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Phylogenetic Analysis and Toxigenic Profile of *Alternaria* Species Isolated from Chickpeas (*Cicer arietinum*) in Argentina

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Abstract: Chickpeas are a very important legume due to their nutritional richness and high protein content and they are used as food for humans and as fodder for livestock. However, they are susceptible to fungal infections and mycotoxin contamination. The *Alternaria* genus was among the main fungi isolated from chickpea samples in Argentina. The species within this genus are able to produce several mycotoxins such as alternariol (AOH), alternariol monomethyl ether (AME), and tenuazonic acid (TA). So, the objectives of this study were to identify the *Alternaria* spp. found in the chickpea samples and to determine their toxigenic potential in vitro. A phylogenetic analysis of 32 *Alternaria* strains was carried out based on the combined sequences of the *tef1*, *gpd*, and *Alt a1* genes. All *Alternaria* strains clustered into the section *Alternaria* and were identified as *A. alternata* and *A. arborescens*. Further, the toxigenic profile of each strain was determined in a ground rice–corn steep liquor medium and analysed by HPLC. Most strains were able to co-produce AOH, AME, and TA. These results indicate a potential risk for human health when consuming chickpeas since this legume could be contaminated with *Alternaria* and its mycotoxins, which are not yet regulated in food.

Keywords: alternariol; alternariol monomethyl ether; tenuazonic acid; section *Alternaria*; allergen alt1a; glyceraldeyde-3-phosphate dehydrogenase; translation elongation factor 1α

Citation: Nichea, M.J.; Cendoya, E.; Romero, C.J.; Humaran, J.F.; Zachetti, V.G.L.; Palacios, S.A.; Ramirez, M.L. Phylogenetic Analysis and Toxigenic Profile of *Alternaria* Species Isolated from Chickpeas (*Cicer arietinum*) in Argentina. *Diversity* **2022**, *14*, 924. https://doi.org/10.3390/d14110924

Academic Editors: Isabel Antonieta Iturrieta-González and Ipek Kurtboke

Received: 24 September 2022
Accepted: 23 October 2022
Published: 29 October 2022

1. Introduction

Since ancient times, chickpeas (*Cicer arietinum* L.) have been a major legume crop, particularly in Asia. Chickpeas are the world's most important crop, mainly used as food for humans and valued for their nutritious seeds with high protein content, which is of comparable or better quality than other legumes. Chickpeas can also be used as fodder for livestock [1]. Chickpeas are cultivated in a wide variety of agro-ecological conditions worldwide [2]. In Argentina, the crop is cultivated in fall–winter with low water requirements in comparison to other crops. Chickpea crops are susceptible to fungal contamination with both pathogens and saprophytes, some of them mycotoxin producers, so there is a potential risk of contamination with said agents. This can affect the vigor and longevity of the seeds [3]. The grain quality achieved by Argentina has enabled the country to be an important exporter and to be competitive in the international market. In 2021, in Argentina, chickpea cultivation spanned 81.236 ha, reaching 84.709 tons. The major portion of Argentina´s production is exported, although over the last few years, chickpea production has decreased due to unfavourable weather conditions, but a rise in output and export is expected for the next growing seasons [4].

The chickpea is susceptible to over 25 well-documented fungal pathogens and is often attacked—before and after harvest—by fungi, which greatly affects productivity. Some of these fungi have been identified as weak parasites as well as important contaminants and can damage the seeds during storage due to their nutritional richness and the texture of

the seed coat. The most widespread fungi in chickpea plants are species belonging to the genera *Aspergillus*, *Fusarium*, *Penicillium*, *Alternaria*, and *Rhizopus* [5].

