agronomy

MDPI

Article

Biodiversity of *Rhizoctonia solani* in *Phaseolus vulgaris* Seeds in East Delta of Egypt

Khalid M. Ghoneem [1], Deiaa A. El-Wakil [1], Mohamed I. M. Ahmed [1], Hisham M. Kamel [1], Ehsan M. Rashad [1], Abdulaziz A. Al-Askar [2,*], Elsherbiny A. Elsherbiny [3] and Amira A. Ibrahim [4,*]

[1] Seed Pathology Research Department, Plant Pathology Research Institute, Agricultural Research Center, Giza 12619, Egypt
[2] Botany and Microbiology Department, Faculty of Science, King Saud University, Riyadh 11451, Saudi Arabia
[3] Department of Biology, Rheinland-Pfälzische Technische Universität-Landau (RPTU), 67663 Kaiserslautern, Germany
[4] Botany and Microbiology Department, Faculty of Science, Arish University, Al-Arish 45511, Egypt
* Correspondence: aalaskara@ksu.edu.sa (A.A.A.-A.); amiranasreldeen@sci.aru.edu.eg (A.A.I.); Tel.: +966-503-461-390 (A.A.A.-A.); +20-106-667-7539 (A.A.I.)

Abstract: Seventy-eight common bean (*Phaseolus vulgaris* L.) seed samples were collected from twenty-one field locations across six governorates (Ismailia, Sharkia, Port Said, South Sinai, North Sinai, and Suez) in the East Delta region of Egypt between 2021 and 2022. Thirty-five *Rhizoctonia solani* Kühn were isolated. Sharkia had the highest percentage of *R. solani* isolates (RS) from the collected samples (59%), followed by Ismailia, South Sinai, Suez, North Sinai, and Port Said (49.7%, 36.7%, 33%, 29.2%, and 27.8%, respectively). Pathogenicity tests proved that the seven highest-virulence strains of *R. solani* had a pre-emergence damping off ranging from 17% to 30% and a post-emergence damping off ranging from 11% to 20%. The seven most pathogenic isolates were identified using the Internal Transcribed Spacer (ITS) with code numbers as follows: *R. solani* RSA1, *R. solani* RIT2, *R. solani* RIF3, *R. solani* RSoR4, *R. solani* RNB5, *R. solani* RSzA6, and *R. solani* RPS11. Principle component analysis (PCA), which depends on the pathogenicity result and soil variables, revealed that soil texture and soil water holding capacity (MWHC) were the most effective factors in grouping and clustering the studied *R. solani* isolates from the different locations into two groups. From this study, it can be established that the biodiversity of *R. solani* in the East Delta area of Egypt depends on changes in the environmental conditions and soil variables. The soil type and pH variables have the greatest impact on *R. solani* diversity, followed by the humidity-holding ability and soil porosity.

Keywords: *Phaseolus vulgaris* L.; East Delta; Egypt; *Rhizoctonia solani* Kühn; biodiversity; pre- and post-emergence damping off

Citation: Ghoneem, K.M.; El-Wakil, D.A.; Ahmed, M.I.M.; Kamel, H.M.; Rashad, E.M.; Al-Askar, A.A.; Elsherbiny, E.A.; Ibrahim, A.A. Biodiversity of *Rhizoctonia solani* in *Phaseolus vulgaris* Seeds in East Delta of Egypt. *Agronomy* **2023**, *13*, 1317. https://doi.org/10.3390/agronomy13051317

Academic Editors: Ana I. Ribeiro-Barros and Susana Redondo-Gómez

Received: 24 March 2023
Revised: 25 April 2023
Accepted: 5 May 2023
Published: 8 May 2023

1. Introduction

The common bean (*Phaseolus vulgaris* L.) has consistently received significant attention due to its high nutritional and economic value, and it is considered one of the most vital nutritional legumes in Africa [1,2]. *P. vulgaris* and its bean pods are reservoirs of numerous dietary nutrients, such as vitamins, anthocyanins, flavonoids, carbohydrates, proteins, amino acids, polyphenols, and minerals [3–6]. *P. vulgaris* is very sensitive to climate change, which comprises variations in temperature, water availability, light intensity, alterations in atmospheric constituents such as CO_2 and O_3, and biotic stress, including sensitivity to fungal pathogens and herbivores [7–9], and numerous insect and nematode pests, such as bean pod weevil (*Apion godmani* Wagner), tropical bruchid (*Zabrotes subfasciatus* Boheman), leafhoppers (*Empoasca kraemeri* Ross and Moore), and nematode pests, such as root-knot nematode (*Meloidogyne* spp.), can cause high yield losses ranging from 35 to 100% globally [10]. Plant pathogenic fungi, including representatives from the genera *Rhizoctonia*, *Fusarium*, *Alternaria*, *Uromyces*, *Colletotrichum*, *Phaeoisariopsis Macrophomina*, *Pythium*, and

Phoma, attack common beans, resulting in disease infection and crop production loss [2]. Viruses that attack *P. vulgaris* include the bean common mosaic virus (BCMV) and the bean common mosaic necrosis virus (BCMNV), and these can cause up to 100% damage to bean crops [11]. Bean crops are also extremely vulnerable to common bacterial blight (CBB) caused by *Xanthomonas axonopodis* pv. *phaseoli* and its fuscans variant, *Xanthomonas fuscans* subsp. *fuscans* [12]. In Egypt, the land area cultivated with common beans is estimated to be 39,665 hectares, with a total annual dry bean production of 98,132 tonnes in 2017 [13]. *P. vulgaris* root rot and damping off, caused by *Rhizoctonia solani* Kühn (teleomoph *Thanatephorus cucumeris* (Frank) Donk), is one of the most devastating diseases [14]. The pathogen first attacks below-ground parts of the plant, such as seeds, hypocotyls, and roots, but it can also infect aerial plant parts, including pods, fruits, leaves, and stems. Damping off is the most prevalent symptom of *Rhizoctonia* infection, which is characterised by the failure of severely infected seeds to germinate. Seedling mortality can also occur with this disease, while seedlings that survive usually have brown to reddish lesions on stems, roots, and the lower hypocotyl [15]. In this respect, yield losses of 5 to 10% are common, but 69% yield losses under severe field conditions have been reported in Egypt [16].

Environmental and soil conditions at crop planting and the inoculum density have been found to strongly affect the variation in yield losses from one season to another and among fields in the same area [17]. Unlike other legume crop pathogens, *R. solani* is able to infect legumes over a wide range of soil temperature and moisture conditions [18]. In this respect, Mayo-Prieto et al. [19] reported high aggressiveness of the *R. solani* pathogen at temperatures between 15 and 18 °C and in moist soils, where it was associated with severe symptoms of seed rot and seedling mortality, stunting, and yellowing of bean plants. All stages of common bean growth are subject to fungal infections, which impede seed development, progression, and plant growth and thus affect the amount and value of the crop. These phytopathogenic fungi can be categorised into two types, one of which is transmitted by seeds and the other by roots, fruits, and flowers [20]. The biotic stresses that infect common beans and that are transmitted through seeds are described as "seed-borne pathogens" [21]. Numerous phytopathogenic fungi are seed-borne, which can result in massive crop reduction. Seed-borne fungi can result in seed rot, necrosis, seed abortion, reduced seed development, and seedling death, all of which result in significantly lower economic outputs [22]. Four intraspecific groups (ISGs), designated AG1-IA to AG1-ID, are collectively grouped as AG1 *R. solani*. Some asteroideae and soybeans are susceptible to the infections caused by AG1-IA and AG1-IB [23]. In these connections, most isolates of *R. solani* (92%) from bean seed and soil samples were found to belong to the anastomosis group (AG) AG 4, comprising seven AG 4 HG-I and nine AG 4 HG-III isolates [24].

Earlier investigators stated that *R. solani* is a mutual seed-borne fungus resulting from the damping off of bean seedlings and manifests as seed abortion, contracted seeds, decreased seed size, seed rot, seed necrosis, seed yellowing, decreased germination capability, and metabolic deviations in bean seed [20,25]. *R. solani* is considered the most vital species in the genus *Rhizoctonia* and is categorised as a soil-borne fungus with extensive variation in its cultural characteristics, host range, and fierceness. *R. solani* has been described as a damaging plant fungal pathogen of economically essential crops all over the world [26] and causes a widespread range of plant diseases, including damping off, brown patch, root rot, and belly rot, with different virulence ranks depending on the host plant [27]. *Rhizoctonia* infections increase in frequency in response to various environmental and management factors [28]. These include those factors that appear to affect the *Rhizoctonia* inoculum in the soil, such as soil type and structure, crop rotation patterns with *Rhizoctonia* hosts, tillage techniques, and the microbial community of the soil. These additional factors appear to be independent of the host plant's availability and weather conditions [29].

