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CHARACTERIZATION OF ITS1 SECONDARY STRUCTURE IN TEN SPECIES OF COLUTEOCARPEAE (BRASSICACEAE) AND ITS TAXONOMICAL UTILITY

KURTULUŞ ÖZGİŞİ

Department of Biology, Faculty of Science and Letters, Eskişehir Osmangazi University, Eskişehir, Turkey

ABSTRACT. Utility of the internal transcribed spacers (ITSs) of ribosomal RNA sequences to infer phylogenetic relationships among organisms have been proven. Although ITS1 and ITS2 are highly variable in sequence, they have conserved structures that have a key function in the processing of rRNA gene transcripts. Determining of such a conserved motif can help to identify relationships between organisms. Since ITS2 has much more conserved secondary structure, structural properties of ITS1 are generally neglected by researchers. In this study, ITS1 secondary structures of ten representative species, which were once assigned under different genera, of tribe Coluteocarpeae were determined. Also taxonomical utility of ITS1 secondary structure was also tested. Analyses indicate that there are four different types (4-, 6-, 7- and 8 hairpin) of secondary structures. On the other hand, each sequences have a conserved region that is common among land plants. Since previous studies reveals other species, that belong different tribes or lineages of Brassicaceae show similar ITS1 secondary structure, it is not a useful delimitation tool for investigated species in terms of taxonomy.

1. INTRODUCTION

Advanced technology in molecular studies provide new insights on systematic studies [1]. Limited number of orthologous sequences are widely used to infer phylogenetic relationships of organisms. Because of its ubiquitous presence; sufficient synapomorphic characters and costeffectiveness, the internal transcribed spacers (ITSs) of ribosomal RNA have been frequently used as a molecular marker for phylogenetic studies in plants including several taxonomic levels [2].

Keyword and phrases. Coluteocarpeae, Brassicaceae, secondary structure, ITS1

kurtulusozgisi@gmail.com
0000-0002-7344-6666

Ribosomal RNA gene cluster composes seven different components. Three (18S, 5.8S, and 26S) of them are rRNA gene regions that are processed to produce the mature RNAs. These highly conserved rDNA exons are preceded by an external transcribed spacer and each gene are separated by two internal transcribed spacers (ITS1 and ITS2), respectively [3].

Although mature cytoplasmic ribosomes do not contain ITS regions, they have important roles in maturation of rRNA. For example, blocking the formation of 40S subunits is depend on the deletion of the central portion of ITS1 [4]. Also deletions in the 5'-terminal region of ITS2 block the maturation of 26S rRNA. These processes are highly related with the specific cleavage and a conserved secondary structure of ITS regions are necessary for the maturation of the ribosomal subunits. Although ITS regions can have a high mutation rate, due to point mutations and indels, conservation of the secondary structure is critical for rRNA processing [5].

The conserved characteristics of ITS regions are also used to infer the boundaries of the species [6]. Nucleotide changes at both sides of the paired bases, called compensatory base changes (CBCs), in ITS2 secondary structure is widely used to detect the relationships between species [7]. Experimental study of Coleman and Vacquier [8] indicate that CBCs (even by just 1 CBC) in the conserved pairing positions of the ITS2 secondary structure block intercrossing among taxa. Since this finding is compatible with biological species concept, CBCs in the ITS2 secondary structure are used as a delimitation tool by many researchers [9, 10, 11].

Contrary to ITS2, high number of possible ITS1 secondary structures makes this region less informative than ITS2 [12] in terms of detecting the relationships between organisms. Nues et al. [13] showed that ITS1 secondary structure is not folded into a compact formation whereas ITS2 tightly folded than ITS1. But utility of ITS1 secondary structure at different taxonomical ranks was tested by different studies and considered as a useful tool to separate different groups [12, 14].