Recently (in the 2018 harvest season), we analysed 70 asymptomatic samples of chickpea from a local cereal storage company located in the northern region of the Córdoba province (the main chickpea production area) in Argentina. The analysis of the mycobiota present in the samples showed that species of the genus *Aspergillus* (54%) and *Alternaria* (22%) were isolated as the predominant ones. In addition, contamination by mycotoxins was determined in 10 chickpea samples using LC-MS/MS for preliminary screening. While *Aspergillus* was the predominant fungal genus isolated, with *A. flavus* being the most common species identified, we did not detect aflatoxins; instead, we detected *Fusarium* mycotoxins such as beauvericin, deoxynivalenol, and zearalenone. Among the *Alternaria* mycotoxin analysed, we detected alternariol monomethyl ether (AME) in all samples, at concentrations ranging from 0.7 to 14.5 ng/g, and alternariol (AOH) in 30% of samples at concentrations ranging from 1.4 to 2.3 ng/g [6].

Alternaria's contamination of crops is particularly relevant due to their ability to produce a wide variety of mycotoxins that can have adverse effects on human and other animals' health. The toxins produced by *Alternaria* species can be classified into host/non-host specific toxins [7] or grouped depending on their chemical structure [8], which is a preferred approach in matters of food security. The dibenzo-α-pyrone derivative group contains alternariol (AOH) and many modified forms, including alternariol mono methylether (AME), altenuene (ALT), isoaltenuene (isoALT), and altenuisol (ATL). Then comes the group derived from tetramic acid with tenuazonic acid (TA), followed by the perylene quinone derivative altertoxin I, II, and III (ATX-I, -II, -III); alterperylenol, also called alteichin (ALP); and stemphyltoxin III (STTX-III). The fourth group consists of various structures such as tentoxin (TEN), altenuic acid III (AA-III), and infectopyrone (INF), which was originally isolated from *A. infectoria* [3,9]. The toxins within these four groups belong to the group of non-host specific toxins and can be formed by different strains of *Alternaria* in a wide range of living host plants and agricultural products, such as harvested wheat grains, vegetables, cereal products, fruits, and oil seeds [10–12]. While there are currently no global regulations setting limits for such toxins in food and feed, the European Food Safety Authority (EFSA) expresses public health concerns regarding *Alternaria* mycotoxins [8,13].

The taxonomy of the genus *Alternaria* is an extremely controversial subject. The classification of the genus *Alternaria* has long been based solely on morphological traits, for example, colony morphology, mycelial growth, sporulation patterns, and conidia size and shape in accordance with Simmons' proposed taxonomy key [14], who introduced the concept of species-groups to make identification easier. This approach is, however, not always efficient because of the strong influence of growing conditions on the morphological characteristics, the high level of similarity between certain species, and the presence of several strains with intermediate features [15]. As a consequence, many studies were devoted to *Alternaria* and related genera, often confirming a merged taxonomic background, which resulted in a continuous process of taxonomic revision. Conversely, multigenic phylogenetic analyses strongly supported the redefinition of the *Alternaria* genus. Several sequences from genetic regions such as glyceraldehyde-3-phosphate dehydrogenase (*gpd*), Alternaria major allergen (*Alt a1*), endopolygalacturonase (*endoPG*), translation elongation factor 1-alpha (*tef1*), calmodulin, plasma membrane ATPase (*ATPase*), the second largest subunit of RNA polymerase II (*RPB2*), and others have been applied to delimit the genus [16–22]. In recent studies, both morphological and molecular analyses have been used to delineate the genus *Alternaria*, which has been divided into 28 sections and eight monotypic lineages. The small-spored *Alternaria* species belongs to the section *Alternaria* under the species type *A. alternata* [20,23–25]. *Alternaria* species' numbers have been continuously growing after redescriptions and new discoveries [26–29]. Coincidentally, several phylogenetic lineages have strongly supported morphology-based sections, while others have not [14,30].

For food-supplying countries, such as Argentina, contamination with mycotoxins can result in a negative impact or problems in trade in terms of rejection, restrictions, or

unjustified demands. Thus, determining the distribution of the small-spored *Alternaria* in chickpea production areas is the initial step in determining the mycotoxigenic potential of crop contaminants and the risk in the final product.