The related pathogens can be found using the highly efficient, specific, and sensitive PCR method [30]. Recently, it has become possible to create primers to capture a pathogen's ribosomal DNA (rDNA) by an internal transcribed spacer (ITS); this method is extremely

helpful in discriminating between the various diseases [31,32]. To examine *R. solani* and other fungi, several researchers have also employed various PCR techniques. Johanson et al. [33] developed several pairs of ITS primers (ITS1/GMRS-3, ITS1/GMRS-4, GMRO-3/R635, ITS1/GMROS-2, and GMROS-6/R635) to distinguish and identify *R. solani* and *R. oryzae*. Fungal DNA sequences made with the primers for the large subunit (nrLSU-26S or 28S), small subunit (nrSSU-18S), and the entire internal transcribed spacer region (ITS1, 5.8S, and ITS2; about 0.45–0.80 kb) started a new era of molecular phylogenetic sequence identification in the kingdom of fungi [34]. To assess genetic variation and characterise AG groups of *Rhizoctonia* isolates, the ITS between the 18S and 28S ribosomal RNA genes has been routinely used. This ITS includes the 5.8S rRNA gene. An anastomosis reaction occurs between isolates of distinct subgroups within the same group [33], and the resolution of hyphal anastomosis analysis is insufficient to discriminate subgroups within AG 1, AG 2, and AG 4 [35]. In order to break through this barrier, ITS sequence analysis is essential [32]. The main goal of the present research was to study the biodiversity of *R. solani* in *P. vulgaris* seeds in the East Delta of Egypt and to determine the effect of soil parameters on this biodiversity.

2. Materials and Methods

2.1. Sampling Process

The six Egyptian governorates of Sharkia, Ismailia, Port Said, Suez, North Sinai, and South Sinai are located in the East Delta region between 29°36′ N and 32°01′ N in latitude and 30°33′ E and 32°33′ E in longitude. A total of 78 samples of common bean seeds were collected from this region between 2021 and 2022. The samples were taken in a zigzag pattern over a 50 m by 50 m area. The ripe common bean pods were collected from cultivated fields, placed in paper bags, and given numbers before being stored at 4 °C until testing. A global positioning system (GPS) was used to georeference the location of each sampling site, and field data were recorded. The weather conditions for the sample sites are illustrated in Tables S1–S9.

2.1.1. Determination of Physical and Chemical Properties of Soil Samples

Soil samples were weighed, and the bulk density was measured after drying at 105 °C, according to Chambers et al. [36]. Additional soil samples were air-dried, crushed, and sieved through a 2 mm sieve to collect measurements of soil porosity, pH, water holding capacity (WHC), and mechanical composition (silt, sand, and clay). The pH of the soil was then evaluated using the potentiometric method using Titralab AT1000 and KF1000 (Hach MENA, Dubai, United Arab Emirates), and the mechanical composition of the soil was determined using a specific gravitymetre using MGS-6 (Micro-g LaCoste, Lafayette, CO 80026, USA).

2.1.2. Isolation and Identification of *R. solani*

Common bean seed samples (400 seeds per sample) were surface-sterilised in a 1% NaOCl solution (for 4 min), washed twice with sterilised distilled water, and dried on sterilised filter papers at room temperature. For each sample, the sterilised *P. vulgaris* seeds were cultivated on plain agar medium containing 0.06 mg/mL chloramphenicol (PDAC) in sterile Petri dishes (10 cm diameter) at 10 seeds per Petri dish. Culture plates were left in the incubator for one week at 27 °C and monitored daily [36,37]. Growing hyphae were sub-cultured at 27 °C for five days on PDAC; then, the growing hyphal tips were picked and purified carefully by following the hyphal-tip technique [20], and the morphological identification was followed according to the *R. solani* isolates' hyphal branching, the variation in colonies, sclerotia colour, and mycelial growth, and finally kept in a refrigerator at 4 °C for further studies [38–40]. After purification, the frequency of the *R. solani* fungus was calculated with the following equation:

$$\text{Frequency of } R.\ solani = \frac{\text{Number of } R.\ solani - \text{infected samples}}{\text{Total number of sample tested}} \times 100$$

2.2. Molecular Identification

After being inoculated with 3 discs from the PDA plate, *R. solani* was grown on 30 mL of potato dextrose broth media (PDB) and kept at 25 °C for 4 days for molecular identification. ITS-PCR products were purified using Qiagen DNA Measurement's Qi Aqick PCR Purification Kit (Qiagen, Hilden, Germany). Using a UV spectrophotometer (Agilent Technologies, Santa Clara, CA, USA) [41], the quantity and quality of DNA isolated from each isolate were evaluated (Thermo Scientific, Karlsruhe, Germany). Thereafter, until usage, the DNA was kept at −20 °C. Universal primers for the partial ITS region were used in PCR amplification: ITS1 (F): TCCGTAGGTGAACCTGCGG [42]; ITS4 (R): TCCTCCGCTTATTGATATGC [43]. The 50 µL PCR reaction mixture contained the following components: 1 buffer (Promega, Madison, WI, USA), 15 mM $MgCl_2$, 0.2 mM dNTPs, 20 pmol of each primer, 1 µL Taq DNA polymerase (GoTaq, Promega, Madison, WI, USA), and 40 ng DNA. After a 5-min denaturation cycle at 95 °C, a Perkin-Elmer/GeneAmp® PCR System 9700 (PE Applied Biosystems Waltham, MA, USA) ran 35 cycles of PCR. Each cycle included 30 s of denaturation at 95 °C, annealing at 51 °C, and extension at 72 °C; the final cycle extension was 7 min at 72 °C. A 1.5% agarose gel with 0.5 µg/mL ethidium bromide in 1× TBE buffer was run at 95 volts to resolve the amplification products. A Gel Documentation System was used to photograph gels using UV light (BIO-RAD 2000, Dubai Branch, United Arab Emirates). The Big Dye terminator cycle sequencing kit (Applied BioSystems, Waltham, MA, USA) was used for the sequencing, and the results were analysed using automated DNA sequencing equipment (Applied Biosystems Model 3730XL) (Applied BioSystems, McCormick, SC, USA).

Using the Basic Local Alignment Search Tool (BLAST), the retrieved nucleotide sequences were then aligned and compared to sequences from other fungal isolates published in the National Centre for Biotechnology Information (NCBI) database (http://www.ncbi.nlm.nih.gov/BLAST, accessed on 20 August 2022). The nucleotide sequences underwent multiple sequence alignments, and phylogenetic trees were created using MEGA-11 software based on the neighbour-joining method (https://www.megasoftware.net/, accessed on 20 August 2022) [44–47].

2.3. Pathogenicity of R. solani Isolates

The pathogenicity test was performed at the Al-Qasasin Research Station, Agricultural Research Centre, Egypt. The inocula of *R. solani* isolates were grown on PDA plates and incubated at 25 ± 2 °C for 5 days. After inoculation, the mycelium discs were transferred to a sterilised medium of maize, coarse sand, and water (2:1:2 *v*/*v*) and incubated at 25 ± 2 °C for two weeks. Pots (25 cm in diameter) were filled with disinfected soil (clay:sand ratio was 2:1 *v*/*v*) and singly infested by the previously prepared *R. solani* inoculum at a rate of 0.4% (*w*/*w*). The soil of each pot was thoroughly mixed with the inoculum, then regularly watered to near field capacity with tap water and left for one week. Pots infested with the uninoculated medium were used as a control. Surface-sterilised bean seeds (CV Nebraska) were sown at a rate of 5 seeds per pot, with 10 replicates for each isolate. Pots were frequently watered to near-field capacity with tap water. The pots were arranged in a one-way randomised block design and kept under greenhouse conditions. The ratios of pre- and post-emergence damping off bean plants were documented 15, 30, and 60 days after sowing and calculated according to Carling et al. [39] (Figures 1 and 2).

Figure 1. *Rhizoctonia solani* root rot symptoms on common bean plants.

Figure 2. Disease severity index of common plants infected with *Rhizoctonia solani* using 0–4 symptom scale obtained by Carling et al. [39], in which 0 = no damage, 1 = minor discoloration of hypocotyl, 2 = discoloration plus small necrotic lesions (<1 mm in diameter) on hypocotyl, 3 = discoloration with large necrotic lesions (≥1 mm in diameter) on hypocotyl, and 4 = death of the seedling.

