Molecular Characterization of Some Saprophyte Agrobacterium Strains Isolated from Root Nodules of *Cicer arietinum* L. Cultivated in Central Anatolia Region of Turkey

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Abstract: In the present study, twelve plant-associated bacteria which isolated from root nodules of *C. arietinum* L. collected from Central-Anatolia region of Turkey were identified with current molecular techniques. None of the isolates produced root nodules or showed pathogenic effects (gall or hairy root) on the original host as the result of authentication and pathogenicity tests, respectively. These results have suggested that all these isolates are root endophytic bacteria. Additionally, PCR amplifications for nodD and virA genes did not reveal any bands. These results showed that these isolates have not to harbour symbiotic (pSym) or pathogenicity (Ti, Tumour inducing or Ri, Root inducing) plasmids which are necessary for nodulation and virulence, respectively. TP-RAPD analysis revealed three patterns indicating three genetically distinct groups within the isolate collection. From each pattern, one representative isolate was selected for further molecular analyses. Phylogenetic analyses depending on nucleotide sequences of 16S rDNA and recA genes assigned representative isolates of Pattern-A (*n*: 4) and Pattern-B (*n*: 6) as *A. radiobacter*. On the other hand, the representative isolate of Pattern-C (*n*: 2) appeared as related to *A. nepotum*. As a result, this study presents the first phylogenetically identified root-endophytic *Agrobacterium radiobacter* and *A. nepotum* isolates from root nodules of *C. arietinum* L. grown in Central Anatolia part of Turkey. Additionally, the first molecular data of *A. radiobacter* for Turkey also presented.

Keywords: *Agrobacterium*, Chickpeas, Nodulation, Phylogeny, Virulence.

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**Türkiye’nin İç Anadolu Bölgesinde Yetiştirilen *Cicer arietinum* L.’nin Kök Nodüllerinden İzole Edilmiş Bazı Saprophyt *Agrobacterium* Suşlarının Moleküler Karakterizasyonu**

Bu çalışmada Türkiye’nin İç Anadolu bölgesinden toplanan *C. arietinum* L. bitkisinin kök nodüllerinden izole edilmiş on iki farklı ilişkili bakteri moleküler teknikler kullanarak teşhis edilmiştir. Bu izolatların hiç birisi hırçın otantıksayı ve patojenite testlerinin sonucunda orijinal konak üzerinde nodül oluşturmuş veya patojenik etki (gall oluşumu veya saçak kök oluşumu) göstermemiştir. Bu sonuçlar bu izolatların kök endofitik bakteriler olduğunu ortaya koymıştır. Ek olarak nodD ve virA genleri için yapılan PCR amplifikasyonları herhangi bir bant ortaya koymamıştır. Bu sonuçlar bazı izolatların nodülasyon veya virülans için gerekli olan srasıyla simbiyotik (pSym) veya patojenite (Ti: Tümör oluştururan veya Ri: Saçak kök oluştururan) plazmidlerini taşımadıklarını göstermiştir. TP-RAPD analizi izolat koleksiyonu içerisinde genetik olarak ayrık üç gruba işaret eden üç şablon ortaya koymıştır. İleri moleküler analizler için her şablonda bir temsili izolat seçilmişdir. 16S rDNA ve recA genlerinin nükleotid dizilerine dayalı filogenetik analizler Şablon-A (*n*: 4) ve Şablon-B (*n*: 6) temsili izolatları *Agrobacterium radiobacter* türü ile ilişkilendirmiştir. Diğer tarafından, Şablon-C’nin (*n*: 2) temsili izolat *A. nepotum* olarak ortaya çıkmıştır. Sonuç olarak bu çalışma Türkiye’nin İç Anadolu Bölgesinde yetiştirilen *C. arietinum* L.’nin kök nodüllerinden filogenetik olarak tanımlanmış ilk kök endofitik *Agrobacterium radiobacter* ve *A. nepotum* izolatlarını sunmaktadır. Ek olarak, *A. radiobacter* için Türkiye’den ilk moleküler verileri de sunmaktadır.

