Agronomy Research **23**(1), 401–414, 2025
https://doi.org/10.15159/AR.25.027

Molecular Identification and Characterization of *Botrytis* spp. from Strawberry in Morocco

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Received: January 13th, 2024; Accepted: April 7th, 2025; Published: April 22nd, 2025

Abstract. *Botrytis cinerea* is a highly destructive infection, responsible for gray mold in small fruit crops such as strawberries, it causes pre and post-harvest losses. Identification of this pathogen is the first step to control it. The purpose of the present study was to identify *Botrytis* species, characterize and assess genetic diversity of gray mold pathogen populations from strawberry in Morocco using molecular markers. Sixty-eight isolates were obtained from infected fruits of 14 different geographic origins in Morocco, DNA was extracted and the isolates were identified using $Bc108^{+}/Bc563^{-}$ and NEP2 sequences variability. To differentiate between groups N and S genotypes of *Botrytis* spp., two primer pairs were used (BcinN-in-F/R and Mrr1-spez-F/R). Presence of transposable elements (TE) *boty*, *flipper* and 9 microsatellites (SSR) were used to examine genetic diversity of the isolates. In our population, forty-seven isolates were identified as *B. cinerea*. Data indicated that 26% of isolates were attributed to groups S and 31% to N. It also showed that 32% of the isolates possessed *flipper* genotype, followed by *transposa* (28%), *boty* (21%) and *vacuma* (19%). Analysis of 68 *Botrytis* spp. isolates by SSR showed a high level of genetic diversity indices among populations of which the isolates clustered into seven genetic groups. Data showed genetic diversity in *Botrytis* spp. populations from Morocco. Identification of the pathogen and knowledge of its genetic diversity enable optimal and effective disease management.

Key words: *Botrytis cinerea*, genetic diversity, grey mold, microsatellites, strawberries, transposable elements.

INTRODUCTION

Fragaria ananassa (Strawberry), one of most popular fruits with a production of more than 9.5 million tons per year worldwide with a harvested area of 397,603 ha (FAOSTAT, 2023). In Morocco, the area occupied by strawberries during 2022/23

campaign was 3,800 ha (*Agri-Mag*, 2023). Several pathogens, including viruses, bacteria, nematodes and fungi, can affect strawberry plants. Fungi, which have the greatest economic impact, can infect all plant parts, causing extensive damage or death (Garrido et al*.,* 2011).

The necrotrophic fungus *Botrytis cinerea* Pers: Fr. (perfect stage as *Botryotinia fuckeliana*), is a pathogen that causes gray mold infection on a wide variety of plants worldwide (Williamson et al., 2007). It can infect more than 1,000 species of plants, as the *Botrytis* genus contains about 35 species (Elad et al., 2016). This pathogen has a significant economic impact on various important crops including strawberry, grape, tomato and japanese quince (*Chaenomeles* spp.) (Dean et al., 2012; Jakobija et al., 2020). It may cause obvious symptoms of disease in the preharvest period or remain dormant until the postharvest period (Fillinger & Elad, 2016). On strawberry, various *Botrytis* spp. can cause gray mold disease. Among them, *B. pseudocinerea*, *B. mali*, *B. cinerea* and *B. fragariae* (Plesken et al., 2015; Dowling & Schnabel, 2017; Hassan et al., 2021; Vielba-Fernández et al., 2023). The severity of the disease depends on organs attacked and plant stage. For strawberry, gray mold, affects petioles, leaves, fruits, stems, flowers and frequently begins early as blossom blight (Williamson et al., 2007). The infection and disease development depend essentially on climatic factors, with relative humidity of 80 to 90% and temperatures between 17 and 23 °C, especially during winter (Wang et al., 1986; Wilcox & Seem, 1994). *B. cinerea* populations are very diverse, structurally complex, depending on cultivation system and host plant (Walker & Fournier, 2013). Strategies for managing grey mold in strawberries rely on an integrated approach to crop protection, which involve the use of fungicides. In Morocco, a wide range of fungicides have been used to control grey mold, but their effectiveness remains limited due to intensive use in the fields. For best application of fungicides, it's crucial to understand distribution and populations structure of pathogens (Rasiukevičiūtė et al., 2018). In Morocco, genetic analysis of *Botryti*s spp. populations in *faba bean* (*Vicia faba* L.) revealed no correlation between genetic diversity and geographical origin (Aouzal et al., 2022).