2.4. Statistical Analysis

The obtained data from in vitro and maintain greenhouse studies were arranged in a randomised block design. After performing a one-way ANOVA, mean averages were compared based on the Tukey test at a probability ≤0.05. The software CoStat (version 6.450, CoHort Software, Birmingham, UK) was applied [48]. Canonical correspondence analysis (CCA) and principal component analysis (PCA) were used to examine the relationship between the pathogenicity of seeds infected with beans and the natural variations in soil. PAST software (ver. 4, Past Software, University of Oslo, Oslo, Norway) Using all data variables (pathogenicity, soil factors, and the SYSTAT genetic data marker), an unweighted pair group method with arithmetic mean (UPGMA) cluster dendrogram of *R. solani* collected from several study locations was produced (Ver. 13.2, Systat Software, Inc., San Jose, CA, USA).

3. Results

3.1. Isolation of R. solani

Thirty-five *R. solani* were isolated from seeds of seventy-eight samples collected in the East Delta region of Egypt. Table S1 andshow the total *R. solani* isolates from each location, as well as the percentage of isolates per seed sample. The number of isolates was twelve (Sharkia), eleven (Ismailia), three (Port Said), two (Suez), four (North Sinai), and three (South Sinai), corresponding to 59%, 49.7%, 27.8%, 33%, 29.2%, and 36.7%, respectively.

3.2. Morphological Identification

The morphological growth shown in Table 1 indicates statistical differences among the *R. solani* isolates in the rate of linear growth, the number of sclerotia, the colour of the sclerotia after three and five days, as well as the mechanism of growth. The linear growth ranged from 65.8 to 90 mm, with a high linear growth recorded at 90 mm after 5 days for RSA1, RIT2, RSoR4, and RSzA6. Alternatively, the number of sclerotia was between 23 and 83.33, and the maximum number (83.33) was recorded for RSoR4. Based on the location of sclerotia, these isolates were categorised into two groups. The first group included those isolates where sclerotium formed within the aerial mycelium (RSA1, RIT2, and RNBS). The second group included those isolates where sclerotia formed at the surface of the mycelium (RIF3, RSoR4, RSzA6, and RPS11), as shown in Table 1 These results are related to many studies that show the initial growth was off-white to cream-coloured and turned brown with maturation on a PDA medium.

Table 1. Morphological characteristics of *R. solani isolates*.

Isolates No.	Linear Growth (mm)	Number of Sclerotia	Sclerotial Formation Days		Sclerotial Location
			White	Brown	
RSA1	90.0 a *	23.33 e	3.0 a	5.0 a	Aerial
RIT2	90.0 a	25.00 d,e	3.0 a	5.0 a	Aerial
RIF3	65.8 c	73.33 b	5.0 a	7.0 a	Surface
RSoR4	90.0 a	83.33 a	4.0 a	6.0 a	Surface
RNB5	83.3 b	26.66 d,e	4.0 a	6.0 a	Aerial
RSzA6	90.0 a	39.33 c	5.0 a	7.0 a	Surface
RPS11	90.0 a	29.33 d	5.0 a	7.0 a	Surface
p value at 0.05	0.000	0.000	0.0210	0.0159	

* Values with different letters indicate significant differences between the studied isolates, and the same letter(s) within a column indicates the non-significant difference at $p \leq 0.05$ based on Tukey's test.

3.3. The Virulence of R. solani Isolates

Table 2 shows that *R. solani* isolates caused emergence damping off disease in both pre-emergence (17–30%) and post-emergence (11–20%) beans. The isolates with the highest

percentages of rotten bean seeds were RNB5, RIF3, RIT2, and RSoR4 (30, 29, 26.67, and 25%, respectively), followed by RSzA6, RSA1, and RPS11 (22, 20, and 17%, respectively) as compared to the control. Conversely, symptoms of seedling mortality induced by isolates RPS11, RIT2, RSzA6, RSA1, and RSoR4 recorded the highest infection percentages (20, 18, 17, 15.8, and 15%, respectively). Disease symptoms were characterised as lesions on stems, roots, and lower hypocotyl tissue that varied from light brown to reddish. These reddish-brown lesions of the root were sunken and girdled the stems, killing the plant. These results proved the presence of a high virulence of *R. solani* on beans.

Table 2. Virulence of different *R. solani* isolates on common bean plants under greenhouse conditions.

Isolates Code	Disease Incidence %		
	Pre-Emergence Damping Off *	Post-Emergence Damping Off *	Survival *
RSA1	20.00 c,d **	15.8 a–c	64.20 b
RIT2	26.67 a,b	18.0 a	55.33 b
RIF3	29.00 a	11.0 c	60.00 b
RSoR4	25.00 a–c	15.0 a–c	60.00 b
RNB5	30.00 a	12.0 b,c	58.00 b
RSzA6	22.00 b–d	17.0 a,b	61.00 b
RPS11	17.00 d	20.0 a	63.00 b
Control (without infection)	0.000 e	0.00 d	100.0 a
p value at 0.05	0.000	0.000	0.000

* Affected plants with different *R. solani* isolates were recorded during seedling growth stage as Pre-emergence damping off = seed/seedling death before emergence; Pre-emergence damping off = seed/seedling death before emergence; Post-emergence damping off = seedling death after emergence; survival = living plants two months after planting. ** The same letter(s) within a column indicate the non-significant difference ($p \leq 0.05$) based on Tukey's test.

3.4. Molecular Characterization

The pathogenic *R. solani* isolates were identified molecularly using the ITS region (Tables S2–S9). The investigated *R. solani* isolates displayed 99.72% similarity with their former nearby relative isolates from anastomosis groups. The phylogenetic cluster tree of the *R. solani* isolates is shown in Figure 3. The tree grouped the tested fungal species into two clades. The first clade comprised *R. solani* RSoR4 from South Sinai, while the second clade had two subclades with 97% support. The initial sub-clade includes Japan's seven clades' anastomosis group (AG-4 HGIII RR2) (I–VII). The first group includes *R. solani* RNB5 from North Sinai, while the second group was also divided into two subgroups with bootstrap percentages of 76%. The first subgroup had a Korean anastomosis group (AG-Fa isolate Y1053), and the second had two clusters. The first cluster includes *R. solani* RPS11 from the Port Said alignment and two Chinese anastomosis groups (AG-4, HGIII, HL-J31, and XJ-19). The second cluster had two branches: Ismailia's *R. solani* RIT2 and Sharkia's RSA1. The second branch includes *R. solani* RIF3 from Ismailia and RSzA6 from the Suez alignment, with two Chinese anastomosis groups (AG-F).

3.5. Relationship Studies

Canonical correlation analysis (CCA) was used to study *R. solani* pathogenicity and farmed soil environmental variables (Figure 4a). The first and second CCA axes have variances of 96.77% and 3.23%, respectively, with eigenvalues of 0.013 and 0.080. The CCA diagram placed the seven *R. solani* species being studied alongside the soil environmental parameters, such as pH, silt, sand, clay, soil permeability, and moisture-holding capacity (MWHC). The CCA found that soil type (sand, silt, and clay), pH, porosity, and humidity-holding capacity were most relevant. The length and direction of the arrows denote the

strongest and most variable soil factors, respectively. The CCA ordinance states that the four *R. solani* isolates in the right quadrant of the plot correlated with MWHC, soil porosity, and soil type variables, while the isolates in the left quadrant (RSA1, RSoR4, and RSzA6) correlated with pH and sand/soil texture. To show grouping and correlation among the examined *R. solani* isolates, principal component analysis (PCA) was performed on soil variables, pathogenicity, and percentage of isolates. In Figure 4b, it is evident that PCA1 accounts for 98.23% of the variance. The most important factors, including the proportion of isolates per seed in the sample, the soil texture, and the soil water holding capacity, were used to separate the isolates. The group included three *R. solani* isolates (RSA1, RIT2, and RSZA6), and the other four isolates were in a separate group (Figure 4b). Based on soil environmental variables and pathogenicity percentages, a hierarchical cluster analysis using the unweighted pair group method with arithmetic mean (UPGAMA) and Pearson correlation was used to study the genetic diversity among the studied isolates of *R. solani*. As shown in Figure 4c, the hierarchical cluster analysis agreed with PCA. The isolates studied were divided into two groups: the first group included isolates RSA1, RIT2, and RSZA6, and the second group included the remaining four isolates.

