering the tight linkage between the markers employed and their respective QTLs (Iruela *et al*., 2006, Madrid *et al*., 2012b), this genotype may actually carry different genes or QTLs for resistance to AB. For example, QTL_{AR3} on linkage group II was associated with AB resistance and was reported to be tightly linked to a microsatellite marker (STMS TA194) (Iruela *et al*., 2007).

It is important to mention that several QTLs controlling AB resistance or other biotic stresses, such as several races of FW in chickpea, are tagged by STMS markers (Iruela *et al*., 2007; Halila *et al*., 2009). STMS markers are described as having extensive polymorphisms within species because of mutations that occur in the number of repeating microsatellite motifs. Thus, resistance allele prediction with STMS is difficult and even not feasible compared to specific markers, hence they are not recommended for screening germplasm collections for biotic stresses (Collard *et al*., 2005). These findings confirm the usefulness of the allele specific marker (CaETR) and the codominant $SCAR17_{590}$ marker for chickpea breeding programs. In fact, applying CaETR and $SCY17_{590}$, to

target QTL_{AR1} and QTL_{AR2} in advanced lines helped to discard those presenting susceptible alleles and allowed the selection of chickpea lines with better allele combinations in order to ensure a durable resistance for AB. This process will be extended also for chickpea lines in the early $F_{3:4}$ generations. This will reduce the number of selected individual plants in each generation and consequently, notably decrease costs for field trials to identify resistant materials.

It is noteworthy that the advanced line V10, which possesses the CaETR resistance allele, was heterozygous for the $SCAR17_{590}$ marker. V10 was derived from a cross 'X96TH61' originated from ICARDA and was evaluated under Tunisian conditions for the double resistance to AB and FW. It is necessary to proceed with the selection of a line from V10 that is homozygous for resistance based on $SCAR17_{590}$. Consequently, V10 combining the two resistance alleles, could be a very interesting genotype to be selected and then registered as a new resistant variety to AB in different chickpea cropping environments. This would be a good example of the application of these molecular markers by assisting chickpea breeding programs.

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