

Genetic and pathogenic characterization of *Rhizoctonia solani* AG-4 isolates obtained from common bean

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ABSTRACT

Common bean (*Phaseolus vulgaris* L.) is the most important grain legume. Crop production of common bean is affected by a number of diseases, such as *Rhizoctonia* root rot, being detected worldwide. Herein, we aimed to genetically and pathologically characterize *Rhizoctonia solani* isolates obtained from different provinces in Turkey. Anastomosis groups (AG) and subgroups of isolates were identified based on the sequence analysis of the rDNA-ITS region. The most prevalent subgroup was AG-4 HGIII, according to BLAST analysis and the phylogeny of resultant sequences, followed by AG-4 HGI and AG-4 HGII, respectively. iPBS retrotransposons highly supported the phylogenetic tree and provided a high level of genetic variability among isolates to discriminate AG subgroups. The results indicated that the iPBS DNA marker system based on retrotransposons could be used to discriminate *Rhizoctonia* isolates relying on AG grouping. The virulence of the pathogen isolates changed from 3.67 to 5 based on the agar-plate assay on the susceptible cv. Gina. The reactions of thirty common bean cultivars were also evaluated against the most aggressive isolate in each subgroup. The reaction assay showed significant differences among both isolates and cultivars; the highest disease severity among cultivars was observed to AG-4 HGI, followed by AG-4 HGIII and AG-4 HGII, respectively. None of the cultivars showed resistance to all AG subgroups. Screening of AGs among the isolates and selecting cultivars are critical to managing *Rhizoctonia* disease due to the range of host suitability according to AG subgroups.

1. Introduction

Common bean (*Phaseolus vulgaris* L.) is one of the most significant grain legumes widely grown in many countries and preferred in human nutrition due to their high protein content and health benefits. Turkey is the world's fifth-largest green bean producer with an annual yield of 547.349 t/yr⁻¹, and the twentieth dry bean producer with 279.518 t/yr⁻¹ [1]. Root rot caused by *Rhizoctonia solani* Kühn (Teleomorph: *Thanatephorus cucumeris*) is one of the most economically important root and hypocotyl diseases of beans worldwide. The pathogen initially causes small, elongate, sunken, reddish-brown lesions on hypocotyl and roots of seedlings as well as web blight symptoms. Severe infections result in pre- or post-emergence damping-off, plant stunting, premature death and ultimately significant yield losses [2,3].

Rhizoctonia genus is a complex soil-borne pathogen with a vast host range and shows significant differences in cultural, molecular and biochemical characteristics. The pathogen is classified into different

anastomosis groups (AG) based on the interactions of isolates with hyphal adaptability that are genetically diverse [4–6]. To date, thirteen AGs (between AG1-1 and AG-13) are classified in different plants. Some of them have been further divided into subgroups due to the colony morphology, host range, virulence, disease symptoms, hyphal fusion, pathogenicity and molecular properties [7–12]. AG-4 is the most common AG group, causing web blight and root rot in bean plants worldwide, followed by AG-1 and AG-2 [13–15]. Similarly, researchers in Turkey informed that *R. solani* AG-1, AG-2, AG-3, AG-4, AG-5, AG-6, AG-9, AG-10 and AG-11 were found in bean production areas, but AG-4 was the most prevalent [16–21]. However, the distribution of AGs has not been determined in some important bean areas of Turkey, such as Ankara, Tokat and Zonguldak, where the most beans are produced.

Rhizoctonia solani AG-4 is also separated into three subgroups, HGI, HGII, and HGIII, based on the hyphal anastomosis. Classification of subgroups is usually performed by molecular techniques such as the sequencing of the internal transcribed spacer (ITS) region between the

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18S and 28S ribosomal RNA genes, restriction fragment length polymorphisms (PCR-RFLP), random amplified polymorphic DNA (RAPD), inter simple sequence repeat (ISSR), DNA base sequence homology [8, 22–28]. DNA profiling method based on inter-primer binding site (iPBS) provided the informative polymorphisms for the evaluation of genetic variations in *R. solani* isolates from different hosts at the intraspecific level [20,29,30]. iPBS analysis is performed by DNA amplification of regions in long-terminal repeat (LTR) which has a highly conserved tRNA priming binding site [31,32]. Replication of these regions provides information about the sequences of the PBS region and is an excellent resource for investigating the genetic differences of pathogens. PCR-based DNA fingerprinting method has been successfully applied for screening genetic polymorphisms on many eukaryotes [33–35]. Investigation of genetic differences of *R. solani* at the level of subgroups contributes to the development of disease-resistant cultivars. Using the iPBS method for this purpose may facilitate the development of resistant varieties in breeding studies. However, commercially resistant bean cultivar was absent against *R. solani* worldwide, bean cultivars showed significant differences in their disease reactions [36–38]. Little information was available about the evaluation of different resistance sources against the disease in Turkey [16–18]. This study aimed respectively: (i) to determine AGs of *R. solani* isolates obtained from diseased bean plants, (ii) to evaluate genetic variation among *R. solani* AG-4 subgroups based on iPBS amplification, (iii) to determine differences in virulence of *R. solani* AG-4 isolates, and (iv) to evaluate disease reaction of thirty common bean cultivars widely grown in Turkey against the most virulent isolates belonging to different AG-4 subgroups.

2. Material and methods

2.1. Fungal materials

Infected plants showing the symptoms of wilting, yellowing, root rot, and death were randomly collected from bean growing areas in Ankara, Samsun, Tokat, and Zonguldak provinces of Turkey during the 2019–2022 growing seasons (Fig. 1, Table 1). Symptomatic root tissues were washed with water, kept in 1% sodium hypochlorite for 1 min for surface disinfection, rinsed with sterile water again, and dried on filter papers. Disinfected tissues were placed on potato dextrose agar (PDA, Merck, Darmstadt, Germany), amended with 100 µg/ml streptomycin sulfate and incubated at 24 °C for four days. After the incubation, the cultures were dissected under the microscope, and a hyphal tip of each *Rhizoctonia*-like isolate was transferred with a sterile needle to PDA medium for purification. Purified isolates were examined for their

Table 1

Isolate IDs, locations, AGs, and GenBank accession numbers of *Rhizoctonia solani* isolates used in this study.