As a result of the lack of information in our country on the contamination of this legume by species of the genus *Alternaria*, the objective of this study was to identify by phylogenetic analysis the *Alternaria* spp. found in samples of chickpea plants and to determine their toxigenic potential in vitro.

2. Materials and Methods

2.1. Isolates of Alternaria spp.

Out of 100 putative small-spored catenulate *Alternaria* strains previously isolated from chickpea grains (asymptomatic) in Argentina during 2018 harvest season [6], 32 single-spore strains were randomly selected for phylogenetic and toxicogenesis analyses. Isolates were morphologically characterized according to Simmons [14], mostly with respect to three-dimensional sporulation patterns. All the strains are deposited at the Universidad Nacional de Río Cuarto (UNRC) culture (RC) collection and preserved as spore suspensions in 15% glycerol frozen at −80 °C.

2.2. DNA Extraction, PCR Amplification, and Sequencing

Each strain was inoculated as a spore suspension in Erlenmeyer flasks containing 50 mL of Wikerman medium [31] and incubated at 25 °C under shaking (150 rpm) for three days. After this period, mycelia were obtained by filtration through non-gauze milk filters (Ken AG, Ashland, OH, USA) and, once dried, were stored frozen at −20 °C until ground with liquid nitrogen. The cetyltrimethylammonium bromide (CTAB) method [32] was used to obtain the fungal DNA. Its quality was analysed by electrophoresis and it was quantified using a spectrophotometer (model ND-1000; NanoDrop Technologies Inc, DE, USA).

The *gpd*, *Alt a1,* and *tef1* gene regions were selected for a molecular characterisation. For PCR reactions, the following specific primer pairs were used: gdp1/gdp2 [33], alt-for/alt-rev [17], and Alt-tef1/Alt-tef2 [34].

The amplification of the three fragments was performed in a PTC-2000 Thermal Cycler (MJ Research Inc., Watertown, MA, USA) with the following parameters: 94 °C for 1 min, then 31 cycles at 94 °C for 30 s, 56 °C for 45 s, and 72 °C for 1 min, followed by 72 °C for 5 min and at 4 °C. After electrophoretic separation in 1X TAE buffer on 1.5% agarose gel, the PCR products were visualized with UV. Then, these PCR products were purified and sequenced by Macrogen, Inc., Seoul, South Korea, using the same primers previously used for the PCR amplification. Using BioEdit Sequence Alignment Editor 7.1.3.0 [35], sequences were edited and compared with reference sequences available on GenBank databases for identification of the field isolates.

2.3. Phylogenetic Analyses

According to BLAST searches, sequences of 32 field strains were aligned with 13 reference sequences downloaded from the National Center for Biotechnology Information (NCBI) and Somma et al. [34]. The present 32 strains and other 13 species were used during the phylogenetic analysis with *A. infectoria* CBS 210.86 as the outgroup (Table 1).

Nucleotide sequences of amplicons of *gpd*, *Alt a1,* and *tef1* were aligned with the MAFFT online version 7 [36]. Ends of alignments were cut in order to avoid regions with missing data. Each locus and the combined data set were subjected to Bayesian phylogenetic inference using MrBayes 3.2.6 [37]. The best substitution model was determined for each data set using JModelTest [38] and scored following the Akaike Information Criterion (AIC). The General Time-Reversible (GTR) substitution model was used for *tef1*, GTR + gamma-distributed rate variation across sites (G) was used for *gpd,* and GTR + proportion of invariable sites (I) was used for *Alt a1*. For the combined data set, each gene was treated as a separate partition with independent parameter estimations. Two runs with

four chains each were run for ten million generations with a sampling frequency of every 100 generations. Trees after the initial 25% trees of each run were discarded as burn-in.

Tree topologies were adjusted using FigTree v1.4.3. DNA sequences generated in this study were deposited in GenBank under accession numbers (Table 1).