Traditionally, the following criteria have been used to identify *Botrytis* species related on colony morphology, measurement of conidia and conidiophores. Nevertheless, molecular characteristics have recently been added to the morphological observations to enhance further identification (Notte et al., 2021). Molecular tools facilitate quick and accurate species identification. Several primers have been developed to identify *Botrytis* spp. such as species specific primers ($Bc108^{+/}Bc563^{-}$) (Rigotti et al., 2006) and NEP2 sequences (Rupp et al., 2017). Moreover, *B. cinerea,* a fungus with a broad range of hosts, exhibits significant genetic diversity. In Germany, a variant of *B. cinerea* was identified in strawberry fields, named *Botrytis* group S. This variant displayed notable distinctions from previously characterized *B. cinerea* strains and a high frequency of resistance to multiple fungicide compared to *B. cinerea* N in German berry fields (Leroch et al., 2013). Four transposable elements (TEs) genotypes were described: *boty, flipper*, *transposa*, and *vacuma* (Diolez et al., 1995; Giraud et al., 1997; Levis et al., 1997). Various studies showed a significant variation in frequency distribution of TE between *Botrytis* samples obtained from diverse host plants or geographic regions (Samuel et al., 2012; Wessels et al., 2016). This characteristic has proven to have a limited impact on genetic classification. However, there are indications

suggesting that *transposa* and *vacuma* genotypes exhibit diverse adaptations to different host plants and fluctuations in their relative frequencies across seasons (Martinez et al., 2005; Samuel et al., 2012). Microsatellite (SSR) by specific genetic markers allows for rapid detection of pathogen polymorphism (Fournier et al., 2002). Objectives of this research were to identify *Botrytis* spp. isolates from strawberry, characterize them, and evaluate their genetic diversity in Morocco using molecular markers.

MATERIALS AND METHODS

Collection of *Botrytis* spp. samples

Sixty-eight *Botrytis* spp. isolates were obtained from strawberry fruits with gray mold symptoms collected from 14 growing regions in Morocco (Table 1). For pathogen isolation, the infected fruits were cut into small pieces (0.5 cm), put in 3% sodium hypochlorite (NaOCl) for 3 min, rinsed three times with sterile distilled water, dried on a sterile paper and incubated for 16 to 24 hours at 23 °C. Using a sterile needle, a small amount of *Botrytis* spp. mycelium was then picked from the fungal growth on the surface of the rotten fruit, sub-cultured onto Potato Dextrose Agar(PDA) media and incubated for 7–10 days at 23 °C. A pure culture was obtained using the single-spore method (Muñoz et al., 2019). Isolates from each region were considered as a population in this study. The fungus was lyophilized and stored at -20 °C until needed.

Table 1. *Botrytis* spp. isolates from different strawberry varieties

Geographic Origin	Populations	Number of isolates	Strawberry variety
Chewafaàe Ouled Msahel, Moulay-Bousselham	Pop1	5	Marquise, Sabrina
Cooperative A'nasr Drdara	Pop2	3	Sabrina
Dlalha	Pop3	13	Camarosa, Victoria, Fortuna and Festival
Douar Lamaronami, Oulad Aikid, Moulay-Bousselham	Pop4	5	Lusa and Lara
Gla-Chewafaae, Moulay-Bousselham	Pop5	2	Fortuna
Laaouamra	Pop6	4	Sabrina and Fortuna
Laghwazi-Laaouamra	Pop7	3	Sabrina, San Andreas and Victoria
Lalla Mimona, Laanabssa	Pop8	9	Sabrina and Fortuna
Larache Under Greenhouse	Pop9	3	Inconu
Larache-Laouamra	Pop10	3	Victoria, Sabrina and Calinda
Ouled Hammou – Laaouamra	Pop11	3	Fortuna, Victoria
Ouled Laghmari R'mal Laaoumra	Pop12	10	Victoria, Sabrina and Calinda
Inra Rabat Guiche Greenhouse	Pop13	1	Palmeritas
Zone Bargha Under Greenhouse	Pop14	4	Calinda and Fortuna

DNA extraction

A genomic DNA extraction kit (Thermo Fisher Scientific, Lithuania) was used to extract genomic DNA from the fungal simples. Subsequently, DNA was dissolved in 100 μl^{-1}×TE buffer, and kept at -20 °C. Its quality was evaluated on 1% agarose gel using a UVP gel imager (Bio-Rad), while concentration was assessed with a Nanodrop 8000

spectrometer (ThermoFisher Scientific, USA). Genomic DNA were diluted (25 ng μl^{-1}) and preserved at -20 °C until usage.

Molecular identification and characterization of *Botrytis* isolates

Botrytis species were identified using specific primers : $Bc108^{+}$/ $Bc563^{-}$ (Rigotti et al., 2006) and NEP2 sequences (Rupp et al., 2017) (Table 2). NEP2 gene was chosen due to its multiple regions with varying nucleotide sequences among 3 species.