**Anahtar kelimeler:** *Agrobacterium*, Filogeni, Nodülasyon, Nohut, Virülans.
INTRODUCTION

The genus Agrobacterium (Class: Alphaproteobacteria, Order: Rhizobiales, Family: Rhizobiaceae) has comprised of gram negative soil borne bacteria with various life strategies including saprophytism, parasitism (causes crown gall or hairy root diseases) and mutualism (induces root nodules on legume plants) (Zahradnik et al., 2018). Since it was first proposed by Conn, (1942) many changes have been taken place in Agrobacterium systematics both in generic and species levels. Till the end of 20th century, Agrobacterium strains were classified within species A. radiobacter, A. tumefaciens, A. rhizogenes, A. rubi and A. vitis. In the last twenty years, as the result of investigations of new strains obtained from different hosts and locations many other species and genomic species (e.g. A. larrymoorei, A. albertimagni, A. arsenijeveci, A. deltaense, A. salinolerans, A. rosae and A. bohemicum) have been added to the genus (Bouzar et al., 1995; Salmassi et al., 2002; Karakaya & Özcan, 2000; Akbulut et al., 2008). On the other hand, there are only a couple of studies on the species diversity of Agrobacterium and the diseases they caused, in Turkey. Argun et al., (2002) characterized some Agrobacterium vitis (currently Alorhizobium vitis) samples collected from grape cultivars in the Central Anatolia region of Turkey using some PCR based molecular techniques (ITS RFLP) besides biochemical features. Aysan & Sahin, (2003) reported a crown gall diseases to outbreak caused by A. tumefaciens on rose, from the Mediterranean part of Turkey.

Additionally, studies on biotechnological applications like Agrobacterium-mediated transformation of economically important plants, including chickpeas, are also common (Karakaş & Özcan, 2000; Akbulut et al., 2008). The purity of the isolates was examined using gram staining. Nodulation ability of the isolates on their original host at 14h light and 10h dark and with three replicates for each isolate. Chickpeas (Cicer arietinum L.), the host plant investigated in the study, is the second most cultivated pulse crop in the World and it is in the first rank in Turkey (FAOSTAT 2016; Gülümser, 2016). Although some strains of Agrobacterium causes crown gall or hairy root diseases on many host plants, there have been no records for the Chickpeas so far. One Agrobacterium species, A. pusense, isolated and identified from rhizosphere of chickpea but there is no data for its pathogenicity capability on the host (Panday et al., 2011). Contrarily, some strains of the genus Agrobacterium have been reported as root-nodulating symbionts of chickpeas from several countries like Tunisia and Iran (Saidi et al., 2011; Rouhrazi & Khodakaramian, 2015). Additionally, some root endophytic Agrobacterium isolates, which are not able to produce root nodules when reinoculated to the original host, were reported from root nodules of C. arietinum L. (Romdhane et al., 2009). Despite their ecological and economical importance, there are few studies on Agrobacterium in Turkey when compared to the World. Most of these studies are about some in-vivo applications to determine the susceptibilities of various crops including chickpeas, against pathogenic Agrobacterium strains (mostly foreign strains). Additionally, studies on biotechnological applications like Agrobacterium-mediated transformation of economically important plants, including chickpeas, are also common (Karakaş & Özcan, 2000; Akbulut et al., 2008). On the other hand, there are only a couple of studies on the species diversity of Agrobacterium and the diseases they caused, in Turkey. Argun et al., (2002) characterized some Agrobacterium vitis (currently Alorhizobium vitis) samples collected from grape cultivars in the Central Anatolia region of Turkey using some PCR based molecular techniques (ITS RFLP) besides biochemical features. Aysan & Sahin, (2003) reported a crown gall diseases to outbreak caused by A. tumefaciens on rose, from the Mediterranean part of Turkey.

The goal of this study is to characterize some Agrobacterium isolates obtained from root nodules of C. arietinum L. collected from five cities of the Central Anatolia region of Turkey with current molecular techniques.