Table 1. Source information of *Alternaria* strains used in the phylogenetic analyses.

Species	Strain	Accession Numbers		
		tef1	*gpd*	*Alt a1*
A. alternata	EGS 34.016 (ATCC 66891, BMP 0269, CBS 916.96)	-	AY278808	MN975270
	RC-CR 1	OP501716	OP501743	OP501686
	RC-CR 94	OP501717	OP501744	OP501687
	RC-CR 105	OP501718	OP501745	OP501688
	RC-CR 132	OP501719	OP501746	OP501689
	RC-CR 199	OP501720	OP501747	OP501690
	RC-CR 279	OP501721	OP501748	OP501691
	RC-CR 293	OP501722	OP501749	OP501692
	RC-CR 307	OP501723	OP501750	OP501693
	RC-CR 329	OP501724	OP501751	OP501694
	RC-CR 455	OP501725	OP501752	OP501695
	RC-CR 482	OP501726	OP501753	OP501696
	RC-CR 487	-	OP501754	OP501697
	RC-CR 539	OP501727	OP501755	OP501698
	RC-CR 573	OP501728	OP501756	-
	RC-CR 610	OP501729	OP501757	OP501699
	RC-CR 720	OP501730	OP501758	OP501700
	RC-CR 734	OP501731	OP501759	OP501701
	RC-CR 746	OP501732	OP501760	OP501702
	RC-CR 762	OP501733	OP501761	OP501703
	RC-CR 764	OP501734	OP501762	OP501704
	RC-CR 767	OP501735	OP501763	OP501705
	RC-CR 777	OP501736	OP501764	OP501706
	RC-CR 830	OP501737	OP501765	OP501707
	RC-CR 835	OP501738	OP501766	OP501708
	RC-CR 847	OP501739	OP501767	OP501709
	RC-CR 869	OP501740	OP501768	OP501710
	RC-CR 902	OP501741	OP501769	OP501711
	RC-CR 911	OP501742	OP501770	-
A. arborescens	EGS 39.128 (CBS 102605)	JQ672481	AY278810	MN975269
	RC-CR 88	OP501712	-	OP501683
	RC-CR 639	OP501713	OP501680	-
	RC-CR 908	OP501714	OP501681	OP501684
	RC-CR 910	OP501715	OP501682	OP501685
A. brassicicola	ATCC 96,836 (EGS 42.002, CBS118699)	-	KC584103	KP993538
A. capsica	BMP 0180 (EGS 45-075)	ACSCTG01642	AY562408	AY563298
A. carthami	BMP 1963 (CBS 635.80)	-	KJ717981	KJ718649
A. citriarbusti	BMP 2343 (EGS 46.140)	ACSCTG01642	ACSCTG00332	ACSCTG04746
A. crassa	BMP 0172	JQ672489	AY278804	AY563293
A. gaisen	BMP 2338 (EGS 90–0512)	ACRCTG02961	ACRCTG04221	ACRCTG04151
A. infectoria	CBS 210.86 (EGS 27.193)	JQ672436	AY278793	FJ266502
A. longipes	BMP 0313 (EGS 30.033, CBS 540.94)	ADTCTG24504	AY278811	AY563304
A. tagetica	BMP 0179 (EGS 44–044)	JQ672490	AY562407	AY563297
A. tenuissima	BMP 0304 (EGS 34–015, ATCC 96828)	ALGCTG00260	ALGCTG02071	ALGCTG02124
A. tomatophila	BMP 2032 (CBS 109156)	ATMCTG00738	GQ180085	GQ180101

ATCC: American Type Culture Collection, Manassas, VA, USA; BMP: BM Pryor, School of Plant Sciences, University of Arizona, Tucson, Arizona 85721; CBS: Culture collection of the Centraalbureau voor Schimmelcultures, Fungal Biodiversity Centre, Utrecht, the Netherlands; E.G.S.: Personal collection of Dr. E.G. Simmons.