Table 2. Primer sequences employed for identification and genetic diversity of *Botrytis* spp.

PRIMERS	PRIMERS SEQUENCES (5'–3')	TM (°C)	SIZE RANG (PB)	REFERENCE
BC108+	ACCCGCACCTAATTCGTCAAC	50	360; 480	Rigotti et al., 2006
BC563–	GGGTCTTCGATACGGGAGAA			
CFM_NEP2	GTAGGAACAGTTTATGAG	60		Rupp et al., 2017
C_NEP2	GACCCATTGAGTGATCGACG		592	
F_NEP2	TAGTTTGGATCTGTAAGGAGGTGA		332	
M_NEP2	ACCACTAAGAAACGTTAGAGACATG		186	
F300	GCACAAAACCTACAGAAGA	60	1,250	Samuel et al., 2012
F1550	ATTCGTTTCTTGGACTGTA			
BOTYF4	CAGCTGCAGTATACTGGGGGA	68	533	
BOTYR4	GGTGCTCAAAGTGTTACGGGAG			
BCINN.IN.F	GCGACCTCATCGTTCTTTCAC	55	182	Plesken et al., 2015
BCINN.IN.R	GGCTCTCGATGAGCTGTTTC			
MRR1.SPEZ.F	TATCGGTCTTGCAGTCCGC	60	165	Leroch et al., 2013
MRR1.SPEZ.R	TTCCGTACCCCGATCTTCGGAA			
BC1-F	AGGGAGGGTATGAGTGTGTA	50	245–281	Fournier et al., 2002
BC1-R	TTGAGGAGGTGGAAGTTGTA			
BC2- F	CATACACGTATTTCTTCCAA	53	161–205	
BC2- R	TTTACGAGTGTTTTTGTTAG			
BC3- F	GGATGAATCAGTTGTTTGTG	50	197–229	
BC3- R	CACCTAGGTATTTCCTGGTA			
BC4- F	CATCTTCTGGGAACGCACAT	59	98–125	
BC4- R	ATCCACCCCCAAACGATTGT			
BC5- F	CGTTTTCCAGCATTTCAAGT	53	143–163	
BC5- R	CATCTCATATTCGTTCCTCA			
BC6- F	ACTAGATTCGAGATTCAGTT	50	88–158	
BC6- R	AAGGTGGTATGAGCGGTTTA			
BC7- F	CCAGTTTCGAGGAGGTCCAC	59	113–131	
BC7- R	GCCTTAGCGGATGTGAGGTA			
BC9- F	CTCGTCATAACCACGCAGAT	50	150–194	
BC9- R	GCAAGGTCTCGATGTCGATC			
BC10- F	TCCTCTTCCCTCCCATCAAC	59	158–189	
BC10- R	GGATCTGCGTGGTTATGACG			

A universal forward primer, CFM_NEP2, was paired with three reverse primers (M_NEP2, F_NEP2, and C_NEP2), each specific to *B. mali, B. fragariae,* and *B. cinerea,* respectively. Polymerase chain reaction (PCR) amplification was conducted in a reaction volume of 10 µl containing 1 µl 10× Tag buffer, 1 µl d NTP (Thermo Fisher Scientific, Mix 2 mM each), 0.5 µl for each primer, 0.2 µl MgCl2 (25 mM), two µl of DNA, 0.2 µl

Taq DNA polymerase (MyTaq DNA polymerase, 5 U μl^{-1}, ThermoFisher Scientific) and 4.6 µl DNase/RNase-free water. A PCR was performed using a thermal cycler with specific protocols. For Bc108+/Bc563− sequence protocol included: an initial denaturation at 95 °C for 1 min, then 35 cycles of denaturation at 94 °C for 45 s, annealing for 45 s at 50 °C, an extension for 45 s at 72 °C and a final extension for five min at 72 °C. While for NEP2 sequences, amplification process began with: an initial denaturation for 5 min at 95 °C, 35 cycles of denaturation for 30 s at 95 °C, annealing for 30 s at 60 °C, extension for one min at 72 °C and 5 min of final extension at 72 °C.

In order to distinguish between group S and group N genotypes of *B. cinerea*, 2 primer pairs were used. Mrr1-spez-F/R to identify an indel of a 21 pb fragment in the Mrr1 gene (Leroch et al., 2013), while BcinN-in-F/R to detect an indel of 18pb fragment in the same gene (Plesken et al., 2015). PCR amplification was performed in 5 min initial denaturation at 95 °C, then followed by 35 cycles of denaturation for 30 s at 95 °C, annealing temperature for 30 s at 60 °C or for 45 s at 55 °C (Table 2), extension for 30 s at 72 °C and final elongation for 7 minutes at 72 °C.