In present, It is aimed to characterize the ITS1 secondary structure of representatives of the tribe Coluteocarpeae (Brassicaceae).Tribe Coluteocarpeae is a member of Expanded Lineage II which is one of the four main Brassicaceae lineages (Aethionemeae, I, expanded II, and III). According to some authors [15, 16, 17, 18], tribe consists of many different genera whereas some [19, 20] indicate that these genera should be assigned under genus *Noccaea* Moench. The members of the genus were treated within the genus *Thlaspi* L. until the studies of Meyer [15, 16] who divided *Thlaspi* into 12 segregate genera. But many researchers [21, 22, 23] rejected Meyer’s concept and the genus systematics retained its complexness until the generic

study of Al-Shehbaz [19]. Al-Shehbaz [19] suggested an expanded *Noccaea* concept and treated all of the species and segregates under the generic name *Noccaea* with the exception *Thlaspi s.str* and *Noccidium.* Although phylogenetic studies of Özüdoğru et al. [20] supports generic delimitation of Al-Shehbaz [19], some authors [17, 18] have chosen to follow different concepts.

Structural characterization of ITS2 and utility of CBC species concept among genus members were tested by Özgişi [24]. But characterization of ITS1 seconder structure of Coluteocarpeae members have not been done, yet. For this purpose, 10 different Coluteocarpeae members which were once assigned under different genera by Meyer [15, 16] were used to characterize ITS1 seconder structure. Besides detecting structural properties of ITS1, using it as a delimitation tool for Coluteocarpeae members were also tested and discussed in this study.

2. Materials and Methods

1.1. Plant samples, DNA extraction, amplification and sequencing

Leaf materials of target taxa were obtained from the field and HUB herbarium. A detailed locality list is given in the table. Vouchers were identified in accordance with the keys and descriptions that were proposed by Meyer [15, 16, 25] and Al-Shehbaz [19]. Total genomic DNA was isolated using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. The ITS region was amplified using primers ITS1 and ITS4 [26]. Amplification of the ITSs was performed following the protocol of Warwick et al. [27]. Purification and sequencing were performed by MedSanTek (İstanbul, Turkey). Also ITS sequences of *Noccidium hastulatum* (DC.) F.K. Mey. and *Raparia bulbosa* (Spruner ex Boiss.) F.K. Mey were obtained from Genbank (accession number are MG944843 and MG944902, respectively).

1.2. Inference of the secondary structure

Sequences were edited with the Codon Code Aligner (CodonCode Corporation) and aligned with MUSCLE v.3.6 (EMBL-EBI, Cambridgeshire, UK) [28]. Since the main goal of this study is to predict ITS1 secondary structure, 5.8S gene region and ITS2 are removed during alignment by using ITS sequences of *Alliaria petiolata* (M.Bieb.) Cavara & Grande which was obtained from Genbank (accession number is AF283492).

ITS1 secondary structures were inferred with Mfold Web Server [29]. Default parameters for folding temperature (37 °C), percent suboptimality (5%), upper bound of computed foldings (50), window parameter (25), and maximum distance

between paired bases (no limit) are used to detect characteristics of ITS1 secondary structures. Lowest free-energy conformation approach that was proposed by Xia et al. [30] is adopted to infer possible secondary structures.

TABLE 1. Collection information of materials used in the analysis. Taxon names followed the concept presented in BrassiBase (https://brassibase.cos.uniheidelberg.de/).

Taxa	Locality, collector, and herbaria information
Callothlaspi lilacinum (Boiss. & A.Huet) F.K. Mey	Turkey, Erzincan, Refahiye, *Ozudogru 3660*, HUB
Kotschyella cilicica (Schott & Kotschy ex Boiss.) F.K. Mey	Turkey, Kahramanmaraş, Küçükçamurlu Village *Ozudogru 3657*, HUB
Microthlaspi perfoliatum (L.) F.K. Mey.	Turkey, Kırklareli, Dereköy, *K.O 1129*, HUB
Masmenia rosularis (Boiss. & Balansa) F.K. Mey.	Turkey, Hatay, Kızıldağ, *K.O 1027*, HUB
Neurotropis platycarpa (Fisch. & C.A. Mey.) F.K. Mey.	Turkey, Hatay, Dörtyol, *K.O 1081*, HUB
Noccaea sintenisii (Bornm.) F.K. Mey	Turkey, Bayburt, Karakaya Mountain, *Ozudogru 3694*, HUB
Syrenopsis stylosa Jaub. & Spach	Turkey, Kütahya, Gediz, *K.O 1048*, HUB
Thlaspiceras elegans (Boiss.) F.K. Mey.	Turkey, Adana, Pozantı, *Ozudogru 3609*, HUB