2.4. Mycotoxins Production and Extraction

A 4 mm diameter agar disk of each *Alternaria* strain grown on synthetic nutrient agar (SNA) [39] for 7 days was used to centrally inoculate Petri plates containing ground rice–corn steep liquor medium (GRCS; ground rice 50 g, corn steep liquor 5 g, agar 15 g, and 1000 mL distilled water). The plates were incubated for 14 d at 25 °C in darkness [40]. The extraction method consisted of a three-step extraction procedure based on a microscale extraction [41,42]. First, from the edge of each colony, 3 agar plugs (4 mm diameter) were

cut from every Petri plate and were extracted using 1.5 mL chloroform/methanol (2:1 *v*/*v*) for 60 min in an ultrasonic bath. After that, the extract was transferred to clean 4 mL amber vials and evaporated to dryness (air, 50 °C). In a second step, the same plugs were extracted in 1.3 mL ethyl acetate with 1% formic acid for 60 min in an ultrasonic bath. This second extract was transferred to the amber vial with the first dried extract and evaporated. Finally, the plugs were extracted a third time with 1.5 mL of 2-propanol for 60 min using a ultrasonic bath, and then the extract was transferred to the amber vial with the two previous extracts and evaporated. Before the HPLC analysis, the pooled, dried extract was ultrasonically re-dissolved in 1 mL of methanol and 1 mL of acetonitrile:water (25:75 *v*/*v*) and filtered through a 0.45 µm filter.

2.5. HPLC Analysis

The HPLC system consisted of a Hewlett Packard model 1100 pump (Palo Alto, CA, USA) connected to a Hewlett Packard 1100 Series variable wavelength detector and a data module Hewlett Packard Kayak XA (HP Chem Station Rev. A.06.01, Palo Alto, CA, USA). Chromatographic separations were performed on a Symmetry C18 (100 × 4.6 mm i.d., 5 µm particle size) connected to a guard column Security Guard (20 × 4.6 mm i.d.) filled with the same phase. The mobile phase consisted of two consecutive isocratic mobile phase mixtures containing acetonitrile:0.027 M sodium dihydrogen phosphate solution (25:75, *v*/*v* Sn A) and acetonitrile:0.027 M sodium dihydrogen phosphate solution (50:50, *v*/*v* Sn B). Solvent A was pumped for 3.5 min at 1.0 mL/min followed by solvent B which was pumped for 16.5 min at 1.0 mL/min. The detector was set at 256 nm for AOH and AME and 279 nm for TA. Injection volume was 50 µL and the retention times were 11.8, 17.5, and 7.0 min for AOH, AME, and TA, respectively. Quantification was relative to external standards of 0.5, 1.0, 2.0, and 3 g/mL in acetonitrile:0.027 M sodium dihydrogen phosphate solution (25:75, *v*/*v*).

A recovery experiment was performed in GRCS medium at 0.1 to 10 µg/g levels of AOH, AME, and TA, respectively. Mean recovery and repeatability (relative standard deviation) ranged from 85 to 98% (0.2 to 1.4%), from 88 to 97% (0.1 to 2%), and from to 86% to 92% (0.5 to 2.5%) for AOH, AME, and TA, respectively. For the three toxins, the limit of detection (LOD; signal-to noise ratio 3) was 0.01 µg/g, and the limit of quantification (LOQ) was established as three times the detection limit.