MATERIAL AND METHOD

In this study, Cicer arietinum L. plants were collected from five different cities of Central Anatolia region of Turkey during May-August of 2015-2018 (Table 1). Isolations of the bacteria from root nodules were performed according to the method of Vincent, (1970). Standard YMA (Yeast Extract Mannitol Agar) medium was used both for isolations and purifications (by consecutive inoculation), of the bacteria (Vincent, 1970). The purity of the isolates was checked by colony morphology and microscopic examinations using gram staining. Nodulation tests were performed using the method of Vincent, (1970), to determine the nodulation capability of the isolates on their original host (C. arietinum L.). Tests were performed in a growth chamber at 14h light and 10h dark and with three replicates for each isolate. Nodulation ability of the isolates were evaluated after 5 weeks of incubation. Pathogenicity tests, to visualize...
gall and hairy-root formation, were performed according to the method of Karakaya & Özcan, (2000). Tests were performed with three replicates for each isolate with the following modification; a growth chamber at 14h light and 10h dark was used for the tests, YMB medium was used for growing the bacterial isolates as inoculum.

Table 1. Source information, TP-RAPD profiles and GenBank accession numbers of Agrobacterium isolates obtained in this study.

| Isolate | Locality | TP-RAPD Profile | GenBank Accession Numbers |
|---------|----------|-----------------|--------------------------|
| Agro-Ca-1 | Ankara/Kırıkkale | A | MT91269, MT919277 |
| Agro-Ca-2 | Ankara/Hatay | A | - |
| Agro-Ca-3 | Konya/Kulu | A | - |
| Agro-Ca-4 | Konya/Beyşehir | A | - |
| Agro-Ca-5 | Şirvan/Vidyeli | B | MT912700, MT919278 |
| Agro-Ca-6 | Şirvan/Uşak | B | - |
| Agro-Ca-7 | Ankara/Nallihan | B | - |
| Agro-Ca-8 | Kayseri/Manisa | B | - |
| Agro-Ca-9 | Kayseri/Ceyhanlı | B | - |
| Agro-Ca-10 | Konya/Ezili | B | - |
| Agro-Ca-11 | Kayseri/Kızılapınar | C | MT912701, MT919279 |
| Agro-Ca-12 | Konya/Yahyalı | C | - |

Fresh bacterial cultures for the genomic DNA extractions were prepared using TY (Tryptone Yeast Extract) broth media (Ditta et al., 1987) with the conditions explained in Gurkanli et al., (2013). Genomic DNA extractions from broth cultures were conducted using CTAB/NaCl miniprep method and extracted DNA was stored in -20°C prior to use (Temizkan & Arda, 2004). A TP-RAPD (Two primers random amplified polymorphic DNA) analysis was performed for discriminating between the distinct species (or genotypes) within our isolate collection.

The analysis was performed using the primer set 879F / 1522R (Rivas et al., 2001) with conditions given in Table 2. PCR amplifications were performed using a Technie TCP-PLUS (Bibby Scientific, Stone, UK) thermal cycler. Presence of virulence (virA, protein kinase) and nodulation (nodD, autoregulatory) genes were investigated in the bacteria to determine the symbiosis type (mutualism or parasitism) between the bacteria and the host plant, C. arrietinum L. Primer sets, VirA1F/virA2R (Velazquez et al., 2005) and Y5/Y6 (Zeze et al., 2001) were used for PCR amplifications of virA and nodD, respectively. The conditions used for PCR amplifications are given in Table 2. Consequently, one representative isolate from each TP-RAPD pattern observed was chosen for the further molecular analyses. The symbiosis types and geographical sources of the isolates were also considered as a criterion during the selection process. Characterizations of the representative isolates were conducted with phylogenetic analyses depending on nucleotide sequences of, 16S rDNA and a housekeeping gene, recA. Primer sets, fd1 / rd2 (Zhang et al., 1999) and recA-Forward / recA-Reverse (Gaunt et al., 2001) were used for PCR amplifications of 16S rDNA and recA genes, respectively. The conditions used for both PCR amplifications are stated in Table 2.