3. RESULTS AND DISCUSSION

The ITS1 secondary structures of 10 species were modelled. The ITS1 regions had variable lengths, a mean length of 263 bp (max = 273 bp; min = 222 bp), and a mean of 4.1 possible secondary structures. Approximately 48.27% of all of the nucleotide positions were paired and a mean of 6.2 total hairpins (median=6). The GC content of the ITS1 sequences ranged from 48.33% to 54.94%, with an average of 50.71%.

ITS1 sequences exhibited different seconder structures whose hairpins are highly variable, between 4 to 8 hairpins. *Noccidium hastulatum* (DC.) F.K. Mey. has 4 fingered secondary structure (Figure 1) whereas *Thlaspiceras elegans* (Boiss.) F.K. Mey. has 8 hairpin in ITS1 secondary structure (Figure 2). Most frequent ITS1 secondary structure is 6 fingered that *Callothlaspi lilacinum* (Boiss. & A.Huet) F.K. Mey, *Kotschyella cilicica* (Schott & Kotschy ex Boiss.) F.K. Mey, *Microthlaspi*

perfoliatum (L.) F.K. Mey., *Masmenia rosularis* (Boiss. & Balansa) F.K. Mey., *Neurotropis platycarpa* (Fisch. & C.A. Mey.) F.K. Mey. and *Noccaea sintenisii* (Bornm.) F.K. Mey exhibit (Figure 3). ITS1 seconder structures of *Raparia bulbosa* (Spruner ex Boiss.) F.K. Mey and *Syrenopsis stylosa* Jaub. & Spach have 7 hairpins (Figure 4).