Transposable Elements (TEs) detection

Presence of TEs *Boty* and *Flipper* were detected using the primer Boty-F4/Boty-R4, while *Flipper* was detected using the primer F300/F1500 (Diolez et al., 1995; Levis et al., 1997) (Table 2). Amplification started with an initial denaturation for 5 min at 95 °C, followed by 40 cycles. In each cycle, denaturation occurred at 94 °C for 1 min, annealing at either 60 °C or 68 °C (depending on primer pair: F300/F1500 and BotyF4/BotyR4, respectively) for 1 min, extension and final extension at 72 °C for 1 min and 10 min respectively.

Microsatellite analysis

Genetic diversity of *Botrytis* spp. was examined using nine microsatellites (SSR) developed by Fournier et al., 2002 (Bc1 to Bc7, Bc9 and Bc10). PCR amplification used a reaction volume of 10 µl, as previously mentioned. PCR amplification started with: an initial denaturation for 3 minutes at 94 °C, 30 cycles of denaturation at 94 °C (30 s), annealing temperatures of 50 °C, 53 °C or 59 °C (Table 2) for 30 s and extension at 72 °C (30 s) (Fournier et al., 2002).

Gel electrophoresis

PCR products were electrophoresed in a polyacrylamide gel (6%) and stained with ethidium bromide. Subsequently, the gel was observed and photographed under UV light in molecular Imager Gel DocTM XR System. The marker used was GeneRuler 1 kb or 0.5 kb DNA Ladder (Thermo Fisher Scientific).

Statistical analysis

Amplified bands from each microsatellite were scored using Image Lab Software as present or absent (1 or 0, respectively) of homologous alleles to create a binary matrix for each primer. Genetic diversity analysis within populations was conducted using GenAlEx ver. 6.5 software. This involved calculating various parameters such as genetic distance among isolates, Nei's gene diversity (*He*), polymorphic loci percentage *(%P*), number of alleles observed (*Na*), effective alleles number (*Ne*) and diversity index of Shannon *(I*) (Peakall & Smouse, 2012). Number of permutations for significant tests was

established at 1,000. In addition, to assess genetic relationships between populations, the Dice inter-individual distance coefficient matrix was used for a principal coordinate analysis (P CoA). A clustering analysis was conducted using unweighted pair group method with arithmetic averages (UPGMA), relying on Jaccard's similarity index. GENSTAT5.5 software was used to conduct this analysis.

RESULTS AND DISCUSSION

Molecular identification and characterization of isolates

Sixty-eight *Botrytis* isolates recovered from different strawberry infected fruits with gray mold in 14 growing regions in Morocco. The identification was based on the use of two specific primers (Fig. 1). The first primer, specific to *B. cinerea* (Bc108+/ Bc563-), while the NEP2 sequence vary between 3 species: *B. mali*, *B. fragariae* and *B. cinerea*. PCR results showed that only 47 out of 68 isolates were identified as *B. cinerea*, while no *B. fragariae* or *B. mali* species were detected in our populations.

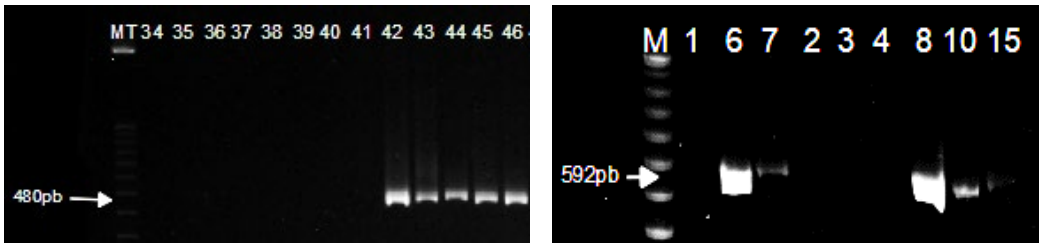

Figure 1. Electrophoretic profile of *Botrytis* spp. isolates, obtained by primers $Bc108^{+}$/ $Bc563^{-}$ (left), CFM–NEP2/C–NEP2 (right).

Botrytis isolates were used to differentiate between two genotypes N and S. The sizes of PCR fragments were examined using 2 primer pairs flanking 18 and 21 bp indels within *mrr*1 gene. Data suggested that 26% of isolates belong to groups S, while 31% belong to group N. TEs analysis indicated the presence of four genotypes: *flipper*, *transposa*, *boty* and *vacuma*. The *flipper* genotype was most prevalent (32%), followed by *transposa* (28%), *boty* (21%), and *vacuma* (19%). The distribution of TEs was similar across the different locations studied.