Isolate IDs	Locations	Year	AGs	Subgroups	GenBank Accession Nos.
A1	Beypazarı-Ankara	2022	AG-4	HGII	OQ932789
A2		2022		HGIII	OQ934054
A3		2022		HGII	OQ934056
A4		2022		HGII	OQ932826
A5		2022		HGI	OQ934058
T1	Niksar-Tokat	2019	AG-4	HGIII	OQ781880
T2		2019		HGI	OQ932787
T3		2022		HGIII	OQ934054
S1		2022		HGI	OQ934057
S2	Çarşamba-Samsun	2022	AG-4	HGIII	OQ934055
S3		2022		HGI	OQ934059
S4		2022		HGII	OQ932829
S5		2022		HGIII	OQ934053
Z1		2022		HGI	OQ940443
Z2	Gökçebey-Zonguldak	2022	AG-4	HGIII	OQ940449

cultural characteristics and number of nuclei in the cells of young vegetative hyphae. All isolates were stored on colonized wheat grains at room conditions and PDA slants at 4 °C.

2.2. DNA extraction

Genomic DNA isolation was performed using the method introduced by Diversity Arrays Technology (DArT) (<http://www.diversityarrays.com>). Mycelial mats from 7-day-old PDA cultures were gently scraped with a sterile scalpel, placed into the 2 ml Eppendorf tubes, and crushed in 650 µl extraction buffer solution (125 mM Tris-HCl pH 8.0, 25 mM EDTA pH 8.0, 0.8 M NaCl, 1% CTAB, 1% sarcosyl, 2% PVP-40 (K29-32), 0.5% sodium disulphite). After an incubation period at 65 °C for 45–60 min, the suspension was mixed strongly with an equal volume of chloroform: isoamyl alcohol (24:1) and then centrifuged at 10,000 g, 4 °C for 20 min. The supernatant was transferred to a clean 1.5 ml Eppendorf tube and mixed with the same volume of cold isopropanol. After the centrifugation at 10,000 g for 10 min, the supernatant was discarded, and the DNA pellet was washed with cold ethanol, air-dried, and suspended with 100 µl of ultra-pure distilled water. DNA concentration of each sample was determined spectrophotometrically, diluted to 20 ng/µl, and stored at –20 °C until used.



Fig. 1. Map showing the geographical distribution of *Rhizoctonia solani* isolates in Turkey.

2.3. DNA sequencing and phylogeny

The ribosomal DNA-ITS region of each isolate was amplified using ITS1 and ITS4 primers [39]. PCR reactions were carried out with a total volume of 50 µl containing 10 × reaction buffer, 1.5 mM MgCl₂, 200 µM dNTPs, 0.2 µM primer, 1.5 U *Taq* DNA polymerase (Thermo Fischer Scientific, Waltham, MA, USA), and 30 ng fungal DNA. All DNA amplifications were performed in an Arktik™ thermal cycler (Thermo Fischer Scientific, Waltham, MA, USA) programmed as follows; initial denaturation at 94 °C for 5 min; 35 cycles of denaturation at 94 °C for 1 min, annealing at 56 °C for 1 min, extension at 72 °C for 1 min and a final extension step at 72 °C for 5 min. The amplified products were visualized on 1.2% agarose gel using 1 × TAE buffer, and Gene Ruler 100 bp DNA Ladder Plus (Thermo Fischer Scientific, Waltham, MA, USA) was used for determining the molecular weight of PCR products. Amplicons were sequenced in both directions using the same primers by the Macrogen company (Seoul, Korea). Sequences were edited with DNASTAR's Lasergene sequence analysis software version 7.1.0 (DNASTAR Inc., Madison, WI, USA), and examined by BLAST analysis on the NCBI website (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). Also, sequence alignments of the isolates obtained in this study and the reference isolates retrieved from GenBank (AG-4 HGI: AY154307, DQ102445; AG-4 HGII: AY154308; AG-4 HGIII: AY154659, AY152813, AY154309, AY152811, DQ021449, AF354075) were carried out in MEGA-X software based on CLUSTAL W alignment method [40]. Phylogenetic tree was inferred using the neighbour joining (NJ) method (Tamura-Nei distance calculation), and the robustness of phylogeny was estimated with 1000 bootstrap replications.

2.4. iPBS analysis

Thirty-two iPBS primers described by Kalendar et al. [31] were tested in the preliminary study, and iPBS primers providing the informative polymorphism were used to determine intra-specific variation among *R. solani* isolates. iPBS amplification was performed in a volume of 25 µl containing 1 × Green Buffer, 1 µM primer, 0.2 mM dNTPs, 1.5 U Dream *Taq* DNA polymerase, and 0.04 U *Pfu* DNA polymerase (Thermo Fischer Scientific, Waltham, MA, USA). The PCR reactions were performed by setting initial denaturation at 95 °C for 3 min, followed by 35 cycles of denaturation step at 95 °C for 15 s, at annealing temperature for 1 min, and at 70 °C for 1 min, and final extension at 72 °C for 5 min (Table 2). PCR products were analyzed by electrophoresis in 1.4% agarose gels with Tris-acetate-EDTA (TAE) buffer at 100 V for 90 min, staining with ethidium bromide, and visualized under UV light.

Scorable bands obtained from iPBS primers were recorded as present (1) or absent (0). The cluster analysis based on the Dice's similarity coefficient was created via the unweighted pair group methods (UPGMA) analysis in NTSYS-pc program ver.2.2 [41]. The values of polymorphism information content (PIC) and resolving power (RP) were computed according to the formula described by De Riek et al. [42] and Prevost & Wilkinson [43], respectively. Principal coordinate analysis (PCoA) was constructed using the GenAlEx software program [44], while the evaluation of the genetic structure of *Rhizoctonia* isolates was

performed in the program STRUCTURE v.2.3.4 [45]. Ten independent runs (K) from 2 to 10 were implemented for each number of clusters, and then STRUCTURE HARVESTER was used to determine the number of populations representing the best grouping as described by Evanno et al. [46].