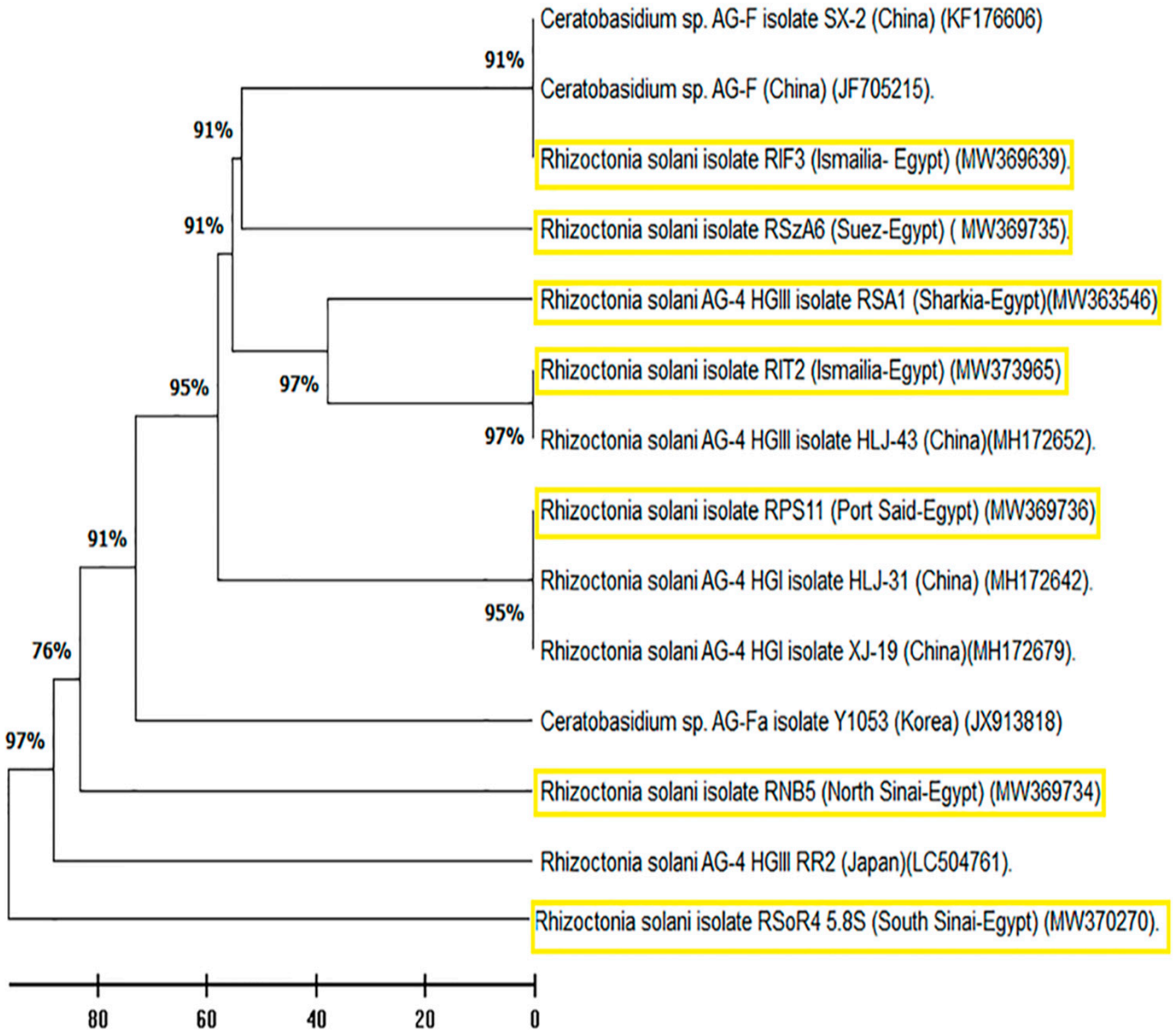

Figure 3. Phylogenetic tree of the investigated *R. solani* isolates (yellow colour).

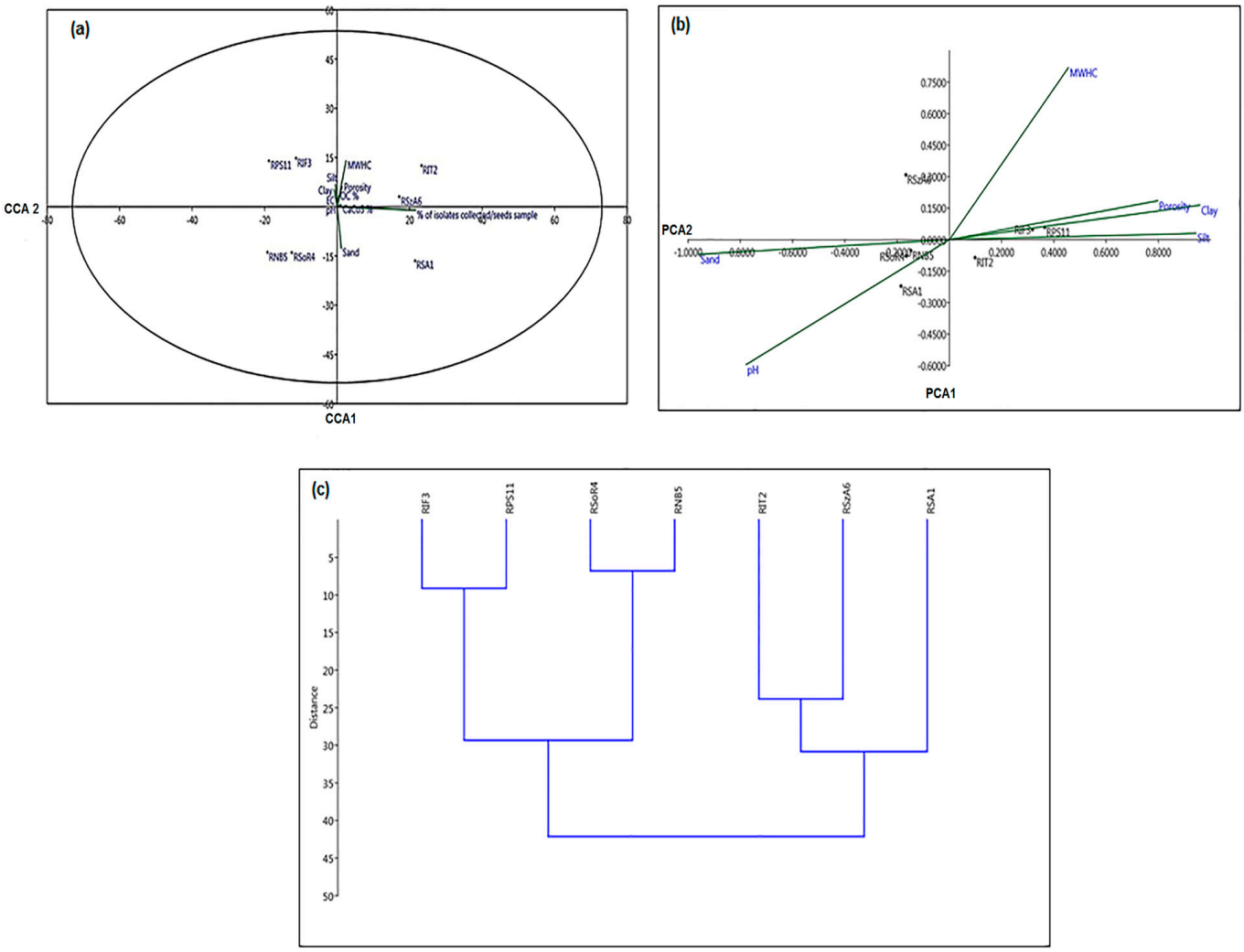

Figure 4. The correlation among the studied *R solani* in different sites; (**a**) CCA ordination diagram of *R. solani* virulence and soil ecological differences; (**b**) principal component analysis (PCA) of the pathogenicity and soil variables of *R. solani*; (**c**) UPGAMA cluster of *R. solani* based on all studied variables. RSA1: *R. solani* collected from Sharkia, RIT2 and RIF3: *R. solani* collected from Ismailia, RSoR4 and RNB5: *R. solani* collected from South Sinai, RSzA6: *R. solani* collected from Suez, RPS11: Port Said.

As shown in Figure 5, generally, there was a high correlation among the different isolates of *R. solani*. The Pearson correlation among the different pathogenicity and soil variables studied revealed that the highest positive correlation was 0.91 between precipitation and relative humidity, followed by 0.8 between the temperature and wind speed variables that affect the incidence of the *Rhizoctonia* pathogen. The highest negative correlation was −0.79 between the post-emergence damping off and pre-emergence damping off, and the lowest negative correlation was −0.21 between precipitation and temperature.