Microsatellite analysis

The population of 68 isolates, including both *B. cinerea* and unidentified *Botrytis* spp., were used to capture a broader genetic diversity and ensure a more comprehensive assessment of the diversity within these isolates across the different Moroccan regions. Analysis of *Botrytis* spp. by 9 microsatellite (SSR) markers demonstrated significant genetic diversity indices within populations. The nine SSR markers exhibited between 3 to 11 alleles, with an average 5.33 alleles/marker in the analysed *Botrytis* spp. isolates (Table 3).

Table 3. Characteristics of nine microsatellite (SSR) loci.

Primer	BC1	BC 2	BC3	BC4	BC5	BC6	BC 7	BC9	BC 10
Number of alleles	4	4	5	3	11	6	3	6	6
Size rang (pb)	202–240	155–200	220–260	116–138	150–450	91–668	126–145	146–484	180–202

Percentage of polymorphic loci varied from 29.17% (pop2) to 77.08% (pop 12) with average value of 44.05%. Observed alleles (*Na*), Nei's diversity gene (*He*), Shannon's diversity index (*I*) and Unbiased Expected Heterozygosity *(UHe)* were higher in pop 8 compared to other populations. The genetic diversity indices had lowest values in population 13 (*Na* = 0.333; *He* = 0; *I* = 0 and *UHe* = 0) (Table 4).

Table 4. Genetic diversity indices for *Botrytis* spp. revealed by microsatellite (SSR)

Population	*%P*	*N*	*Na*	*Ne*	*I*	*He*	*UHe*
Pop1	52.08	5.000	1.104	1.236	0.239	0.152	0.169
Pop2	29.17	3.000	0.646	1.169	0.156	0.103	0.124
Pop3	77.08	13.000	1.563	1.258	0.279	0.170	0.177
Pop4	39.58	5.000	0.833	1.175	0.178	0.112	0.125
Pop5	39.58	2.000	0.854	1.280	0.239	0.164	0.219
Pop6	52.08	4.000	1.063	1.249	0.248	0.158	0.181
Pop7	31.25	3.000	0.729	1.210	0.179	0.121	0.145
Pop8	66.67	9.000	1.354	1.319	0.308	0.198	0.210
Pop9	33.33	3.000	0.792	1.175	0.172	0.112	0.134
Pop10	43.75	3.000	0.958	1.253	0.234	0.155	0.186
Pop11	31.25	3.000	0.792	1.210	0.179	0.121	0.145
Pop12	77.08	10.000	1.563	1.265	0.284	0.173	0.182
Pop13	0.00	1.000	0.333	1.000	0.000	0.000	0.000
Pop14	43.75	4.000	0.958	1.247	0.225	0.148	0.170
Average	44.05	4.857	0.967	1.218	0.208	0.135	0.155

He: Nei's gene diversity; *I:* Shannon's diversity index; *N*: isolates number; *Na:* observed number of alleles; Ne*:* effective number of alleles; *P* (%): polymorphic loci percentage; *UHe*: Unbiased Expected Heterozygosity.

The genetic distance among populations was very high, it varied from 0.011 and 0.271. Populations 3 (Dlalha origin) and 6 (Laaouamra origin) exhibited lowest genetic distance, whereas greatest genetic distance was seen between 12 and 13 populations from Ouled Laghmari R'mal Laaoumra and INRA Rabat Guiche greenhouse, respectively (Table 5).

Table 5. Pairwise *Botrytis* spp. populations matrix the Nei genetic distance

	Pop1	Pop2	Pop3	Pop4	Pop5	Pop6	Pop7	Pop8	Pop9	Pop10	Pop11	Pop12	Pop13	Pop14
Pop1	0.000													
Pop2	0.048	0.000												
Pop3	0.027	0.043	0.000											
Pop4	0.016	0.027	0.017	0.000										
Pop5	0.042	0.054	0.038	0.044	0.000									
Pop6	0.033	0.038	0.011	0.015	0.044	0.000								
Pop7	0.077	0.070	0.065	0.062	0.090	0.057	0.000							
Pop8	0.026	0.061	0.013	0.021	0.041	0.018	0.061	0.000						
Pop9	0.055	0.093	0.058	0.072	0.061	0.065	0.094	0.045	0.000					
Pop10	0.071	0.124	0.051	0.073	0.098	0.069	0.143	0.050	0.087	0.000				
Pop11	0.124	0.152	0.104	0.117	0.154	0.105	0.077	0.078	0.151	0.175	0.000			
Pop12	0.034	0.039	0.009	0.017	0.040	0.008	0.062	0.016	0.062	0.056	0.117	0.000		
Pop13	0.220	0.369	0.247	0.248	0.276	0.245	0.323	0.206	0.232	0.228	0.253	0.271	0.000	
Pop14	0.050	0.093	0.044	0.053	0.062	0.043	0.074	0.029	0.036	0.071	0.111	0.038	0.237	0.000