3. Results

Thirty-two *Alternaria* strains isolated from chickpea plants were identified through a phylogenetic analysis. Sequences of the *tef1*, *gpd,* and *Alt a1*, genes were generated, yielding fragments of about 560 bp for *tef1*, 578 bp for *gpd,* and 497 bp for the *Alt a1* gene. The combined *tef1*, *gpd,* and *Alt a1* sequence data set consisted of 1635 base alignments. One Bayesian phylogenetic tree was generated for each sequenced gene and for the concatenated sequences of the three genes (Figure 1). The phylogenetic tree obtained showed three well-defined clades with high support values (> 90) corresponding to the sections *Alternaria*, *Porri*, and *Brassicicola*. All *Alternaria* strains isolated from the chickpeas clustered into the section *Alternaria* (100% support value) and were separated into three different groups. Seventeen strains (53%) clustered with the *A. tenuissima* and *A. alternata* reference strains (90% support), showing high homology among them, while four other strains (12.5%) clustered with the *A. arborescens* reference strain (62% support). In particular, the strain RC-CR639 was very close to the reference strain while the three other strains (RC-CR910, RC-CR908, and RC-CR88) were similar among themselves but distant from the *A. arborescens* reference strain. The remaining eleven strains (34.3%) formed a separated cluster, which included the *A. citriarbusti* reference strain (91% support). Within this group, a high variability was observed since most of the strains were closely related to the reference strain but did not cluster with it.