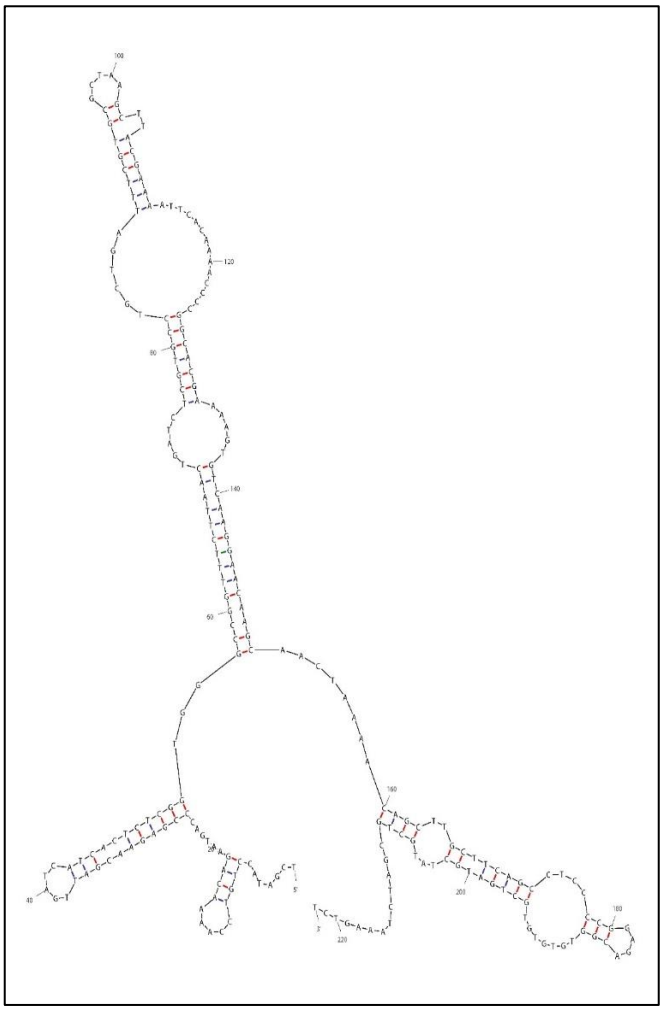

FIGURE 1.4 hairpin structures of *Noccidium hastulatum* observed from ITS1 sequences.

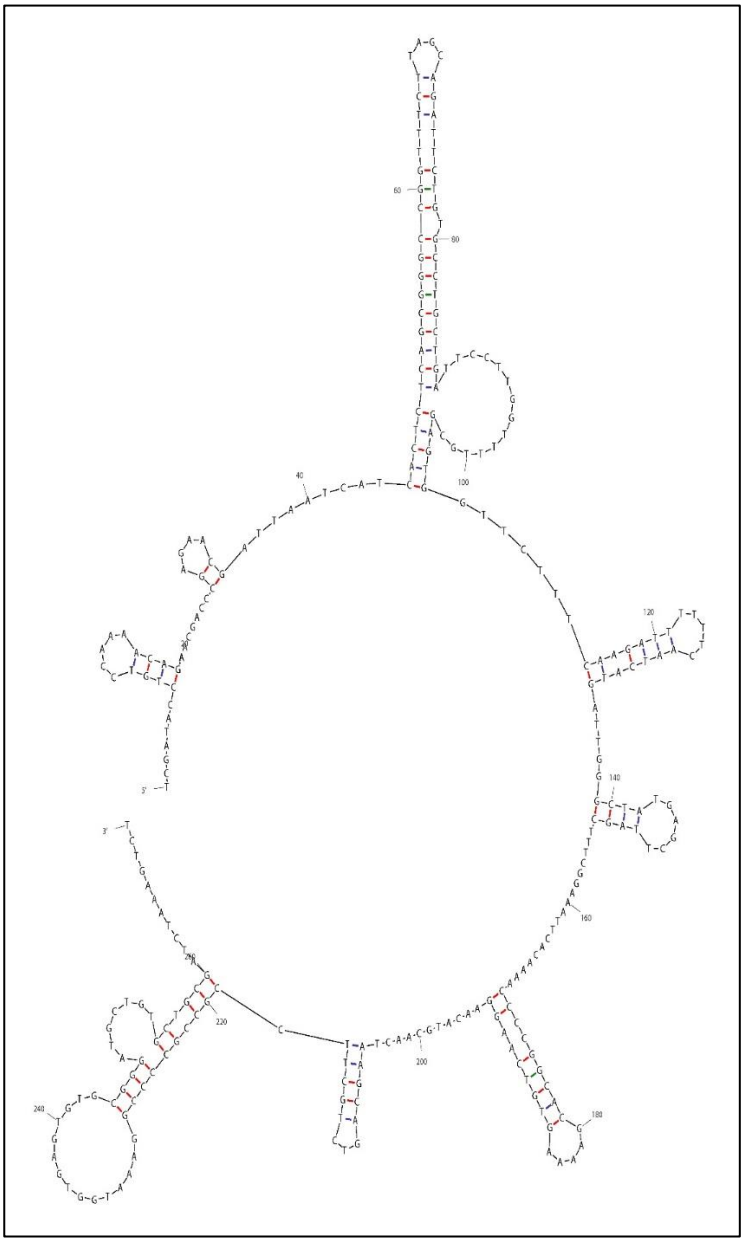

FIGURE 2. 8 hairpin structure which was observed from ITS1 sequences of *Thlaspiceras elegans.*