Cluster analysis, neighbor joining and multivariate PCoA were used to investigate relationships among isolates. Fig. 2 shows the dendrogram constructed with UPGMA illustrating relationship among *Botrytis* spp. isolates entirely genotyped using 9 microsatellites. The similarity between isolates was calculated as Jaccard similarity index, the strains clustered in 7 main clusters at 70% of similarity, but one isolate was distant. The distant isolate was obtained from calinda strawberry from Zone Bargha greenhouse. The number of strains of each cluster, depending on the group from I to VII were respectively: 23, 4, 4, 15, 11, 1 and 10. The results showed that geographical origin of isolates and strawberry variety did not affect genetic diversity of *Botrytis* spp., the isolates from the same geographical origin were found in different cluster groups.

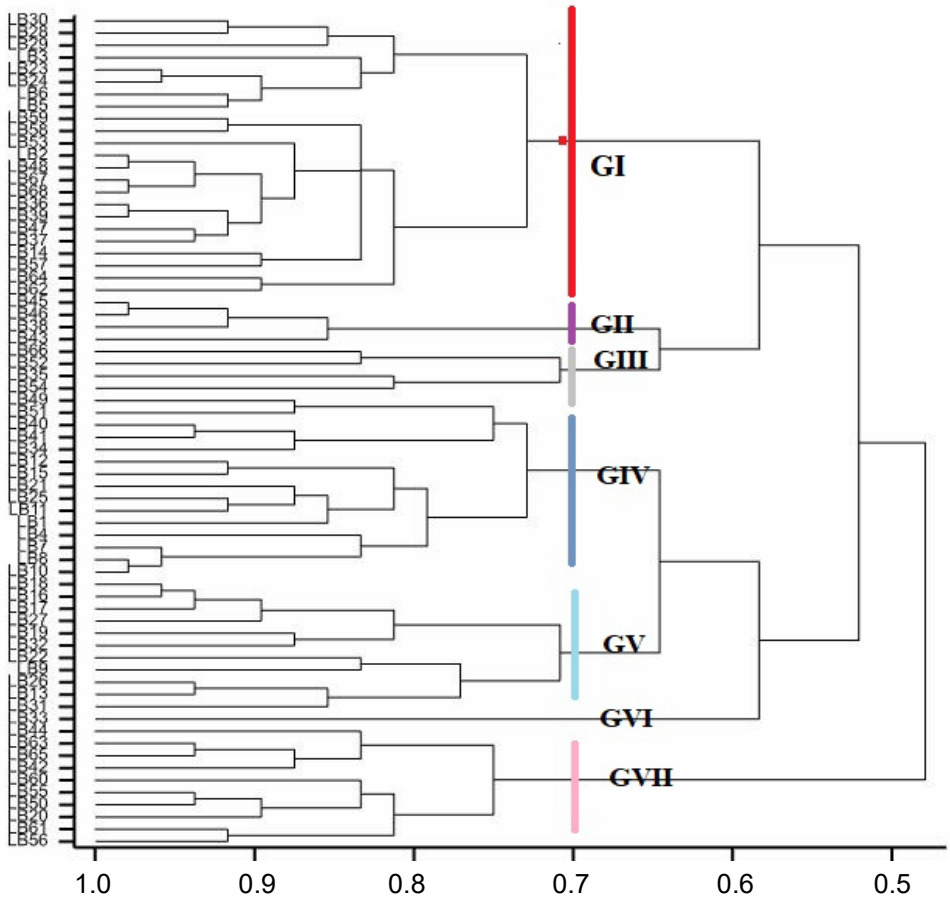

Figure 2. Dendrogram of *Botrytis* spp. isolates from various geographic origins, constructed using UPGMA algorithm.

Fig. 3 shows the PCoA results, highlighting the genetic diversity among isolates from different areas. Two coordinated analysis explained 18.62% and 14.57% of variability. The biplot produced through PCoA shows a broad dispersal of isolates independent of their geographic origins.