2.5. Virulence of *Rhizoctonia* isolates

All isolates from infected common bean plants were tested for virulence degrees on cv. Gina seeds by using the agar-plate assay. A 3 mm mycelial disc taken from 7 day-PDA culture was put in the center of 9 cm Petri dishes, including 1% water agar medium and incubated at 24 ± 1 °C for 3 days. Four bean seeds were disinfected with 1% NaOCl for 2 min, and then placed at the sides of fungal discs. No colonized PDA discs were used for the control treatments. The Petri dishes were incubated with 12 h photoperiod light/dark at 24 ± 1 °C for 9 days. Four replicates were used for each isolate, which was repeated once. The disease severity was evaluated by assessment on individual seeds/seedlings symptoms using 1–5 scale described by Muyolo et al. [47]: 1 = no symptoms, normal root development; 2 = localized tissue root discoloration without necrosis, near-normal root development; 3 = localized with extensive tissue discoloration lesions, near-normal root development; 4 = nearly complete root necrosis, partially restricted root length; 5 = complete root rot, root length severely restricted. The disease severity index (DSI) was calculated for each isolates by using the formula (scale value × number of plants on the scale/total number of plants). Data of disease severity index among isolates were analyzed using Minitab 17 statistical program [48] and the significant differences were separated using LSD test (Least Significant Difference test, $P \geq 0.05$). The isolates were re-isolated from the infected tissues of the seed/seedlings for complete the Koch's postulates.

2.6. Pathogenicity tests and plant height

The reactions of thirty common bean cultivars widely grown in Turkey were tested to the representing isolates of T2 (AG-4 HGI), A3 (AG-4 HGII), and T1 (AG-4 HGIII), which were the most virulent in agar-plate assay. The seeds of common bean cultivars were superficially disinfected in 1% NaOCl for 2 min, washed twice with water, and then air-dried on sterile filter paper. Five seeds were sown into per pot (15 cm diameter), containing the sterile soil mix (peat-perlite; 1:1 v/v). The plants were watered lightly every 2–3 days throughout the assay. Wheat seeds, colonized separately with each isolate, were used as the source of inoculum and placed on the edges of the bean seeds at a rate of 100 mg/kg soil per pot [49]. Then, the pots were covered with approximately 1 cm of sterile peat-perlite mixture and incubated at 23 ± 2 °C with a 12 h photoperiod in the plant growth room. Sterile wheat seeds were used as control. Three replicates were arranged for each cultivar. Fourteen days after inoculation, the plants were gently uprooted, washed with tap water, and evaluated by examining the root and hypocotyl parts according to the 1–5 scale of Muyolo et al. [47]. The values of the disease severity index were compared by the least significant difference test (LSD_{0.05}) using Minitab 17 statistical program [48]. In addition, the

Table 2
The knowledge of iPBS primers used for investigating *Rhizoctonia solani* AG-4 isolates.

iPBS ID	Primer sequences (5'–3')	GC (%)	Annealing (°C)	TB	PB	PIC	RP
2237	CCCCACCTGGCGTGCCA	55	57	14	14	0,11	1,61
2242	GCCCCATGGTGGGCGCCA	77	57	20	20	0,08	1,79
2399	AAACTGGCAACGGCGCCA	61	52	16	16	0,13	2,18
2395	TCCCAGCGGAGTCGCCA	72	53	12	12	0,11	1,43
2077	CTCACGATGCCA	58	55	18	18	0,09	1,72
2232	AGAGAGGCTCGGATACCA	55	55	24	23	0,08	1,98
	Total			104	103		
	Avg./primer			17,33	17,17	0,10	1,79

GC (%) = percentage of guanine-cytosine content; TB = Total band; PB = polymorphic band; PIC = mean polymorphism information content; RP = resolving power.

height of the plants was measured from the root zone, and the % growth rates were evaluated by comparing them with the control plant growth.

3. Results

3.1. AGs and subgroups of *Rhizoctonia* spp. isolates from common bean

Rhizoctonia-like isolates from infected samples were quite similar in terms of cultural characteristics. Fungal hyphae showed branching at right angles and carried multinucleate cells having 3–8 nuclei per cell. Cultures were initially white, gradually turning brown to dark brown. Sclerotia were few or many and dark brown to black. All isolates were identified as multinucleate *R. solani* (Table 1). These findings on morphological characteristics were in accordance with Sneh et al. [50]. Anastomosis grouping of *R. solani* isolates was determined by DNA sequence analysis of the ribosomal DNA-ITS region. PCR amplification with primers ITS1 and ITS4 produced a single amplicon, approximately ranging from 660-bp to 669-bp in length. BLASTn search of the obtained sequences showed 99–100% match with the reference sequences used by Sharon et al. [51] in GenBank. The majority of isolates had identical sequences with the reference isolates. Five isolates showed sequence homology with the reference sequence of HGI, 4 to HGII and 6 to HGIII subgroups of *R. solani* AG-4. Phylogenetic analysis with concatenated sequences based on the NJ method further confirmed the identification and clustering of *R. solani* AG-4 subgroups with 100% bootstrap support (Fig. 2).

3.2. iPBS retrotransposons analysis

Six of iPBS primers tested produced interpretable and variable banding patterns. The other primers did not produce any PCR product or provided a low level of polymorphisms between the isolates. These primers amplified 104 scorable bands, of which a hundred-three (99.04%) were polymorphic for evaluating the extent of genetic variation among *R. solani* AG-4 isolates (Table 2). The total number of fragments amplified with these primers varied from 12 (primer 2395) to 24 (primer 2232), averaging 17.33 per primer. The representative amplification profiles showing the polymorphism between AG-4 subgroups with iPBS primers were given in Fig. 3.