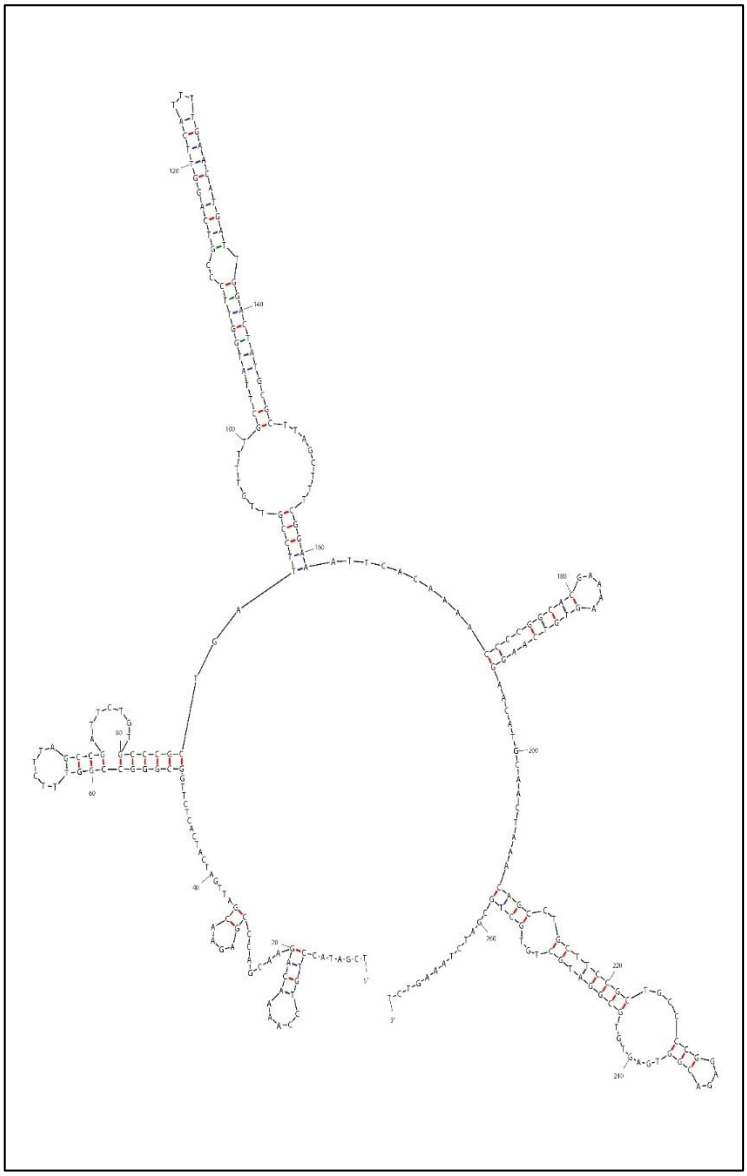

FIGURE 3. 6 hairpin structure which was observed from ITS1 sequences of *Callothlaspi lilacinum*, *Kotschyella cilicica*, *Microthlaspi perfoliatum*, *Masmenia rosularis*, *Neurotropis platycarpa* and *Noccaea sintenisii*.