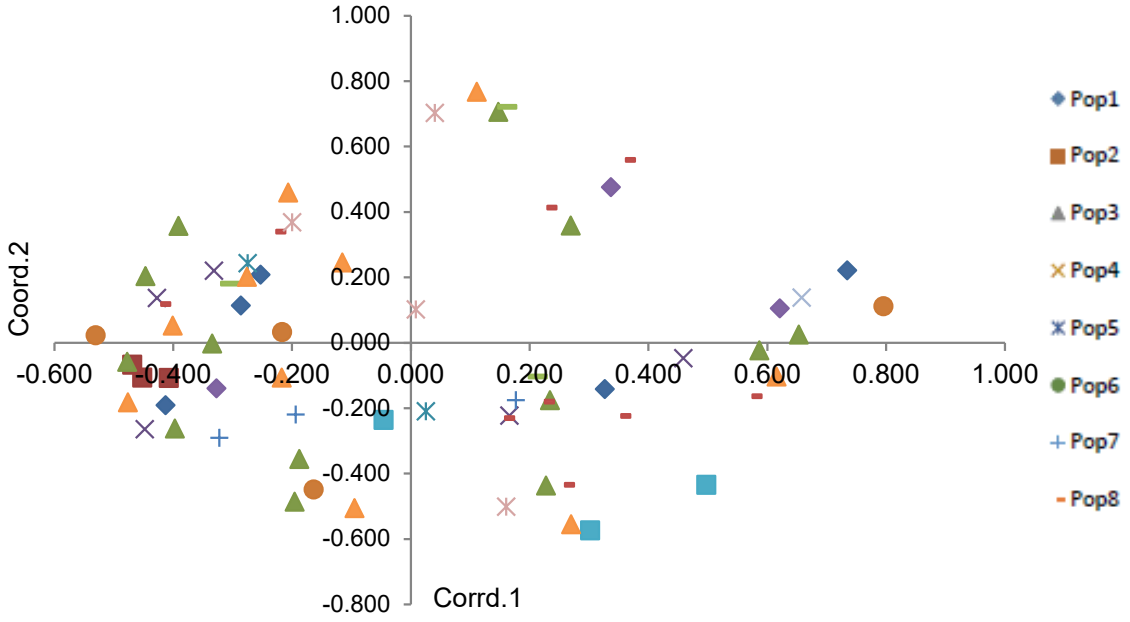

Figure 3. P CoA of *Botrytis* isolates using SSR primer data, classified by geographic origin.

Discussion

This study represents a first investigation to evaluate *Botrytis* spp. genetic diversity from strawberry in Morocco. Out of our 68 isolates, 47 belonged to *B. cinerea*, which is a significant pathogen that impacts strawberry crops in the studied regions. Early research showed that genetic diversity of *B. cinerea* was associated to the presence or absence of TEs (Giraud et al., 1997; Giraud et al., 1999). A later research using microsatellite and IGS (inter-genic spacer)-RFLP markers, showed that TEs only were not effective in differentiating populations (Kretschmer & Hahn, 2008; Fournier & Giraud, 2008). However, in our study we used TEs in combination with SSR markers because SSR offer high resolution for genetic diversity analysis, while TEs provide insight into genomic variation. Together, they allow for a more comprehensive understanding of the genetic structure of *Botrytis* populations. TEs analysis revealed the presence of four genotypes: *Flipper* (39%), the most prevalent, followed by *transposa* (30%), *boty* (24%), and *vacuma* (19%). The data showed that *flipper* isolates did not display any particular specialization in terms of strawberry variety or plant location. In contrast, *boty, transposa* and *vacuma* showed a geographical preference, this differential distribution may be linked to host specificity. Several studies have shown a high incidence of *Flipper* in *B. cinerea* populations from Bangladesh (Isenegger et al., 2008) and Greece (Samuel et al., 2012).The distribution of all genotypes in our study corresponded with findings reported in Egypt (Wagih et al., 2019), India and Nepal (Kumari et al., 2014). However, in another study only *flipper, boty,* and *transposa* types were identified in China (Pei et al., 2019). Additionally, Notte et al. (2021) did not detect any isolate with *flipper* type in Chile. Variances in genotype frequencies have been noted between strawberry, cucumber, tomato and grape populations, with *transposa* genotype dominating in populations, while *vacuma* genotype dominates in apple and kiwi (Samuel et al., 2012). Furthermore, it has been suggested that changes in *vacuma,*

transposa frequencies are probably attributable to variances in saprophytic and pathogenic adaptations (Martinez et al., 2005). Another study also found a correlation between pathogenicity and type of TEs, showing that isolates with *transposa* type displayed a greater potential for causing disease than *vacuma* (Wagih et al., 2019).