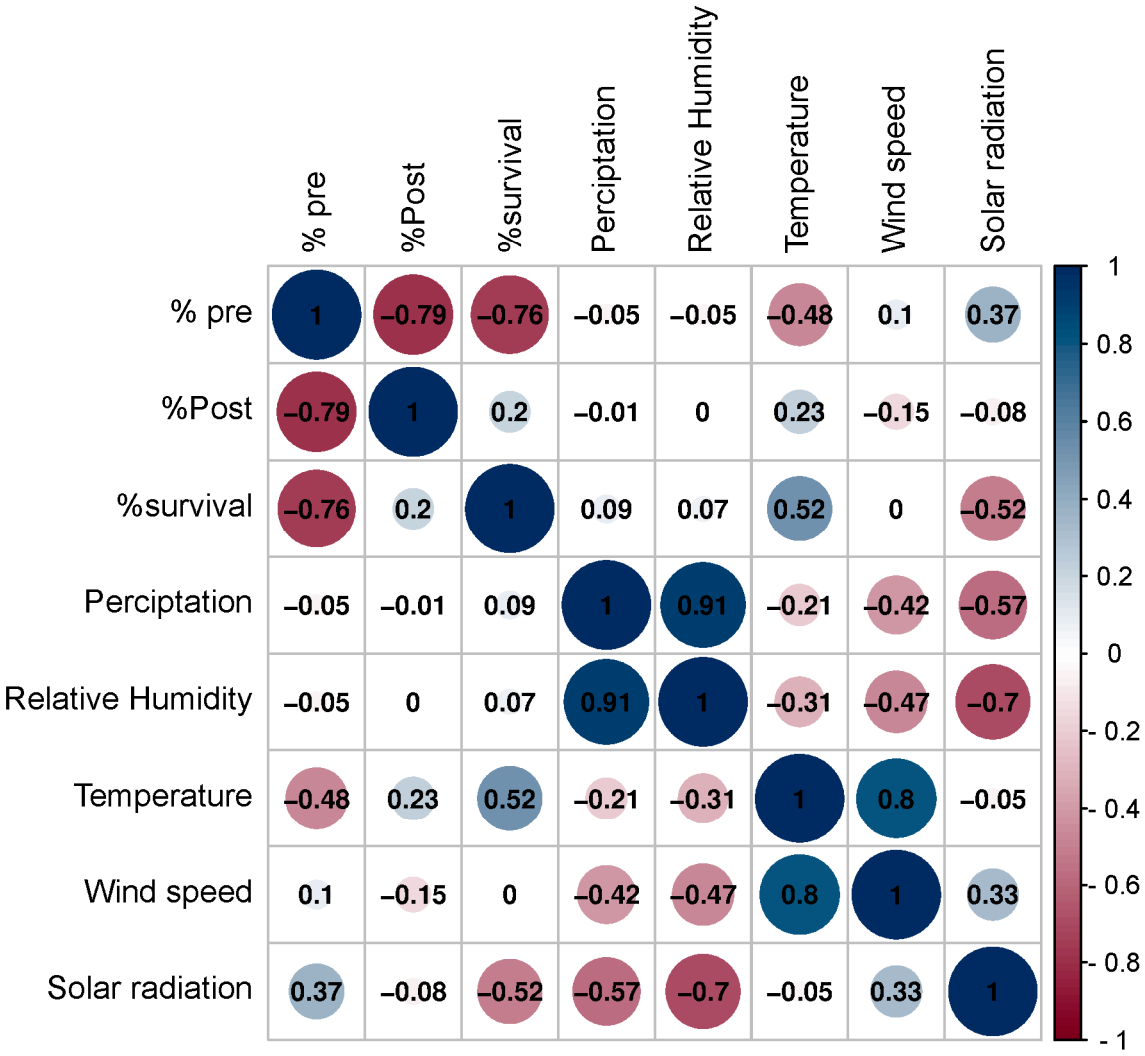

Figure 5. Colour correlation coefficient among the different studied variables; %Pre: Pre-emergence damping off, %post: Post-emergence damping off. The colour scale indicating the variable data ranged from −1 (deep red) to 1 (deep blue).

4. Discussion

Virulence degrees in disease symptoms, host range, and geographical position are crucial features of *R. solani* isolates, which indicate that there are several strains of the pathogen and provide basic information for disease control [49]. The results presented in this study are consistent with the results of previous studies in the same field, which have shown that *R. solani* is one of the most widespread and ubiquitous seed-borne fungi in the world, as well as in Egypt [50–53]. These results are also consistent with previous studies, which have confirmed that there is a major change in the distribution and prevalence of *R. solani* in different proportions in the seeds of beans [51,54]. These results can be explained based on the need of *R. solani* for special ecological conditions that include temperature, pH, and nutrients, which support its development and diversity [55]. In this respect, subnormal rainfall seasons followed by cool weather [56] and high temperatures and soil moisture under greenhouse conditions [57] have both been reported to support *Rhizoctonia* disease infection in soybean. Similar results were obtained by van Bruggen et al. [58], who reported a maximum *Rhizoctonia* infection of the red kidney at 27 °C and 20% soil moisture holding capacity. *Rhizoctonia* sp. diversity in the soil is also influenced by the whole soil's properties, with larger pores increasing their spread and peak effect [59]. *R. solani* isolates have been identified morphologically as well as molecularly. The morphological identification has

shown that *R. solani* grows as an aerial, surface-to-aerial, and surface pathogen, with a linear growth of 65.8 to 90 mm. The number of sclerotia ranged from 23.33 to 83.33 and varied from white to brown in colour. These results are in accordance with Carling et al. [39]. Pathogenicity tests show that *R. solani* had a pre-emergence damping off of 17 to 30% but a post-emergence damping off of 11 to 20%. Previous research has revealed that *R. solani* acts as a major seed-borne phytopathogenic fungus that causes diverse plant infections, with an annual crop damage range of 20–40% [60], sometimes reaching crop losses of 100% [58]. These results and others have shown that *R. solani* is highly virulent for *P. vulgaris* [61–65].

Seven pathogenic fungi of *R. solani* from six different governorates in Egypt were identified molecularly, using the ITS region, as *R. solani* RSA1, *R. solani* RIT2, *R. solani* RIF3, *R. solani* RSoR4, *R. solani* RNB5, *R. solani* RSzA6, and *R. solani* RPS11 (MW363546, MW373965, MW369639, MW370270, MW369734, MW369735, and MW3697369736, respectively). The identified isolates displayed 99.72% similarity with their former nearby relative isolates from anastomosis groups of Japan's seven clades' anastomosis group (AG-4 HGIII RR2) (I–VII), Korean anastomosis group (AG-Fa isolate Y1053), two Chinese anastomosis groups (AG-4 HGIII HL-J31 and XJ-19), and (AG-F). Previous reports showed that AG 4 and AG 1 were the primary causal aggressive isolates for *Rhizoctonia* rot in common beans [66]. Conversely, [15] reported anastomosis groups AG-BI and AG 2.3 to highly attack bean seeds. These results align with [67], which identified AGs of *R. solani* from root-rotted bean plants as AG4, AG4HGII, and AG2-2-2B. The data are also consistent with the finding of Mikhail et al. [68], who recorded severe damping off, root, and hypocotyl rot symptoms in bean seedlings caused by *R. solani* anastomosis group AG-2-2 IIIB or AG-4. Similarly, Rashad et al. [69] found that six out of nine *R. solani* isolates from different plant types, including common bean, broad bean, bell pepper, tomato, and cucumber, belonged to anastomosis group AG 2-2 IIIB and the remaining three to AG 4 HG-I, which have the ability to cause seed rot and seedling mortality symptoms ranging from 13.33 to 100% in bean. Importantly, this study elucidates the correlation between the biodiversity of *R. solani* in the East Delta region of Egypt and *P. vulgaris*, consequently providing a greater opportunity for controlling this disease and protecting bean crops from destruction. According to Mahendra et al. [70], who researched the impact of soil characteristics on isolates of *R. solani* in Meghalaya, the diversity of the analysed pathogen isolates was primarily connected to soil analyses, with the soil type (sand, silt, and clay) and pH variables being the most significant. The fungus thrived in a pH 5.5 nutrition broth [71], but it had a more detrimental impact on mung bean and pea seedlings in neutral and alkaline river sand than it did in sand that had undergone an acidic response. According to Grosch and Kofoet [72], 25 °C and a pH range of 5 to 8 were the optimal conditions for *R. solani* hyphal growth. Temperature and pH had a substantial impact on the development of sclerotia and the expansion of fungal mycelia [73].