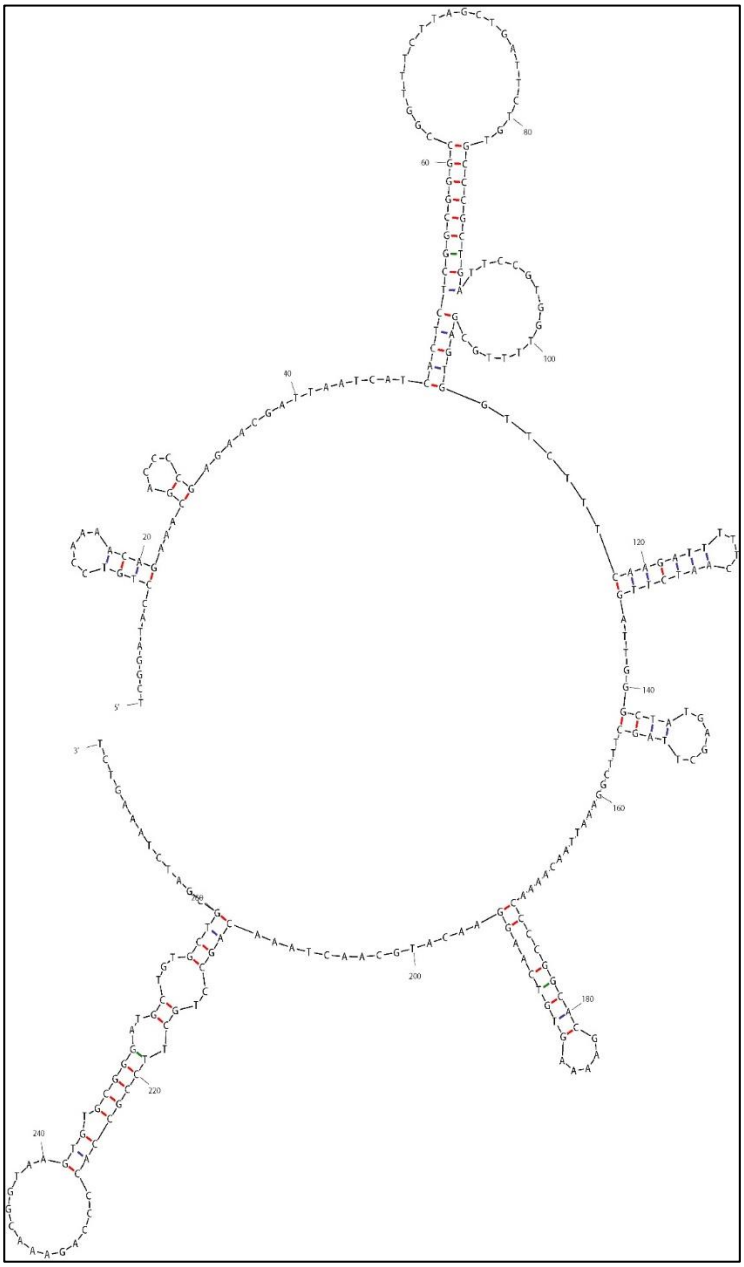

FIGURE 4. 7 hairpin structure which was observed from ITS1 sequences of *Raparia bulbosa* and *Syrenopsis stylosa.*

ITS1 secondary structure analysis showed that secondary structures are highly divergent among investigated species. On the other hand each ITS1 sequences have a conserved AAGGAA motif near the 3' ends. Liu and Schardl, [12] have emphasized the importance of this region during maturation of rRNA. In their study, Liu and Schardl [12] also showed that ITS1 secondary structure of *Arabidopsis thaliana* (L.) Heynh. has two major helices near the 5'- and 3'-ends. But, Edger et al. [31] indicated that ITS1 secondary structure is highly divergent among Brassicaceae members. This study also showed that secondary structures of ITS1 can be different among family members.

Comparisons among species from different genera that were proposed by Meyer [15, 16] did not show conserved patterns on ITS1 secondary structure. So using ITS1 secondary structure to detect relationships between investigated species does not seem to be useful tool. Edgar et al. [31] showed that members of Lineage I and Lineage II generally have six hairpin structure (5 to 7 hairpins) whereas 5 hairpin structures were most common among Lineage III species (4 to 7). Six fingered ITS1 structure is also most frequent among investigated species (expanded Lineage II). So structural properties of ITS1 does not reflect natural and correct relationships of Brassicaceae members.

Parameters (e.g., temperature) that are used in the analysis can be the reason of highly divergent structure of the ITS. Since evolution does not always proceed via the shortest route, parameters which are considered optimal to predict structure thermodynamically cannot reflect the in vivo structure. On the other hand, AAGGAA motif, which is considered unique for land plants [32], shows the importance of ITS1 and further studies should be done to find out the most similar ITS1 structure that reflect the in vivo one.

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