The mean polymorphism information content (PIC) and resolving power (RP) values, which were indicators of the discrimination potential of iPBS primers, varied between 0.08–0.13 and 1.43–2.18, respectively (Table 2). The highest PIC and RP values were recorded for primer 2399. The dendrogram constructed with the iPBS data of *R. solani* isolates showed a similar allocation among the isolates as the dendrogram of ITS sequencing. The UPGMA cluster analysis produced using Dice's similarity coefficient for iPBS data grouped accurately into three major clusters associated with the subgrouping of *R. solani* AG-4 isolates (Fig. 4). Also, isolate Z1 belonging to AG-4 HGI and isolate Z2 to AG-4 HGIII further clustered separate from other isolates within the group, as in phylogenetic analysis based on ITS sequences. Principal coordinate analysis (PCoA) of iPBS profiles showed a similar distribution among *R. solani* AG-4 isolates, confirming the clustering of subgroups in the dendrogram (Fig. 5). STRUCTURE runs were performed for K = 1 to 10

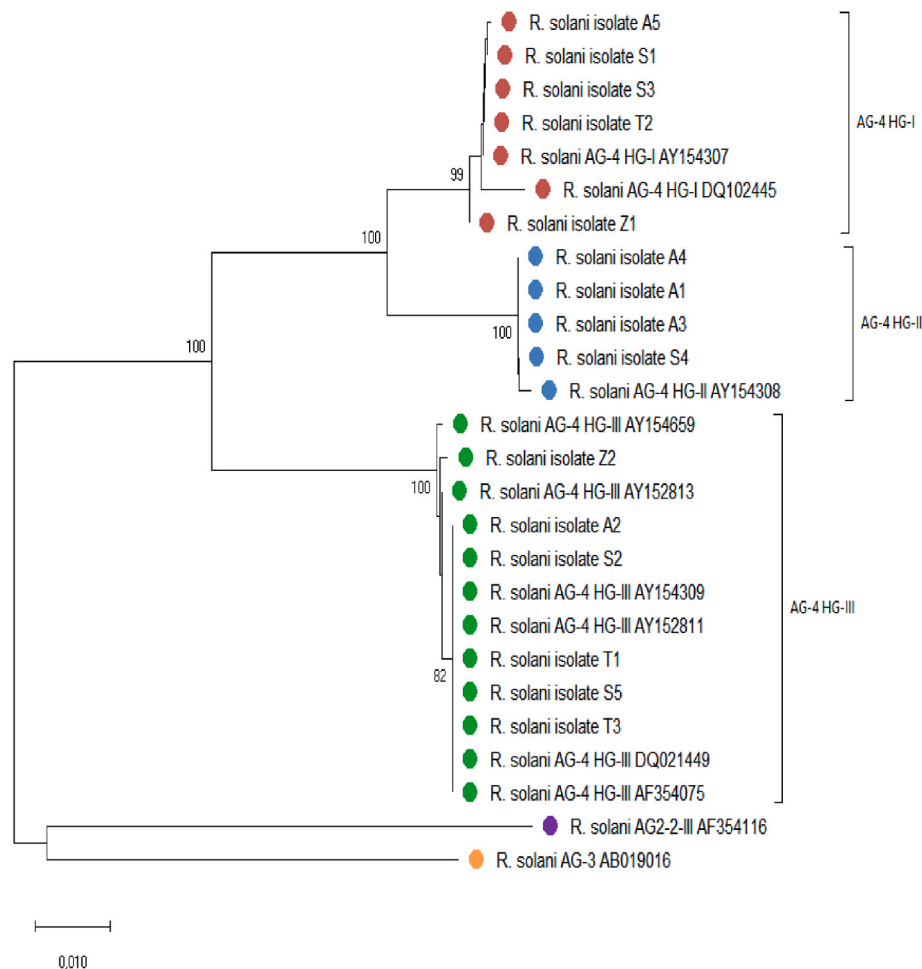


Fig. 2. Neighbour Joining (NJ) tree based on ITS sequences of *Rhizoctonia solani* AG-4 isolates collected in this study and the reference isolates retrieved from GenBank. Bootstrap values were calculated from 1000 replicates.

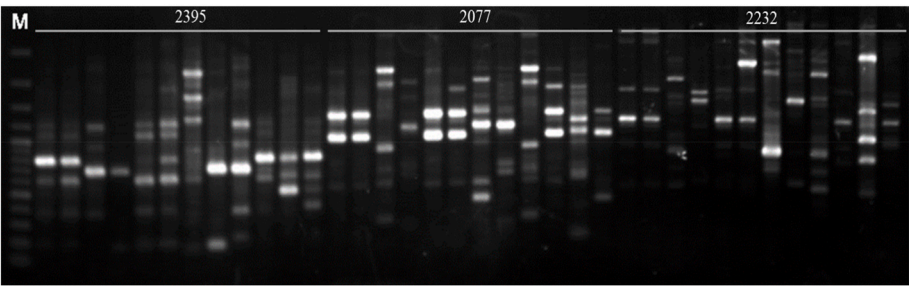


Fig. 3. The amplification profiles of *Rhizoctonia solani* AG-4 isolates obtained with iPBS primer 2395 (lane 1–12), 2077 (lane 13–24), and 2232 (lane 25–36). M: Gene ruler 100 bp DNA ladder.