Table 2. Primers and PCR conditions used for amplifications of genes analysed in this study.

| Gene | Primer | ID | C | D | A | E | FE |
|------|--------|----|---|---|---|---|---|
| TP-RAPD analysis | 879F/1522R (Rivas et al., 2001) | 95°C 5 min | × 35 | 94°C / 1 min | 45°C 1 min | 72°C / 2 min | 72°C / 10 min |
| 16S rDNA | pApF (Zhang et al., 1999) | 95°C 3 min | × 35 | 94°C / 1 min | 55°C 1 min | 72°C / 1 min | 72°C / 5 min |
| recA | recA-Forward / recA-Reverse (Gaunt et al., 2001) | 95°C 3 min | × 35 | 95°C / 45 s | 50°C 1 min | 72°C / 2 min | - |
| virA | VirA1F/virA2R (Velazquez et al., 2005) | 95°C 5 min | × 35 | 94°C / 1 min | 51°C 1 min | 72°C / 75 sec | 72°C / 10 min |
| nodD | Y5/Y6 (Zeze et al., 2001) | 95°C 5 min | × 35 | 94°C / 1 min | 55°C 1 min | 72°C / 1 min | 72°C / 10 min |

*ID*: Initial denaturation, *C*: Numbers of Cycles, *D*: Denaturation, *A*: Annealing, *E*: Elongation, *FE*: Final elongation.

A 50 µl PCR reaction for all amplifications was prepared with, 25 µl of GoTaq® Colorless Master Mix, 2X (Promega Corporation, Madison, WI, USA), genomic DNA=0.5 µg, 0.4 pmol of each primer (in final concentration) and sterile ddH2O (up to 50 µl). PCR products were electrophoresed on 1% (2.5% for TP-RAPD) agarose gel prepared with 1X TBE buffer and visualized using a Photo-Print Digital Imaging System (Vilber Lourmat, France).

Nucleotide sequencings for 16S rDNA and recA genes of the selected isolates were performed commercially by Macrogen Europe (Amsterdam, The Netherlands) with the same primers used for PCR amplifications. Nucleotide sequences obtained from both strands were assembled using BioEdit (Hall, 1999). For phylogenetic analyses, data sets were created for each of 16S rDNA and recA genes using the haplotypes (downloaded from GenBank) given in legends of Figures - 2 and -3, together with our new sequences (Table 1). On the other hand, most Agrobacterium isolates (recA and 16S rDNA sequences) in GenBank are still remaining with the older nomenclature (e.g. Rhizobium nepotum, Rhizobium pusense, Rhizobium skiernewicense etc.) and this situation have causing some confusions, thus should be revised by the original submitters. ClustalX (Thompson et al., 1997) was used for multiple nucleotide sequence alignments. To determine the best fitting substitution models for our data sets, Akaike’s information criterion (AIC) and Bayesian information criterion (BIC) tests were performed using the jModelTest v. 0.1 program (Guindon & Gascuel, 2003; Posada, 2008). Neighbor-joining (NJ), Maximum parsimony (MP) and Maximum likelihood (ML) algorithms were used to determine the phylogenetic relationships among haplotypes. NJ and MP analyses were performed using software program PAUP* v. 4.0b10 (Swofford, 1998) and PhyML 3.0 (Guindon & Gascuel, 2003) was used for the ML analyses. MP analyses were performed using a heuristic search approach with the TBR
swapping algorithm (10 random repetitions). To test the
reliability of the nodes on the trees, Bootstrap tests were
performed with 10000 replications for all analyses.

All new sequences obtained in this study were
deposited in the NCBI data bank under accession numbers
MT912699-MT912701 and MT919277-MT919279 (Table
1).