A non-linear regression model was used to examine the growth of the fungal mycelia, and the results showed that, for all treatments, growth was mostly favourable at a pH of 6.0 and at 30 °C. However, the pH of the soil had no appreciable effect on the ability of the fungus to survive [74]. With this experiment, the effect of pH on *R. solani* growth was also confirmed. The biodiversity of various plant diseases was impacted by soil and environmental factors. The presence and spread of fungal diseases to areas that are uninfected by *R. solani* can be predicted with the aid of relationships between the prevalence of the causal pathogens and the various meteorological variables [75]. A severe plant disease such as *R. solani* reduces the productivity of legume crops all over the world and preys on members of the Araceae, Amaranthaceae, Linaceae, Moraceae, Malvaceae, Fabaceae, Poaceae, Rubiaceae, and Solanaceae plant families. The Fabaceae family of legume crops has been most severely affected by *R. solani* infection. At the genus and species levels, molecular techniques enable more accurate, useful, and reproducible pathogen identification [76].

5. Conclusions

Rhizoctonia solani is widespread in the East Delta region of Egypt, as represented in the governorates of Sharkia, Ismailia, Port Said, Suez, North Sinai, and South Sinai. A high virulence of *R. solani* in *P. vulgaris* seeds was recorded in the isolates in this study. The most virulent isolates were fully characterised. The type of soil, soil acidity, soil moisture retention capacity, and soil porosity all had a significant impact on *R. solani* diversity.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy13051317/s1, Table S1. Number of *R. solani* isolates, GPS and wheatear conditions for the studied sites from East Delta region in Egypt; Table S2. Accession numbers of the *R. solani* isolates; Table S3. Similarity of *R. solani* RSA1 to other isolates in NCBI; Table S4. Similarity of *R. solani* RIT2 to other isolates in NCBI; Table S5. Similarity of *R. solani* RIF3 to other isolates in NCBI; Table S6. Similarity of *R. solani* RSoR4 to other isolates in NCBI; Table S7. Similarity percentage of *R. solani* RNB5 to other isolates in NCBI; Table S8. Similarity percentage of *R. solani* RSzA6 to other isolates in NCBI; Table S9. Similarity percentage of *R. solani* RPS11 to other isolates in NCBI; Figure S1. The percentage of *Rhizoctonia solani* isolates relative to seed samples.

Author Contributions: Conceptualization, K.M.G., A.A.A.-A. and D.A.E.-W.; methodology, M.I.M.A., H.M.K., E.A.E. and E.M.R.; software A.A.I.; validation, A.A.I. and K.M.G.; formal analysis, A.A.I. and K.M.G.; investigation, K.M.G., D.A.E.-W., E.M.R., A.A.A.-A. and M.I.M.A.; resources, K.M.G. and A.A.I.; data curation, A.A.I., A.A.A.-A. and K.M.G.; writing—original draft preparation, A.A.I. and K.M.G.; writing—review and editing, All authors; visualization, K.M.G., A.A.A.-A. and E.A.E.; funding acquisition, A.A.A.-A. All authors have read and agreed to the published version of the manuscript.

Funding: The authors extend their appreciation to the Researcher Supporting Project number (RSP2023R505), King Saud University, Riyadh, Saudi Arabia for funding this work.

Institutional Review Board Statement: Not applicable

Informed Consent Statement: Not applicable

Data Availability Statement: Not applicable

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

References

1. Silva-Cristobal, L.; Osorio-Díaz, P.; Tovar, J.; Bello-Pérez, L. Chemical composition, carbohydrate digestibility, and antioxidant capacity of cooked black bean, chickpea, and lentil Mexican varieties Composición química, digestibilidad de carbohidratos, y capacidad antioxidante de variedades mexicanas cocidas de frijol negro, garbanzo, y lenteja. *CYTA-J. Food* **2010**, *8*, 7–14. [CrossRef]
2. Sendi, Y.; Romdhane, S.B.; Mhamdi, R.; Mrabet, M. Diversity and geographic distribution of fungal strains infecting field-grown common bean (*Phaseolus vulgaris* L.) in Tunisia. *Eur. J. Plant Pathol.* **2019**, *153*, 947–955. [CrossRef]
3. Rabey, J.; Vered, Y.; Shabtai, H.; Graff, E.; Korczyn, A. Improvement of parkinsonian features correlate with high plasma levodopa values after broad bean (*Vicia faba*) consumption. *J. Neurol. Neurosurg. Psychiatry* **1992**, *55*, 725–727. [CrossRef] [PubMed]
4. Broughton, W.J.; Hernandez, G.; Blair, M.; Beebe, S.; Gepts, P.; Vanderleyden, J. Beans (*Phaseolus* spp.)–model food legumes. *Plant Soil* **2003**, *252*, 55–128. [CrossRef]
5. Saha, S.; Singh, G.; Mahajan, V.; Gupta, H. Variability of nutritional and cooking quality in bean (*Phaseolus vulgaris* L.) as a function of genotype. *Plant Foods Hum. Nutr.* **2009**, *64*, 174–180. [CrossRef] [PubMed]
6. Chávez-Mendoza, C.; Sánchez, E. Bioactive compounds from Mexican varieties of the common bean (*Phaseolus vulgaris*): Implications for health. *Molecules* **2017**, *22*, 1360. [CrossRef]
7. Awaad, H.A. Approaches in faba bean to mitigate impact of climate change. In *Sustainable Agriculture in Egypt*; Springer: Berlin/Heidelberg, Germany, 2022; pp. 185–217. [CrossRef]
8. Hashem, A.H.; Abdelaziz, A.M.; Askar, A.A.; Fouda, H.M.; Khalil, A.M.; Abd-Elsalam, K.A.; Khaleil, M.M. *Bacillus megaterium*-mediated synthesis of selenium nanoparticles and their antifungal activity against *Rhizoctonia solani* in Faba Bean Plants. *J. Fungi* **2021**, *7*, 195. [CrossRef]
9. Leisner, C.P.; Potnis, N.; Sanz-Saez, A. Crosstalk and trade-offs: Plant responses to climate change-associated abiotic and biotic stresses. *Plant Cell Environ.* **2022**. [CrossRef]
10. Singh, S.; Schwartz, H. Breeding common bean for resistance to insect pests and nematodes. *Can. J. Plant Sci.* **2010**, *91*, 239–250. [CrossRef]