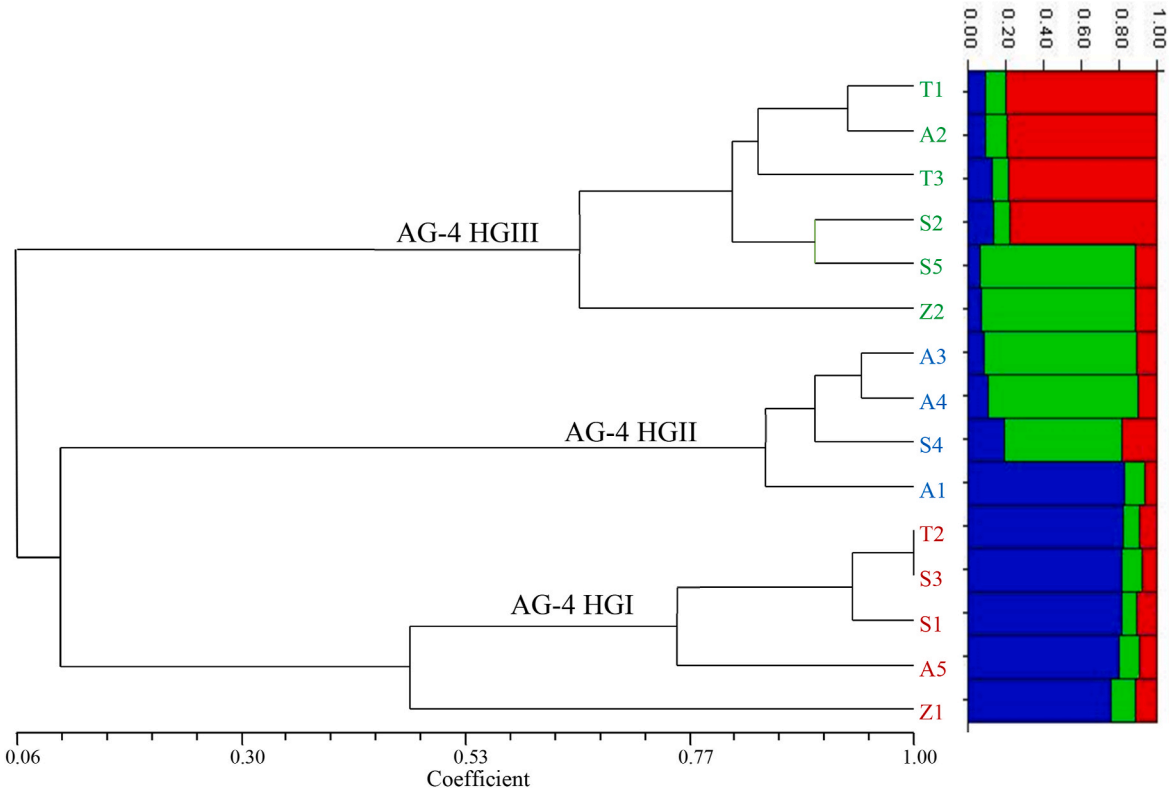


Fig. 4. UPGMA tree based on iPBS data of *Rhizoctonia solani* isolates and STRUCTURE bar plots individual assignment results for K = 3.

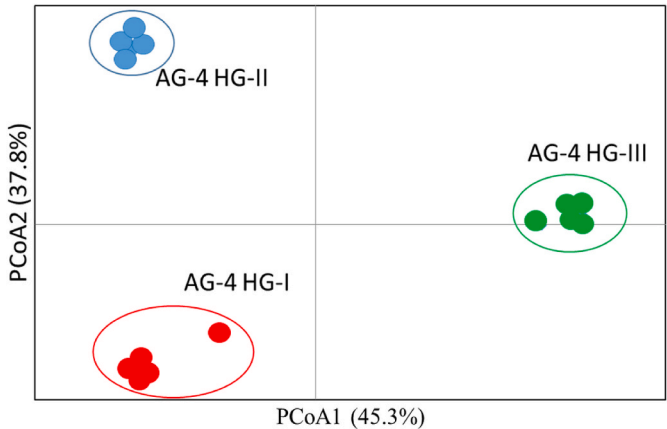


Fig. 5. Association matrix for subgroups of *Rhizoctonia solani* AG-4 based on principal coordinate analysis (PCoA).

using the iPBS data. The analysis generated three clusters with the highest peak of $\Delta K = 3$ ($\text{LnP}(K) = -4485.0$) according to the ΔK criteria of Evanno et al. [46] (Fig. 4).

3.3. Virulence of *Rhizoctonia solani* isolates

All isolates were subjected to the preliminary pathogenicity test on susceptible common bean cv. Gina. The isolates belonging to different AG-4 subgroups showed a large variation for their virulence and disease symptoms. AG-4 HGI isolates generally prevented seed germination with intense mycelial growth on the seeds. AG-4 HGII isolates caused symptoms such as root rot and brown discoloration in the lateral roots, while AG-4 HGIII isolates developed slightly sunken necrotic lesions of different sizes and also brown discoloration in hypocotyl parts (Fig. 6). The results of the disease index led to significant differences in the virulence of isolates ($\text{LSD}_{0.05} = 0.46$) (Fig. 7). Disease severity ranged from 3.67 (Isolate A4) to 5 (Isolate T2) among all isolates. The majority of the tested isolates caused disease severity above the value of 4.50. The virulence of AG-4 HGI isolates varied from 4.25 to 5, while AG-4 HGII isolates were between 3.67 and 4.83. The isolates belonging to AG-4

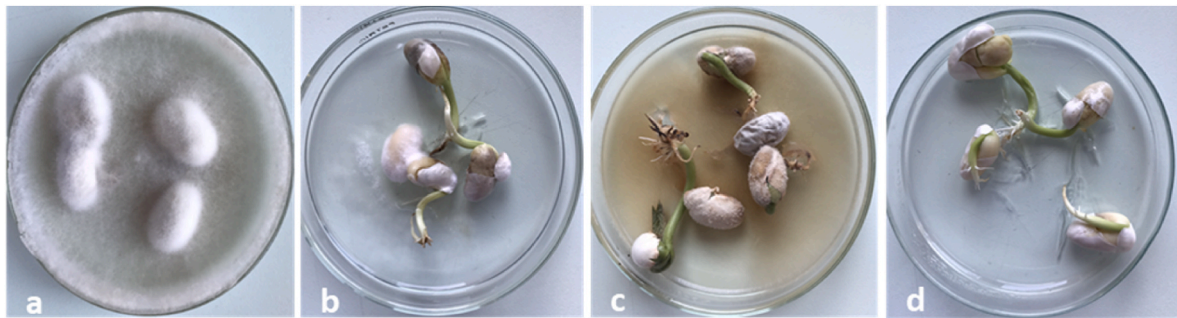


Fig. 6. Disease symptoms caused by different subgroups of *Rhizoctonia solani* AG-4 on cv. Gina in agar-plate assay (a: AG-4 HGI inhibited seed germination, b: AG-4 HGII occurred brown discoloration in the lateral roots, c: AG-4 HGIII caused brown discoloration in hypocotyl parts and slightly sunken necrotic lesions, d: Control).