RESULTS

As the result of isolations, twelve bacterial
isolates were obtained from root nodules of  C. arietinum
L. collected from 5 cities of Central Anatolia part
of Turkey (Table 1). Although the isolates were collected
from active root nodules of  C. arietinum L., none of them
produced root nodules on the original host in the
nodulation tests. Moreover, PCR amplification for nodD
gene from these twelve isolates did not reveal any product.
Similarly, none of the twelve isolates induced tumour-like
structures (galls) on the stems or caused root diseases
(hairy-root) on  C. arietinum L in pathogenicity tests.
Additionally, PCR amplification from these isolates for
virulence gene,  virA, did not produce any DNA band.
Results of TP-RAPD analysis revealed three patterns
suggesting three genetically different groups within our
isolate collection (Figure 1, Table 1). From each TP-RAPD
pattern, also considering the geographical location,
follow ing representative isolates were selected for further
molecular analyses; from Pattern-A: Agr-Ca-1, Pattern-B:
Agr-Ca-5 Pattern-C: Agr-Ca-11.

As the result of nucleotide sequencings, the
variative thus informative first half of 16S rDNA (approx.
900 bp) were obtained from isolates, Agr-Ca-1, Agr-Ca-5
and Agr-Ca-11. Results of BLAST (Basic Local
Alignment Search Tool, https://blast.ncbi.nlm.nih.gov/Blast.cgi) search for our new
16S rDNA haplotypes revealed that all isolates obtained in
this study are belonging to the genus  Agrobacterium. Thus,
a data set was created with the 16S rDNA haplotypes
belonging to type strains of available  Agrobacterium
species and Genomovars (see legend of Figure 2).
Phylogenies were constructed using 850 aligned
nucleotides with 76 variable sites. AIC and BIC tests
suggested the GTR+I+G (I: 0.785, G: 0.624) and TrN+I+G
(I: 0.79; G: 0.655) substitution models, respectively.
Because of their higher bootstrap values, NJ (Figure 2) and
ML (only the bootstrap values are given in Figure 2) trees
created using the GTR+I+G model were prefered in the
study. MP analysis was depending on 56 symapomorphic
sites and yielded 24 equally most parsimonious trees with
117 steps in length (CI: 0.717949, RI: 0.852018, HI:
0.282051). Bootstrap values derived from MP analysis also
given on Figure 2.

Figure 1. TP-RAPD patterns of  Agrobacterium isolates obtained
in this study. 1) Agr-Ca-1, 2) Agr-Ca-2, 3) Agr-Ca-3, 4) Agr-Ca-4, 5) Agr-Ca-
5, 6) Agr-Ca-6, 7) Agr-Ca-7, 8) Agr-Ca-8, 9) Agr-Ca-9, 10) Agr-Ca-10, 11) Agr-
Ca-11, 12) Agr-Ca-12, 13) 1 kb DNA Ladder (Norgen), 14) Negative control.

Figure 2. NJ tree derived from nucleotide sequences of 16S
rDNA haplotypes obtained in this study (Bold) and the ones
downloaded from GenBank (given with GenBank accession
numbers). The tree has rooted with  recA haplotypes of  E.
terargaue and  E. kummerowiae. The bootstrap values (≥50%)
derived from NJ, ML and MP analyses have stated on the tree
with the same order.