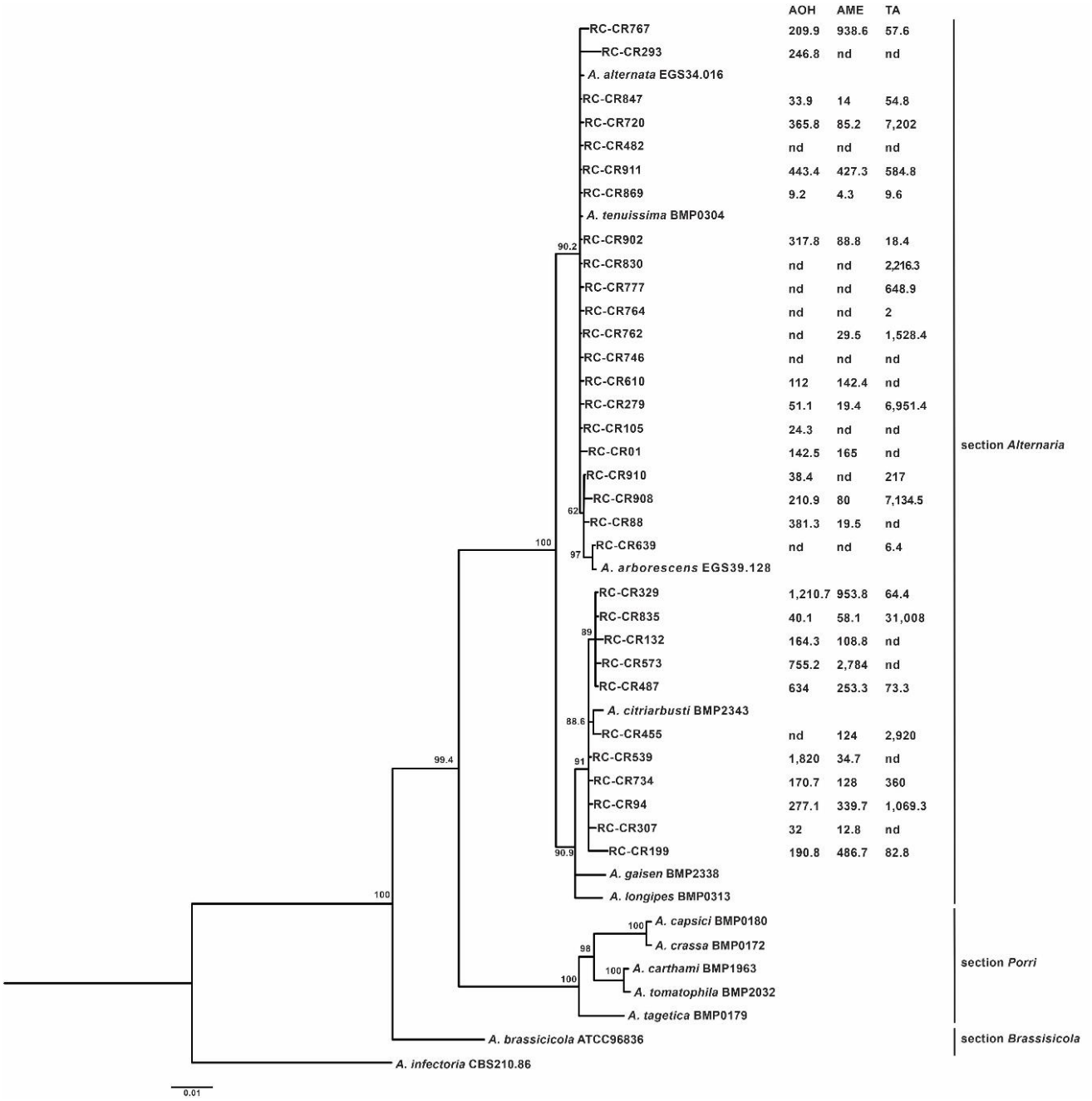

Figure 1. Bayesian phylogenetic tree based on the combined gene sequences of tef, gpd, and Alt a1 of 32 *Alternaria* strains isolated from chickpea. Bayesian posterior probabilities (>50) are shown at nodes. *Alternaria infectoria* CBS 210.86 was used as the outgroup. Mycotoxin profile (μg/g) of *Alternaria* strains is indicated next to each strain. AOH: alternariol, AME: alternariol monomethylether, and TA: tenuazonic acid. nd: not detected (≤LOQ).

The mycotoxin production profile of the 32 *Alternaria* section *Alternaria* stains in the GRCS medium is reported in Figure 1. Among all the *Alternaria* strains tested, 94% of them were able to produce at least one of the mycotoxins evaluated. The most frequent profile found was the co-production of AOH, AME, and TA by 14 strains, followed by AOH and AME profiles found in 7 strains. The percentage of strains that did not produce any of the tested toxins was very low (6%), while TA yielded the highest concentration.

4. Discussion

During the present study, strains of *Alternaria* spp. isolated from chickpea plants in Argentina were phylogenetically identified as *A. alternata* and *A. arborescens*, which belong to the small-spored section *Alternaria.* The taxonomy of the small-spored *Alternaria* spp. has been based mostly on both morphological characteristics and molecular analysis. However, due to the absence of coherent morphological features and the limited variability of the molecular markers, it may be difficult to define a single species based only on the agreement between the morphological and phylogenetic lineages [15,30,43–45]. Our combined phylogenetic tree confirmed the difficulties in the identification of the different morphospecies in this section. In the present study, the phylogenetic analysis of the *Alt a1*, *gpd,* and *tef1* genes demonstrated that all the isolates were grouped into three distinct clades: *A. arborescens* (n = 4), *A. citriarbusti* (n = 11), and a cluster containing *A. alternata* and *A. tenuissima*, which could not be clearly separated and, therefore, were grouped into a big cluster (n = 17). This close relation between *A. alternata* and *A. tenuissima* has also been reported using other molecular loci [15,43,46–48]. As in the phylogenetic analysis, there is no distinct separation between *A. alternata* and *A. tenuissima*; researchers recently suggested that these two species should be combined into a single species—*A. alternata*. Several studies that initially identified *A. alternata* and *A. tenuissima* using morphological features then found that the two species could not be separated by phylogenetic analysis using multiple molecular markers and, therefore, proposed that *A. tenuissima* be referred to as *A. alternata* [15,30,43,44,46,49]. Further, a study conducted by Dettman and Eggertson [50] compared—through a high-resolution, genome-wide study— the species within the section *Alternaria*, and they also proposed to merge *A. alternata* and *A. tenuissima* into one species, namely, *A. alternata*. Consequently, in the present study, the strains grouped into the *alternaria/tenuissima* clade were identified as *A. alternata*. Additionally, *A. arborescens*, defined by Woudenberg et al. [30] and Dettman and Eggertson [50] as a species complex, formed a separate clade from *A. alternata* in our analysis as well. These researchers also suggested that *A. citriarbusti* is synonymous with *A. alternata* species; however, in the present study, the *A. citriarbusti* isolates were not grouped together with *A. alternata* species. The incongruity between our results and the data produced by Woudenberg et al. [30] and Dettman and Eggertson [50] may be attributable to the different genomic regions used for the phylogenetic analysis. Based on our results, we agree with Dettman and Eggertson's [50] conclusion that most existing loci may be able to place an unknown *Alternaria* strain into a section, although section-specific molecular markers are required to differentiate lineages in the section *Alternaria*. Nevertheless, until a common marker is not unanimously defined by the scientific community dealing with the taxonomy of the *Alternaria* genus, the phylogenetic relationships between species will remain confused.