11. Mwaipopo, B.; Nchimbi-Msolla, S.; Njau, P.J.; Mark, D.; Mbanzibwa, D.R. Comprehensive surveys of Bean common mosaic virus and Bean common mosaic necrosis virus and molecular evidence for occurrence of other *Phaseolus vulgaris* viruses in Tanzania. *Plant Dis.* **2018**, *102*, 2361–2370. [CrossRef]
12. Foucher, J.; Ruh, M.; Préveaux, A.; Carrère, S.; Pelletier, S.; Briand, M.; Serre, R.F.; Jacques, M.A.; Chen, N.W.G. Common bean resistance to *Xanthomonas* is associated with upregulation of the salicylic acid pathway and downregulation of photosynthesis. *BMC Genom.* **2020**, *21*, 566. [CrossRef]
13. FAOSTAT© FAO. Statistics Division. *Statistical Yearbook of the Food and Agricultural Organization-FAO*. 2017. Available online: www.fao.org/3/i3107e/i3107e03 (accessed on 25 January 2022).
14. Van Emden, H.; Ball, S.; Rao, M. Pest, disease and weed problems in pea, lentil, faba bean and chickpea. In *World Crops: Cool season Food Legumes*; Springer: Berlin/Heidelberg, Germany, 1988; pp. 519–534. [CrossRef]
15. López-Olmos, K.; Hernández-Delgado, S.; Mayek-Pérez, N. AFLP fingerprinting for Identification of Anastomosis Groups of *Rhizoctonia solani* Kühn from Common Bean (*Phaseolus vulgaris* L.) in México. *Rev. Mex. Fitopatol.* **2005**, *23*, 147–151.
16. Derbalah, A.; Shebl, A.M.; Elgobashy, S.F.; Ahmad, A.A.; Ramadan, N.E.; Behiry, S.I.; Abdelkhalek, A.; Saleem, M.H.; Al-Askar, A.A.; Kamran, M.; et al. Resistance induction and direct antifungal activity of some monoterpenes against *Rhizoctonia solani,* the causal of root rot in common bean. *Life* **2022**, *12*, 1040. [CrossRef] [PubMed]
17. Abawi, G.S. Roots rot. In *Bean Production Problems in the Tropics*; Schwartz, H.F., PastorCorrales, M.A., Eds.; CIAT: Cali, Colombia, 1989; pp. 105–157.
18. Ajayi-Oyetundea, O.O.; Bradley, C.A. *Rhizoctonia solani*: Taxonomy, population biology and management of rhizoctonia seedling disease of soybean. *Plant Pathol.* **2018**, *67*, 3–17. [CrossRef]
19. Mayo-Prieto, S.; Rodríguez-González, Á.; Lorenzana, A.; Gutiérrez, S.; Casquero, P.A. Influence of substrates in the development of bean and in pathogenicity of *Rhizoctonia solani* JG Kühn. *Agronomy* **2020**, *10*, 707. [CrossRef]
20. Elwakil, M.; El-Refai, I.; Awadallah, O.; El-Metwally, M.; Mohammed, M. Seed-borne pathogens of faba bean in Egypt: Detection and pathogenicity. *Plant Pathol. J.* **2009**, *8*, 90–97. [CrossRef]
21. Dell'Olmo, E.; Zaccardelli, M.; Basile, B.; Corrado, G.; Sigillo, L. Identification and characterization of new seedborne pathogens in *Phaseolus vulgaris* landraces of Southern Italy. *Pathogens* **2023**, *12*, 108. [CrossRef]
22. Etebu, E.; Nwauzoma, A.B. A mini-review on the development and emerging perspectives of seed pathology. *Microbiol. Res. Int.* **2017**, *5*, 1–7. [CrossRef]
23. Hane, J.K.; Anderson, J.P.; Williams, A.H.; Sperschneider, J.; Singh, K.B. Genome sequencing and comparative genomics of the broad host-range pathogen *Rhizoctonia solani* AG8. *PLoS Genet.* **2014**, *10*, e1004281. [CrossRef]
24. Spedaletti, Y.; Mercado Cárdenas, G.; Taboada, G.; Aban, C.; Aparicio, M.; Rodriguero, M.; Vizgarra, O.; Sühring, S.; Galíndez, G.; Galván, M. Molecular identification and pathogenicity of *Rhizoctonia* spp. recovered from seed and soil samples of the main bean growing area of Argentina. *Aust. J. Crop Sci.* **2017**, *11*, 952–958. [CrossRef]
25. Neergaard, P. *Seed Pathology*; Revised Edition; The Macmillan Press Ltd.: London, UK, 1979; 1191p.
26. Zrenner, R.; Genzel, F.; Verwaaijen, B.; Wibberg, D.; Grosch, R. Necrotrophic lifestyle of *Rhizoctonia solani* AG3-PT during interaction with its host plant potato as revealed by transcriptome analysis. *Sci. Rep.* **2020**, *10*, 12574. [CrossRef] [PubMed]
27. Erlacher, A.; Cardinale, M.; Grosch, R.; Grube, M.; Berg, G. The impact of the pathogen *Rhizoctonia solani* and its beneficial counterpart *Bacillus amyloliquefaciens* on the indigenous lettuce microbiome. *Front. Microbiol.* **2014**, *5*, 175. [CrossRef] [PubMed]
28. Garrett, K.A.; Forbes, G.A.; Savary, S.; Skelsey, P.; Sparks, A.H.; Valdivia, C.; van Bruggen, A.H.; Willocquet, L.; Djurle, A.; Duveiller, E. Complexity in climate-change impacts: An analytical framework for effects mediated by plant disease. *Plant Pathol.* **2011**, *60*, 15–30. [CrossRef]
29. Anees, M.; Edel-Hermann, V.; Steinberg, C. Build up of patches caused by *Rhizoctonia solani*. *Soil Biol. Biochem.* **2010**, *42*, 1661–1672. [CrossRef]
30. Alhussaini, M.S.; Alghonaim, M.; Al-Ghanayem, A.; Al-Yahya, A.; Hefny, H.; Saadabi, A. Characterization of *Cladosporium* species by Internal Transcribed Spacer-PCR and Microsatellites-PCR. *Pak. J. Biol. Sci.* **2016**, *19*, 143–157. [CrossRef]
31. Dubey, S.C.; Tripathi, A.; Upadhyay, B.K.; Kumar, A. Development of conventional and real time PCR assay for detection and quantification of *Rhizoctonia solani* infecting pulse crops. *Biologia* **2016**, *71*, 133–138. [CrossRef]
32. Lin, Y.-H.; Chang, J.-Y.; Liu, E.-T.; Chao, C.-P.; Huang, J.-W.; Chang, P.-F.L. Development of a molecular marker for specific detection of *Fusarium oxysporum* f. sp. *cubense* race 4. *Eur. J. Plant Pathol.* **2009**, *123*, 353–365. [CrossRef]
33. Johanson, A.; Turner, H.C.; McKay, G.J.; Brown, A.E. A PCR-based method to distinguish fungi of the rice sheath-blight complex, *Rhizoctonia solani*, *R. oryzae* and *R. oryzae-sativae*. *FEMS Microbiol. Lett.* **1998**, *162*, 289–294. [CrossRef] [PubMed]
34. Erper, I.; Ozer, G.; Kalendar, R.; Avci, S.; Yildirim, E.; Alkan, M.; Turkkan, M. Genetic Diversity and Pathogenicity of *Rhizoctonia* spp. Isolates Associated with Red Cabbage in Samsun (Turkey). *J. Fungi* **2021**, *7*, 234. [CrossRef] [PubMed]
35. Sharon, M.; Sneh, B.; Kuninaga, S.; Hyakumachi, M.; Naito, S. Classification of *Rhizoctonia* spp. using rDNA-ITS sequence analysis supports the genetic basis of the classical anastomosis grouping. *Mycoscience* **2008**, *49*, 93–114. [CrossRef]
36. Chambers, F.M.; Beilman, D.; Yu, Z. Methods for Determining Peat Humification and for Quantifying Peat Bulk Density, Organic Matter and Carbon Content for Palaeostudies of Climate and Peatland Carbon Dynamics. *Mires Peat* **2011**, *7*, 1–10.
37. Dhingra, O.; Sinclair, J. Location of *Macrophomina phaseoli* on soybean plants related to culture characteristics and virulence. *Phytopathology* **1973**, *63*, 934–936. [CrossRef]