Lee et al., (Unpublished); Bautista-Zapanta et al., (2009); Kuzmanovic et al.,
(2018); Yan et al., (2017a); E Mougel et al., (Unpublished); F Register & Williams
(Unpublished); G Van et al., (2017b); Pandonay et al., (2011); H Bouzar et al., (1995); I
Goodner et al., (2001); J Mousavi et al., (2015); K Pulawska et al., (2012a);
L Zapanta et al., (2009); M Willems & Collins, (1993); N Pulawska et al., (2012a);
O Pulawska et al., (2012b); P Kuzmanovic et al., (2015a); Q Webber et al., (2011);
R Salmass et al., (2002); S De Lajudie et al., (1994); T Wei et al., 2002.
Although they showed different TP-RAPD patterns, isolates Agr-Ca-1 (representative of Pattern-A) and Agr-Ca-5 (representative of Pattern-B) showed the same 16S rDNA haplotype with each other and also with *A. radiobacter* isolates, IAM 12848\(^8\) (Type strain), B6 and *Agrobacterium* Genomovar 3 isolate CIP107443. This lineage was supported with 84% and 85% bootstrap values in NJ and ML trees, respectively. The lineage composed of *Agrobacterium* Genomovars -5, -7, -13 and *A. deltaense* isolate YIC4121\(^1\), which showed the same 16S rDNA haplotype with each other, appeared as sister to the lineage above (Figure 2). The third isolate, Agr-Ca-11, showed the same 16S rDNA haplotype with *A. nepotum* type strain 39/7\(^2\). The relationship between these two haplotypes was supported with 90%, 88% and 78% bootstrap values in the NJ, ML and MP trees, respectively. *A. arsenijevicii* type strain KFB 330\(^3\) and *Agrobacterium* Genomovar 1 isolate H13.3 also appeared in the same lineage with Agr-Ca-11 (Figure 2). In general, relationships within this lineage were supported with significant bootstrap values (≥65).

To assign our selected isolates to one of the valid *Agrobacterium* species (or genovars), approximately 570 bp of *recA* gene were obtained and subjected to phylogenetic analyses. The data set used for phylogenetic analyses was containing the *recA* haplotypes of type strains of available *Agrobacterium* species. Additionally, *recA* haplotypes of isolates representing available *Agrobacterium* Genomovars as well as *recA* haplotypes some *A. radiobacter* and *A. nepotum* isolates also included to the data set (see legend of Figure 3). Analyses were carried out on 373 aligned nucleotides containing 135 variable sites. Both AIC and BIC tests suggested TIM2+I+G (I: 0.513; G: 0.795) substitution model. MP analysis which gave 17 most parsimonious trees (Length: 458 steps, CI: 0.423581, RI: 0.667925, HI: 0.576419) was used. The ing TIM2+I+G model was given in Figure 3 and analyses have also stated on the tree. The bootstrap values (≥50%) derived from ML and MP algorithms (Figure 3) showed the same 16S rDNA phylogeny. This relationship has supported with 100% bootstrap values in all trees created using NJ, ML and MP algorithms (Figure 3). Nucleotide sequence similarity between Agr-Ca-11 and the type strain of *A. nepotum* (isolate 39/7\(^3\)) was determined as 97.8%.

Figure 3. NJ tree derived from nucleotide sequences of *recA* haplotypes obtained in this study (Bold) and the ones downloaded from GenBank (given with GenBank accession numbers). The tree has rooted with *recA* haplotypes of *E. kummerowiae* and *E. fredii*. The bootstrap values (≥50%) derived from ML and MP analyses have also stated on the tree.

\(^{1}\)Yan et al., (2017a); \(^{2}\)Costecharrey et al., (Unpublished); \(^{3}\)Yan et al., (2017b); \(^{4}\)Kuzmanovic et al., (2015a); \(^{5}\)Goodner et al., (2001); \(^{6}\)Hao et al., (2012); \(^{7}\)Palawiska et al., (2012b); \(^{8}\)Mafakheri et al., (2019); \(^{9}\)Kuzmanovic et al., (2015b); \(^{10}\)Wiberg et al., (2011); \(^{11}\)Kuzmanovic et al., (2018); \(^{12}\)Zahradnik et al., (2018); \(^{13}\)Katano et al., (Unpublished); \(^{14}\)Shams et al., (2013); \(^{15}\)Trumble et al., (2012); \(^{16}\)Lloret et al., (2007); \(^{17}\)Martens et al., (2007).