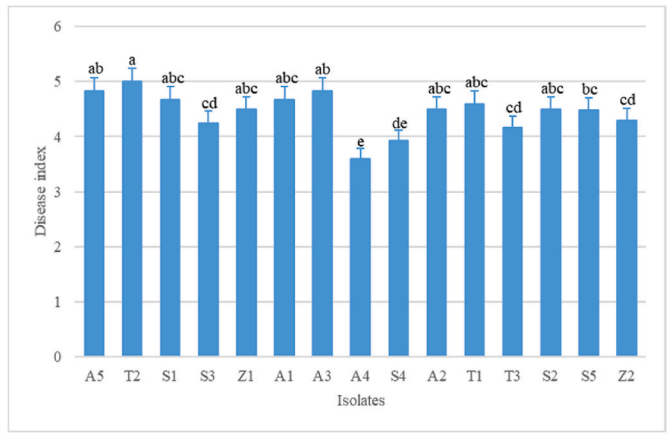


Fig. 7. Disease index of *Rhizoctonia solani* AG-4 isolates obtained from seeds of cv. Gina nine days after inoculation based on 1–5 scale of Muyolo et al. [47]. Values followed by the same letter are not significantly different ($P \geq 0.05$).

HGIII caused disease severity between 4.17 and 4.60. Based on these results, the most virulent isolates representing different AG subgroups were selected to determine the reaction of different common bean cultivars.

3.4. Host response of Turkish common bean cultivars to *Rhizoctonia solani* AG-4 subgroups

Pathogenicity tests revealed statistically significant differences in the disease severity of common bean cultivars against *R. solani* AG-4 subgroups ($LSD_{0.05} = 0.46$) (Table 3). The isolates belonging to different subgroups caused similar disease symptoms to that in the agar-plate assay. The isolate T2 belonging to AG-4 HGI showed high virulence on common bean cultivars and caused prevalently pre-emergence damping off. Severe brown necrotic lesions and deformations were observed in infected plants even if they germinated and formed roots and hypocotyls (Fig. 8a). Disease severity in common bean cultivars, caused by isolate T2 ranged from 4.1 (Nazende) to 5 (Seher yıldızı, Java, Boncuk, Sofia, Özayşe, Miray, Sarıkız, Perolar, Mina). Also, the isolate inhibited the growth of bean plants by 21–100% (Table 4).

The isolate A3, AG-4 HGII had the lowest rates of disease severity among subgroups in common bean cultivars (Table 3). As a result of infection with this isolate, cv. Miray showed the least disease symptoms with 2.4 of disease severity, while the highest value (4.0) was observed in cultivars Asya and Java. Disease severity among other common bean cultivars ranged from 2.6 to 3.6. The isolate caused mostly root infection rather than hypocotyl (Fig. 8b). The inhibition rate of plant height varied between 1.37 and 53.01 among cultivars, with an average of 17.79 (Table 4).

Table 3
Disease reaction of common bean cultivars to different subgroups of *Rhizoctonia solani* AG-4 (T1: AG-4 HGIII, T2: AG-4 HGI, A3: AG-4 HGII).

Bean cultivars	Bean Type	Disease severity index caused by pathogen isolates		
		T1	T2	A3
Zeynebim	Green	3.5 ± 0.23 ^{cd}	4.7 ± 0.12 ^{abcd}	2.7 ± 0.41 ^{cdef}
Seher yıldızı		2.8 ± 0.6 ^h	5.0 ± 0.00 ^{abcd}	3.0 ± 0.68 ^{bdef}
Karabacak		2.9 ± 0.23 ^{gh}	4.5 ± 0.00 ^{defg}	3.0 ± 0.45 ^{bdef}
Fransız		3.5 ± 0.3 ^{cdefgh}	4.6 ± 0.31 ^{cde}	2.6 ± 0.10 ^{ef}
Sazova		2.9 ± 0.41 ^{gh}	4.9 ± 0.12 ^{abc}	3.2 ± 0.46 ^{bde}
Boncuk		3.6 ± 0.2 ^{cdefg}	5.0 ± 0.00 ^a	3.0 ± 0.20 ^{bdef}
40 Günlük		3.6 ± 0.23 ^{cdefg}	4.6 ± 0.12 ^{cde}	3.2 ± 0.58 ^{bde}
Magnum		4.2 ± 0.2 ^{abc}	4.9 ± 0.12 ^{ab}	3.3 ± 0.50 ^{bde}
Asya		3.8 ± 0.34 ^{abcdef}	4.5 ± 0.00 ^{defg}	4.0 ± 1.00 ^a
Java		4.4 ± 0.11 ^{ab}	5.0 ± 0.00 ^a	4.0 ± 0.87 ^a
Volare		3.4 ± 1.05 ^{defgh}	4.4 ± 0.12 ^{efgh}	2.8 ± 0.17 ^{cdef}
Sofia		3.7 ± 0 ^{bdef}	5.0 ± 0.00 ^a	2.7 ± 0.17 ^{cdef}
Özayşe		4.1 ± 0.11 ^{abcd}	5.0 ± 0.00 ^a	3.4 ± 0.32 ^{abc}
Yalova 5		4.5 ± 0.34 ^a	4.2 ± 0.00 ^{gh}	3.3 ± 0.25 ^{bcd}
Yalova 17		3.9 ± 0.2 ^{abcde}	4.8 ± 0.12 ^{abc}	3.0 ± 0.58 ^{bdef}
Hanımteni	Kidney	3.3 ± 0.61 ^{efgh}	4.3 ± 0.50 ^{fgh}	2.8 ± 0.20 ^{cdef}
Miray		3.9 ± 0.61 ^{abcdef}	5.0 ± 0.00 ^a	2.4 ± 0.23 ^f
Gina		4.1 ± 0.6 ^{abcd}	4.5 ± 0.00 ^{defg}	3.6 ± 0.18 ^{ab}
Tavil		3.5 ± 0.3 ^{cdefgh}	4.9 ± 0.12 ^{abc}	3.3 ± 0.66 ^{bde}
Sarıkız		2.9 ± 0.34 ^{gh}	5.0 ± 0.00 ^a	2.9 ± 0.12 ^{bdef}
Perolar		2.9 ± 0.46 ^{gh}	5.0 ± 0.00 ^a	2.8 ± 0.38 ^{cdef}
Gelincik		3.8 ± 0.11 ^{abcdef}	4.7 ± 0.23 ^{abcd}	2.7 ± 0.68 ^{cdef}
Nazende		3.9 ± 0.11 ^{abcde}	4.1 ± 0.12 ^h	2.8 ± 0.25 ^{cdef}
Mina		3.4 ± 0.52 ^{defgh}	5.0 ± 0.00 ^a	2.7 ± 0.10 ^{cdef}
Sırık		3.2 ± 0.2 ^{efgh}	4.5 ± 0.50 ^{def}	3.4 ± 0.15 ^{abc}
Klas		3.8 ± 0.72 ^{abcdef}	4.9 ± 0.12 ^{ab}	2.9 ± 0.31 ^{bdef}
Belinay Sırık		3.1 ± 0.3 ^{fgh}	4.5 ± 0.20 ^{def}	2.9 ± 0.23 ^{bdef}
Selim		4.1 ± 0.57 ^{abcd}	4.9 ± 0.12 ^{abc}	2.6 ± 0.33 ^{def}
Buse Oturak		3.5 ± 0.75 ^{cdefgh}	4.7 ± 0.31 ^{bde}	2.6 ± 0.40 ^{def}
Sembol		3.5 ± 0.41 ^{cdefgh}	4.6 ± 0.00 ^{cde}	2.9 ± 0.43 ^{bdef}