All the strains in this study were characterized through the determination of their *in vitro* mycotoxin production. It is remarkable that most of the strains of the *Alternaria* section were able to produce at least one of the mycotoxins tested, and co-production was the prevalent manner by which this occurred. We failed to differentiate the *A. alternata* strains from the *A. arboresces* strains based on the mycotoxin profiles (types and concentrations); this result is in agreement with that reported by Zwickel et al. [51]. A previous study on the natural occurrence of *Alternaria* toxins in the same chickpea grains detected AME as the most frequent toxin contaminating the samples (100% positive) followed by AOH (30% positive). Thus, the degree of toxin contamination in the samples was in accordance with the metabolite profile of the predominant *Alternaria* species present.

In particular, *A. alternata* is the most important mycotoxin producer within the genus, since this mentioned species is widely reported to produce TA, AME, AOH, ALT, and ATX [52–57]. A recent study performed by Huybrechts et al. [58] has related the chronic exposure of a low-dose of *Alternaria* mycotoxins in food commodities to the onset of colorectal cancer in humans. In addition, consumer exposure and associated toxic effects are plausible due to the absence of regulation in food.

5. Conclusions

In conclusion, the present study contributed to attaining a successful classification of the *Alternaria* strains isolated from chickpea plants using a multi-locus analysis, and the toxigenic profile was also assessed. All the strains belong to the *Alternaria* section *Alternaria*, with *A. alternata* being the most common species found. Additionally, we were able to identify species within the *A. arboresens* species group. The analysis of the secondary metabolite production showed that most of the strains were able to produce at least one of the mycotoxins analysed. We were not able to find any differences between the mycotoxin profiles of the *Alternaria* species studied. The knowledge of the fungal populations infecting crops is a valuable tool for monitoring and establishing mycotoxin controls. Since *Alternaria* section *Alternaria* species are frequently found in chickpeas, and members of this section are well-known mycotoxin producers, food safety authorities should consider the wide diversity of toxic metabolites that may contaminate this pulse when reviewing the risks and safety aspects of *Alternaria* toxins in food and feed.

Author Contributions: M.J.N. and E.C. performed the whole experiment, J.F.H., C.J.R. and V.G.L.Z. performed the analysis of the metabolites, S.A.P., M.J.N. phylogenetic analysis, M.L.R. conceived and designed the experiments. M.L.R., M.J.N., S.A.P. analysed the data and wrote the paper. M.L.R. revised the manuscript. M.L.R. project administration and funding acquisition. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by grants ANPCyT (Agencia Nacional de Promoción Científica y Tecnológica) PICT 2493/2017 and Consejo Nacional de Investigaciones Científicas y Técnicas: PUE 22920200100004.

Institutional Review Board Statement: Not applicable.

Data Availability Statement: All sequence data are available in NCBI GenBank following the accession numbers in the manuscript.

Acknowledgments: C.J.R. and M.J.N. are fellow of CONICET and M.L.R., E.C., V.G.L.Z. and S.A.P. are members of the Research Career of CONICET. The authors are grateful to A. Moretti and M. Masiello for sharing sequences data.

Conflicts of Interest: The authors declare no conflict of interest.

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