The presence of two *Botrytis* spp. genotypes, groups S and N, was analyzed with 26% and 31% of isolates, respectively, showing only a small difference. While the small sample size of our population limits the statistical power and generalizability of these findings, they offer preliminary insights into the potential distribution of *Botrytis* genotypes in strawberry crops. These findings indicate the presence of both genotypes in Morocco, which could have significant implications for disease management strategies of gray mold disease. Leroch et al. (2013) found that the frequencies or distribution of group S was different to the host plant, showing preference for strawberry, sampling regions and country. Subsequent, group S present on strawberry in German (Leroch et al., 2013), blackberry in Carolinas and strawberry plants from several US states (Li et al., 2014; Fernández-Ortuño et al., 2015). On another side, New Zealand grapes (Johnston et al., 2014), eggplant, cucumber, strawberry, green bean and tomato in Cyprus (Kanetis et al., 2017) contains group S. In Morocco, groups S and N were not confirmed in all populations examined as *B. cinerea* which was dominant in strawberry in all sampling areas. This result was similar to those found in Greece by Konstantinou et al. (2015), where *B. cinerea* dominated strawberry populations, whereas group S accounting for a minority of 5.9–21.1%. The presence of *Botrytis* group S in different hosts suggests that this clade doesn't signify a specialized subdivision of *B. cinerea* on host preference (Li et al., 2014). Several studies have shown the difference in the response of group S to fungicides. In Cyprus, resistance *B. cinerea* phenotypes and group S to fungicides in tomatoes were mostly evenly distributed across tested fungicides. However, in strawberries, *B. cinerea* was more prone to developing resistance (Kanetis et al., 2017). In Germany strawberry fields, group S populations predominantly contained isolates exhibiting multidrug resistance (MDR) phenotypes, showing partial resistance to fludioxonil and cyprodinil (Leroch et al., 2013). Nevertheless, they observed that *B. cinerea* populations in both strawberries and tomatoes were more susceptible to resistance against boscalid and pyraclostrobin compared to group S isolates (Konstantinou et al., 2015), this finding is likely due to the use of distinct gray mold management strategies.

Genetic diversity of *Botrytis* spp. was also evaluated using microsatellite marker, all loci were polymorphic. Nine markers exhibited between 3 and 11 alleles in *Botrytis* spp. isolates, Fournier & Giraud (2008) showed that the number of alleles varies between 3 and 6. SSR markers unveiled significant genetic diversity indices among strawberries populations from different areas of Morocco. *He* varied from 0.10 to 0.19 and *UHe* value varied from 0.12 to 0.21 (with average 0.13 and 0.1 respectively). *He* values were lower than *UHe* in our population. Our findings are consistent with Rasiukevičiūtė et al.'s (2018) study**,** who reported that *He* ranged from 0.02 to 0.61, lower than *UHe* indicates a high incidence of inbreeding between isolates. On another hand, high genetic diversity is correlated with high expected heterozygosity of *B. cinerea* (Kumari et al., 2014; Corwin et al., 2016).

The relationship among *Botrytis* spp. isolates using UPGMA, was clustered in 7 main clusters at 70% of similarity, results showed that geographical origin of isolates and strawberry variety didn't effect genetic diversity of *Botrytis* spp. Isolates of the same geographical origin were present in different cluster groups. Similar result was obtained by Fournier et al. (2013), proving that geographical origin of isolates has no impact on genetic diversity of *B. cinerea*. Our study demonstrates a genetic diversity in *Botrytis* spp. populations in Morocco. Because of its significance as a pathogen affecting strawberries and its effects on yield, careful treatment is necessary, one of the tools used in management is the detection and identification of pathogens. The genetic diversity of *Botrytis* spp. on strawberry is important for understanding its adaptability, virulence, and potential resistance to control methods. Understanding this genetic variation allows for more targeted and effective approaches to controlling *Botrytis* spp. in strawberry crops, ensuring long-term sustainability and minimizing the impact of the disease.

CONCLUSION

This study represents an initial assessment of the genetic diversity of *Botrytis* spp. isolated from strawberries in Morocco. PCR confirmed that 47 of isolates were identified as *B. cinerea. Botrytis* populations were composed of 4 TEs genotypes, the data revealed that *flipper* isolates showed no specialization to strawberry variety and plant origin, while *boty, transposa* and *vacuma* revealed a geographical preference. Genetic diversity assessment using microsatellites revealed differences between *Botrytis* spp. populations in Morocco, with a high level of genetic diversity indices, isolates clustered into seven genetic groups. The outcome showed that genetic diversity within Moroccan *Botrytis* spp. populations. Pathogen identification and knowledge of its genetic diversity is crucial for optimal and effective disease management.

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