*Values followed by the same letter are not significantly different ($P \geq 0.05$).

The isolate T1 selected from AG-4 HGIII caused general growth retardation, deformation and damping-off of common bean cultivars at different levels compared to control plants (Fig. 8c). Five cultivars (Seher Yıldızı, Karabacak, Sazova, Sarıkız, Perolar) exhibited disease severity below 3.0, while the disease severity in six cultivars (Yalova 5, Magnum, Java, Özayşe, Gina, Selim) was above 4 (Table 3). The isolate led to a decrease in the lengths of bean plants ranging from 1.1% to



Fig. 8. Disease symptoms caused by different subgroups of *Rhizoctonia solani* AG-4 on common bean cultivars (a: AG-4 HGI, b: AG-4 HGII, c: AG-4 HGIII, d: Control).

Table 4

The rate of % inhibition in plant heights caused by three *Rhizoctonia solani* AG-4 isolates (T1: AG-4 HGIII, T2: AG-4 HGI, A3: AG-4 HGII) on common bean cultivars 14 days after inoculation.

Bean cultivars	Bean Type	Isolate		
		T1	T2	A3
Zeynebim	Green	29.21	62.78	10.06
Seher yıldızı ^a		39.22	–	19.11
Karabacak ^a		24.07	–	38.72
Fransız		5.20	54.00	16.16
Sazova		17.59	40.59	30.43
Boncuk ^a		24.36	–	1.37
40 Günlük		29.02	58.13	3.24
Magnum		39.86	21.48	22.70
Asya ^a		51.46	–	7.50
Java ^a		66.30	–	53.01
Volare		26.14	57.14	2.80
Sofia ^a		3.38	–	25.27
Özayşe ^a		42.77	–	20.86
Yalova 5 ^a		71.63	–	13.19
Yalova 17		27.18	77.06	4.45
Hanımteni		16.31	57.30	17.25
Miray ^a		24.73	–	2.59
Gina ^a		42.06	–	40.57
Tavil ^a		29.56	–	5.42
Sarıköz ^a		34.13	–	23.47
Perolar ^a		24.47	–	23.66
Gelincik		30.14	49.35	4.06
Nazende		1.10	30.76	19.10
Mina ^a	Kidney	15.69	–	4.73
Sırık		32.46	41.85	25.65
Klas		34.13	67.47	14.71
Belinay Sırık		41.94	29.55	13.84
Selim		36.65	21.87	4.62
Buse Oturak		50.15	50.21	22.67
Sembol ^a		53.26	–	42.51

^a Plant heights could not be measured because the T2 isolate caused pre-emergence damping off.

66.3% (Table 4). The highest inhibition was seen in cv. Java with 66.3%, followed by Symbol (53.2%), Asya (51.4%) and Buse Oturak (50.1%). On the other hand, the least inhibition among cultivars was observed in Nazende, Sofia and Fransız, with rates varying between 1.1 and 5.2%. There was no cultivar resistant to all tested isolates considering all results. For example, Seher yıldızı, Sarıköz and Perolar showed moderately resistant reactions to T1 with below 3 scale value, while the same cultivars were susceptible to T2 with 5 scale value.

4. Discussion

Our results showed that all *R. solani* isolates obtained from common bean growing areas in different provinces of Turkey belonged to AG-4. This finding was in accordance with the previous reports indicated that *R. solani* AG-4 was the prevalent AG group in bean production areas in both Turkey and the world [16,17,52–56]. However, different researchers in Turkey and the world reported the presence of AG-1, AG-2-1, AG-2-2, AG-3, AG-5, AG-6, AG-7, AG-9, AG-10 and AG-11 in bean fields [11,16,21,25,57]. Eken and Demirci [17] detected seven anastomosis groups (AG-2-1, AG-3, AG-4, AG-5, AG-9, AG-10 and AG-11) in Erzurum province. Five anastomosis groups (AG-1, AG-4, AG-5, AG-6 and AG-7) of *R. solani* obtained from infected bean plants in Samsun were identified [52]. This study was the first to identify *Rhizoctonia* AGs associated with bean plants in Ankara, Tokat, and Zonguldak provinces, Turkey's important bean production areas. Ribosomal DNA-ITS sequencing provided molecular characterization of *R. solani* AG-4 isolates at the level of subgroups, as indicated in the study of Kılıçoğlu and Özkoç [24]. Similarly, many researchers used successfully sequence variability of ITS region for discrimination of AGs in different hosts such as chickpea, red cabbage, potato, brassica species, sugar beet, and ornamental plants [58–65].