38. Sneh, B.; Burpee, L.; Ogoshi, A. *Identification of Rhizoctonia Species*; American Phytopathological Society Press: St. Paul, MN, USA, 1991; pp. 1–133.
39. Carling, D.E.; Pope, E.J.; Brainard, K.A.; Carter, D.A. Characterization of mycorrhizal isolates of *Rhizoctonia solani* from an orchid, including AG-12, a new anastomosis group. *Phytopathology* **1999**, *89*, 942–946. [CrossRef] [PubMed]
40. Ajayi-Oyetunde, O.O.; Bradley, C.A. Identification and characterization of *Rhizoctonia* species associated with soybean seedling disease. *Plant Dis.* **2017**, *101*, 520–533. [CrossRef]
41. Wilfinger, W.W.; Mackey, K.; Chomczynski, P. Assessing the quantity, purity and integrity of RNA and DNA following nucleic acid purification. In *DNA Sequencing II Optimizing Preparation and Cleanup*; Sudbury, K.J., Ed.; Jones and Bartlett Publishers: Burlington, MA, USA, 2006; Chapter 16; pp. 291–312.
42. Sneh, B.; Jabaji-Hare, S.; Neate, S.; Dijst, G. *Rhizoctonia Species: Taxonomy, Molecular Biology, Ecology, Pathology and Disease Control*, 1st ed.; American Phytopathological Society: St. Paul, MN, USA, 1996; pp. 1–133.
43. White, T.J.; Bruns, T.; Lee, S.; Taylor, J. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In *PCR Protocols: A Guide to Methods and Applications*; Innis, M.A., Gelfand, D.H., Sninsky, J.J., White, T.J., Eds.; Academic Press: London, UK, 1990; Volume 18, pp. 315–322.
44. Vilgalys, R.; Hopple, J.S., Jr.; Hibbett, D.S. Phylogenetic implications of generic concepts in fungal taxonomy: The impact of molecular systematic studies. *Mycol. Helv.* **1994**, *6*, 73–91.
45. Tamura, K.; Nei, M. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Mol. Biol. Evol.* **1993**, *10*, 512–526. [CrossRef]
46. Kumar, S.; Stecher, G.; Li, M.; Knyaz, C.; Tamura, K. MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Mol. Biol. Evol.* **2018**, *35*, 1547. [CrossRef]
47. Shan, Q.; Wang, Y.; Chen, K.; Liang, Z.; Li, J.; Zhang, Y.; Zhang, K.; Liu, J.; Voytas, D.F.; Zheng, X. Rapid and efficient gene modification in rice and *Brachypodium* using TALENs. *Mol. Plant* **2013**, *6*, 1365–1368. [CrossRef]
48. CoStat. *CoHort Software, Version 6.4. 798 Lighthouse Ave. PMB 320*; Cohort Software Ltd: Monterey, CA, USA, 2008; Available online: https://www.cohortsoftware.com/costat.html (accessed on 5 February 2022).
49. Akber, M.A.; Mubeen, M.; Sohail, M.A.; Khan, S.W.; Solanki, M.K.; Khalid, R.; Abbas, A.; Divvela, P.K.; Zhou, L. Global distribution, traditional and modern detection, diagnostic, and management approaches of *Rhizoctonia solani* associated with legume crops. *Front. Microbiol.* **2023**, *13*, 1091288. [CrossRef]
50. Aydin, M.H. *Rhizoctonia solani* and its biological control. *Turk. J. Agric. Res.* **2022**, *9*, 118–135. [CrossRef]
51. El-Benawy, N.M.; Abdel-Fattah, G.M.; Ghoneem, K.M.; Shabana, Y.M. Antimicrobial activities of *Trichoderma atroviride* against common bean seed-borne *Macrophomina phaseolina* and *Rhizoctonia solani*. *Egypt. J. Basic Appl. Sci.* **2020**, *7*, 267–280. [CrossRef]
52. Mahmoud, S.Y.; Hosseny, M.H.; Obiadalla, A.H. Seed borne fungal pathogens associated with common bean (*Phaseolus vulgaris* L.) seeds and their impact on germination. *J. Environ. Sci. Stud.* **2013**, *11*, 19–26. [CrossRef]
53. Abu-Tahon, M.; Mogazy, A.; Isaac, G. Resistance assessment and enzymatic responses of common bean (*Phaseolus vulgaris* L.) against *Rhizoctonia solani* damping-off in response to seed presoaking in *Vitex agnus-castus* L. oils and foliar spray with zinc oxide nanoparticles. *S. Afr. J. Bot.* **2022**, *146*, 77–89. [CrossRef]
54. Valentín Torres, S.; Vargas, M.M.; Godoy-Lutz, G.; Porch, T.G.; Beaver, J.B. Isolates of *Rhizoctonia solani* can produce both web blight and root rot symptoms in common bean (*Phaseolus vulgaris* L.). *Plant Dis.* **2016**, *100*, 1351–1357. [CrossRef] [PubMed]
55. Oladzad, A.; Zitnick-Anderson, K.; Jain, S.; Simons, K.; Osorno, J.M.; McClean, P.E.; Pasche, J.S. Genotypes and genomic regions associated with *Rhizoctonia solani* resistance in common bean. *Front. Plant Sci.* **2019**, *10*, 956. [CrossRef]
56. Tachibana, H. *Rhizoctonia solani* root rot epidemic of soybeans in central Iowa 1967. *Plant Dis. Rep.* **1968**, *52*, 613–614.
57. Liu, Z.; Sinclair, J.B. Isolates of *Rhizoctonia solani* anastomosis group 2-2 pathogenic to soybean. *Plant Dis.* **1991**, *75*, 682–687. [CrossRef]
58. van Bruggen, A.H.C.; Whalen, C.H.; Arneson, P.A. Emergence, growth, and development of dry bean seedlings in response to temperature, soil moisture, and *Rhizoctonia solani*. *Phytopathology* **1986**, *76*, 568–572. [CrossRef]
59. van Bruggen, A.H.; Gamliel, A.; Finckh, M.R. Plant disease management in organic farming systems. *Pest Manag. Sci.* **2016**, *72*, 30–44. [CrossRef]
60. Harris, K.; Young, I.M.; Gilligan, C.A.; Otten, W.; Ritz, K. Effect of bulk density on the spatial organisation of the fungus *Rhizoctonia solani* in soil. *FEMS Microbiol. Ecol.* **2003**, *44*, 45–56. [CrossRef]
61. Ghosh, S.; Kanwar, P.; Jha, G. Alterations in rice chloroplast integrity, photosynthesis and metabolome associated with pathogenesis of *Rhizoctonia solani*. *Sci. Rep.* **2017**, *7*, 41610. [CrossRef] [PubMed]
62. Sun, G.; Zhu, C.; Kramer, M.; Yang, S.; Song, W.; Piepho, H.; Yu, J. Variation explained in mixed-model association mapping. *Heredity* **2010**, *105*, 333–340. [CrossRef] [PubMed]
63. Costa-Coelho, G.R.; de Toledo-Souza, E.D.; Café-Filho, A.C.; Lobo, M. Dynamics of common bean web blight epidemics and grain yields in different tillage systems. *Trop. Plant Pathol.* **2016**, *41*, 306–311. [CrossRef]
64. Bolkan, H.; Ribeiro, W. Anastomosis groups and pathogenicity of *Rhizoctonia solani* isolates from Brazil. *Plant Dis.* **1985**, *69*, 599–601. [CrossRef]
65. Bolkan, H.A.; Butler, E.E. Studies on heterokaryosis and virulence of *Rhizoctonia solani*. *Phytopathology* **1974**, *64*, 13–522. [CrossRef]
66. Mahendra, K.; Baiswar, P.; Chandra, S.; Choudhury, B.; Majumder, D.; Rajesh, T.; Firake, D. Molecular characterization and influence of soil factors on isolates of *Rhizoctonia solani* in Meghalaya. *Indian Phytopathol.* **2016**, *69*, 271.

67. Echávez-Badel, R.; Gómez-Galué, J.E.; Alameda-Lozada, M. Characterization of *Rhizoctonia* spp. isolates collected from *Phaseolus vulgaris* in Puerto Rico. *J. Agrie. Univ. Puerto Rico* **2000**, *84*, 79–86. [CrossRef]
68. Bohlooli, A.; Okhovvat, S.M.; Javan-Nikkhah, M. Identification of anastomosis group of *Rhizotonia solani*, the causal agent of seed root and damping-off of bean in Iran. *Commun. Agric. App. Biol. Sci.* **2005**, *70*, 137–141.
69. Mikhail, M.S.; Sabet, K.K.; Omar, M.R.; Asran, A.A.; Kasem, K.K. Current *Rhizoctonia solani* anastomosis groups in Egypt and their pathogenic relation to cotton seedlings. *Afr. J. Microbiol. Res.* **2010**, *4*, 386–395.
70. Rashad, Y.M.; Abdel-Fattah, G.M.; Hafez, E.E.; El-Haddad, S.A. Diversity among some Egyptian isolates of *Rhizoctonia solani* based on anastomosis grouping, molecular identification and virulence on common bean. *Afr. J. Microbiol. Res.* **2012**, *6*, 6661–6667. [CrossRef]
71. Meinhardt, L.W.; Wulff, N.A.; Bellato, C.M.; Tsai, S.M. Genetic analyses of *Rhizoctonia solani* isolates from *Phaseolus vulgaris* grown in the Atlantic Rainforest Region of São Paulo, Brazil. *Fitopatol. Brasil.* **2002**, *27*, 259–267. [CrossRef]
72. Kataria, H.R.; Grover, R.K. Influence of soil factors, fertilizers and manures on pathogenicity of *Rhizoctonia solani* on Vigna species. *Plant Soil* **1987**, *103*, 57–66. [CrossRef]
73. Grosch, R.; Kofoet, A. Influence of temperature, pH and inoculum density on bottom rot on lettuce caused by *Rhizoctonia solani*/Einfluss von Temperatur, pH und Inokulumdichte auf die Salatfäule verursacht durch *Rhizoctonia solani*. *J. Plant Dis. Prot.* **2003**, *110*, 366–378.
74. Datta, S.; Das, S.; Sarkar, A.; Tarafdar, J.; Chowdhury, A. Assessing the effects of varied temperature and pH on the growth and sclerotial formation of *Rhizoctonia solani* Kuhn, isolated from paddy field: A case study. *Int. J. Life Sci.* **2014**, *8*, 4–9. [CrossRef]
75. Velásquez, A.C.; Castroverde, C.D.M.; He, S.Y. Plant and pathogen warfare under changing climate conditions. *Curr. Biol.* **2018**, *28*, 619–634. [CrossRef]
76. Benson, D.M.; Baker, R. Epidemiology of *Rhizoctonia solani* pre emergence damping-off of radish: Survival. *Phytopathology* **1974**, *64*, 1163–1168. [CrossRef]