**DISCUSSION**

During a survey for rhizobia which nodulates *C. arietinum* L. in the Central Anatolia region of Turkey, a group of (n: 12) bacteria that morphologically dissimilar (much more mucoid and opaque) to classical rhizobia were obtained from root nodules of *C. arietinum* L. TP-RAPD analysis which is a commonly used pre-grouping method in bacteriological studies (Rivas et al., 2001), revealed three patterns within our collection which indicated three

\[ \text{Figure 3. NJ tree derived from nucleotide sequences of } \text{recA} \text{ haplotypes obtained in this study (Bold) and the ones downloaded from GenBank (given with GenBank accession numbers). The tree has rooted with } \text{recA} \text{ haplotypes of } E. \text{kummerowiae and } E. \text{fredii}. \text{ The bootstrap values (≥50%) derived from ML and MP analyses have also stated on the tree.} \]
genetically distinct groups. Phylogenetic analyses depending on nucleotide sequences of two genetic markers, 16S rDNA and recA, designated representative isolates of first two TP-RAPD patterns, Pattern-A (Agr-Ca-1) and Pattern-B (Agr-Ca-5) as A. radiobacter. Although some A. radiobacter isolates have reported from Turkey so far, they were all clinical strains causing infections in humans (Otağ et al., 2007). Thus according to the available literature, there has been only one soil-borne *Agrobacterium* species, *A. tumefaciens*, reported from Turkey (Aysan & Sahin, 2003; Aysan et al., 2003). On the other hand, identifications of the bacteria in all these studies were depending on some morphological and biochemical features, thus were highly doubtful. That is why, isolates, Agr-Ca-1 and Agr-Ca-5, in this study are the first properly (with current molecular phylogenetic techniques) identified soil-borne *A. radiobacter* isolates from Turkey. Phylogenetic analyses designated isolate Agr-Ca-11, the representative isolate of TP-RAPD Pattern-C, as *A. nepotum*. This species was first described by Pulowska et al., (2012b) from tumours on several plant species in Europe. Since then, it has been reported from several countries as pathogen or root nodule endophytes (Mafakheri et al., 2019; El Yemlali, Unpublished). In GenBank, there is one 16S rDNA sequence, submitted from Gaziantep University (Gaziantep-Turkey), belonging to a nodule endophytic *A. nepotum* strain which isolated from root nodules of *C. arietinum* L. in Turkey. On the other hand, there is no available publication on the strain, thus its exact origin and other features are unknown. But together with our finding, it may indicate that *A. nepotum* is one of the common nodule endophytic *Agrobacterium* species in chickpea fields of Central and Southeastern Anatolia. But this presumption must be confirmed with comprehensive investigations. In authentication and pathogenicity tests, none of the twelve isolates produced root nodules or disease symptoms on chickpeas. Additionally, no appropriate DNA bands were observed as the result of nodD and virA amplifications. These results have proved the absence of the genetic accessories necessary for the nodulation or virulence processes in these isolates therefore they all are nodule-endophytes (non-nodulating). Presence of nodule-endophytic bacteria in root nodules have been reported from various legume species including *Melilotus dentatus*, *P. vulgaris* L. and *C. arietinum* L. (Mhamdi et al., 2005; Wang et al., 2006; Ben Romdhane et al., 2009). Although the mechanism underlying this biological phenomenon is unknown, Ben Romdhane et al., (2009) suggested that water deficiency conditions induce the colonisation of chickpea nodules by non-symbiotic or non-specific *Rhizobium* and *Agrobacterium* isolates. If we consider the geographical and climatic features of Central Anatolia during the nodulation period of chickpea seedlings (approx. during June-July), a water deficiency condition is highly possible. This unfavourable situation possibly may have caused the infiltration of nodule-endophytic *Agrobacterium* isolates to chickpea root nodules. Although these bacteria are avirulent (gives no direct harm to the plant) it may indirectly reduce the crop productivity by competing with the actual rhizobial partner of *C. arietinum* L. in the rhizosphere. In this context, it has been reported that *Agrobacterium* isolates may reduce the nodulation of *P. vulgaris* L. by its rhizobial partner, *Rhizobium gallicum* (Mrabet et al. 2006).

As conclusion, this study presents the first root-endophytic *Agrobacterium radiobacter* and *A. nepotum* isolates, which identified using proper molecular phylogenetic techniques, from *C. arietinum* grown in Central Anatolia part of Turkey. Additionally, it gives the first molecular data for *A. radiobacter* for Turkey.

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482
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