A few sequence polymorphisms were observed between the isolates of the same subgroup and the reference isolates. This difference may be due to the plant composition grown in the same area and the anastomosis between closely related isolates [66,67]. Also, Sharon et al. [51] reported that the sequence similarity rates of isolates within the same AG ranged from 96 to 100%. The distribution of AG-4 subgroups showed differences according to the provinces. All AG-4 subgroups were present in Ankara and Samsun provinces, while AG-4 HG II was absent in Zonguldak and Tokat provinces. The results were in agreement with the study of Kılıçoğlu and Özkoç [23], who indicated that the common bean plants in the Black Sea region of Turkey were commonly infected by *R. solani* AG-4 HGI. Similarly, Spedaletti et al. [54] reported that most *R. solani* isolates obtained from bean seeds and soil in Argentina belong to AG-4, including AG-4 HG I and AG-4 HG III. Helmy et al. [68] informed that the most prevalent AG of *R. solani* on faba bean in Egypt was AG-4 HGI. Previous studies also explained that the ecological variation in different provinces affected the formation and prevalence of AGs according to regions [16,20,69,70].

Genetic variation of *R. solani* AG-4 isolates was also analyzed by iPBS retrotransposon, a novel universal DNA fingerprinting method. iPBS primers amplified 103 polymorphic bands that allowed the differentiation of AG-4 subgroups, which was similar to the classification obtained by rDNA sequencing. The results were in agreement with the studies of

Erper et al. [20] and Pourmahdi and Taheri [29], who classified AGs and subgroups of *R. solani* isolates from red cabbage and tomato based on iPBS markers, respectively. Pourmahdi and Taheri [29] examined the genetic variation of 56 isolates obtained from tomato plants. Four iPBS primers provided 106 amplification products, 94% of which were polymorphic. The researchers also stated that the iPBS analysis provided high resolution for distinguishing groups and subgroups of the pathogen. Similarly, iPBS-retrotransposon markers have been successfully used for genetic characterization of a wide range of plant pathogenic fungi [70–75]. To our knowledge, this research is the first characterization of *R. solani* AG-4 isolates obtained from common bean using iPBS retrotransposon markers.

Significant differences were observed among the virulence of the isolates, ranging from 3.6 to 5 based on the agar-plate method. Different researchers widely used this method for evaluating the virulence of *Rhizoctonia* spp. isolates [3,30,47,61]. Also, disease symptoms showed differences among the isolates belonging to distinct AG subgroups. Differences in virulence and symptoms between AG groups have been observed by other researchers [3,16–18,52,66,76]. Balali and Kowsari [77] found that AG-4 and AG-2-2 caused damping-off in the early stage of plants, while they caused severe necrosis of the stem and root tissues in the later stages. Similarly, the virulence of *Rhizoctonia* spp. isolates may vary depending on inoculum density, inoculation type, host resistance, and environmental conditions [47,52,78,79].

The control of *R. solani* was quite challenging to manage due to its high pathogenic and genetic variability, wide host range, and the ability for long-term survival in soil [80,81]. Thus, many studies have been carried out to interpret the reaction of common bean cultivars to different AG groups of *R. solani*, and few cultivars or varieties have been recorded as moderately resistant to some AG groups [11,16–18,52,82]. The present study evaluated the disease reaction of common bean cultivars against the most aggressive isolates representing AG-4 subgroups selected as a result of *in vitro* assay. The pathogenicity studies showed significant differences among the reactions of common bean cultivars depending on the pathogen isolates. In the AG-4 HGI, the T2 isolate was the most aggressive on the cultivars, and no cultivar showed resistance to the pathogen. The lowest disease severity value was observed against the A3 isolate of AG-4 HGII, and Zeynebim, Sofia, Fransız, Boncuk and Miray cultivars showed moderate resistance to the pathogen. Yalova-5, Magnum, Java, Özyaşe, Gina, and Selim cultivars were susceptible to the T1 isolate belonging to AG-4 HGIII, while Seher yıldızı, Karabacak, Sazova, Sarıkız, and Perolar were moderately resistance bean cultivars. Considering all the results, no cultivar showed a resistant reaction against all isolates. In evaluating 6 common bean cultivars to 5 isolates belonging to AG-4, AG-2-2, and AG-5 groups, Karaca et al. [16] reported that none of them were resistant to the pathogen. The disease rate changed depending on the isolate or AGs. The cultivar Horoz was the most susceptible, while Özyaşe was the most resistant. This study regarded Özyaşe and Yalova-5 cultivars as resistant and susceptible to AG-4 isolates, respectively. These findings supported that the host reaction of common bean cultivars was changeable according to the isolates or subgroups of *R. solani*. Similar results were reported by Omar et al. [82], who screened 13 common bean cultivars against *R. solani* AG-4 isolate in Nevşehir province. All cultivars, including Miray and Sarıkız, used in our study were reported to be susceptible to the pathogen.

In conclusion, the present study revealed the characterization and genetic diversity of *R. solani* AG-4 subgroups obtained from common bean in Turkey and the response of common bean cultivars against the pathogen isolates. The iPBS retrotransposon analysis was a simple, reliable and effective tool for analyzing the subgroups of *R. solani* and was compatible with rDNA-ITS sequencing. Also, the pathogenicity results showed that the most aggressive among the subgroups of *R. solani* was AG-4 HGI, followed by HGIII and HGII, respectively. Moreover, none of the tested cultivars was resistant to the subgroups. Thus, future studies should be conducted for integrated control methods, including

the application of chemical and biological fungicides, crop rotation, and the breeding of resistant varieties to control *R. solani*.

CRediT authorship contribution statement

Gülsüm Palacıoğlu: Writing – original draft, Methodology, Investigation, Formal analysis. **Beyza Cankara:** Methodology, Investigation. **Harun Bayraktar:** Writing – review & editing, Visualization, Supervision, Formal analysis. **Göksel Özer:** Writing – review & editing, Supervision, Formal analysis.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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