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SEED MORPHOLOGY IMPROVES DATA FROM PHYLOGENETIC RELATIONSHIPS OF MEDICAGOID TRIGONELLA, MEDICAGO AND MELILOTUS (FABACEAE) SPECIES

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Phylogenetic relationship and seed morphological characters of 11 species belong to Medicagoid *Trigonella*, six non Medicagoid and one species of each genera of *Medicago* and *Melilotus* were estimated before based on ITS sequences by using Baysian inference and maximum parsimony. In this paper, scanning Electron Microscope and stereomicroscope for seed morphological characters were used. Seed morphology results indicated that majority of Medicagoid *Trigonella* species belong to *Bucerates* and *Lunatae* sections placed in two close subclusters and joined together as did by Baysian analysis results. Both studies confirmed that *Medicago radiata* is sister for these species. Our results confirm that using molecular and morphological characters data are reliable evidence in systematic discriminations of the taxa.

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Key words: *Fabaceae*; Medicagoid; Seed morphology; *Trigonella*; Molecular Phylogeny

ریخت‌شناسی دانه اطلاعات قرابت فیلوژنتیک بین گونه‌های Medicagoid Trigonella و Medicago و Melilotus را با استفاده از مارکرمولکولی ITS تکمیل می نماید

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ارتباط فیلوژنتیکی ۱۱ گونه متعلق به Medicagoid Trigonella و ۶ گونه متعلق به Trigonella غیر Medicagoid، یک گونه از هر کدام از جنس‌های Medicago و Melilotus با استفاده از توالی منطقه ITS با روش ماکزیمم پارسیمونی وبایسین قبلاً انجام شد. در این مقاله بررسی‌های ریختی دانه توسط میکروسکوپ الکترونی اسکنینگ و استریومیکروسکوپ انجام گرفت. نتایج بدست آمده از ریخت‌شناسی دانه با نتایج حاصل از

مطالعات ITS همخوانی دارد و گونه‌های Medicagoid Trigonella در یک دندروگرام با حمایت بالا قرار گرفته و منوفیلتیک هستند. همچنین نتایج مطالعات مورفولوژی دانه قرار گرفتن گونه‌های بخشه‌های Bucerates وLunatea متعلق به گروه Medicagoid Trigonella در دو زیر خوشه نزدیک به هم را تایید می‌کند. در هردو مطالعه انجام یافته گونه Medicago radiata به عنوان گروه خواهری گونه‌های مورد مطالعه در نظر گرفته شد. این نتایج هماهنگی نتایج مورفولوژیکی و مولکولی را در حل اختلافات تاگزونومیکی بیان می‌دارد.

INTRODUCTION

The Fabaceae family is the third largest family of flowering plants that commonly is known as Legum family with about 751 genera and 19000 species that are distributed mainly in temprerate and subtropical zones of the world (Mirzaei & al., 2015b). *Trigonella* L. is a large genus with about 135 species from tribe *Trifolieae*. The subtribe *Trigonellinae* from the tribe *Trifolieae* consist of three genera, *Trigonella*, *Medicago* and *Melilotus*. The generic delimitation between *Trigonella* and *Medicago* has long been a problem. Members of this subtribe are united by the consistent presence of pinnately trifoliate leaves and high bootstap support (99%) in phylogenetic analyses using *mat*k (Steele & Wociechoeski, 2003).

Some of the earlier taxonomical studies for delimitation of *Trigonella* and *Medicago* based on floral characters, asymmetric leaves, phenolic variation, stigma morphology, pollen-ovule patterns and seed characters for discriminating between them have been done but with no success (Bena, 2001). Small (1987) based on some floral morophologial characters transferred 23 *Trigonella* species including *Bucerates* and *Lunatea* sections to the genus *Medicago* and called them "Medicagoid" species (Gazara & al., 2001). These 23 species have taxonomic problems in the genus *Trigonella* and have been considered as belonging to the genus *Trigonella*. Baum (1968) introduced them as Medicagoid species because of the floral and seed structure similarities among them and *Medicago* species, but_based on strong similarities between *Trigonella* and these species in the fruit appearance maintained them in the genus *Trigonella* (Bena, 2001).

Phylogenetic analysis of Medicagoid *Trigonella* L. species based on ITS sequence data showed monophylly of Medicagoid *Trigonella* species while *Bucerates* and *Lunatae* sections were placed in two close subclusters (Khandani & al., 2016b).

Seed Morphological characters in tribe *Trifolieae* have been studied by Lersten & Gunn (1982), Small & al., (1990), Tia (2004), Ceter & al. (2012), Salimpour & al. (2013), Turkie & al. (2013).

The aim of this study is to evaluate and compare the phenetic and phylogenetic relationships within Medicagoid and non Medicagoid *Trigonella* species.

MATERIAL AND METHODS

Taxon Sampling

The materials were taken from dried herbarium specimens and new collection from various parts of Iran during May and June 2014. All of the specimens are deposited in TARI and IAUH. The collected samples were studied and identified by using available floras, monographs and papers. In this study, 19 species including 11 species of Medicagoid *Trigonella* and 6 species of non Medicagoids were selected. Also *Medicago radiata* and *Melilotus officinalis* were chosen as outgroups. (Khandani & al. 2016 a & b).

RESULTS AND DISCUSSION

Seed morphology

Total of 8 quantitate/qualitative characters of seeds including length, width, outline, color, testa texture, homogeneity of cell sizes, testa ornamentation and hilum shape were studied and statistical analysis evaluated. Testa patterns terminology is based on Lersten & Gunn (1982), Small & al., (1990), Pinar & al. (2009), Gunes & Cirpici (2011), Ceter & al. (2012), Gunes (2013),Teixeira & al. (2013), Ozbek & al. (2014) and Mirzaei & al. (2015a) and (Khandani & al. 2016a). *Trifolium orthoceras* and *T. persica* (Medicagoid) have both aculate-tuberculate-verrucate testa ornamentation, circular hilum shape, uniform homogenity cell size, creased testa texture, oblong outline, but *T. orthoceras* is green color and has length mean 1.76 mm and 0.74 mm width mean. *Trifoliu persica* is dark brown color and has mean length 1.95 mm, and mean width 0.69 mm. *Trifolium coerulsences* (non Medicagoid) has reticulate – verrucate testa ornamentation, circular hilum shape, uniform homogenity cell size, creased testa texture, dark brown color, ovoid outline, lengh mean 1.47 mm and width mean 0.95 mm. *Trifolium calliceras* (non Medicagoid) has reticulate –verrucate testa ornamentation, elliptic hilum shape, creased testa texture, dark brown color, ovoid outline, lengh mean 1.77 mm and width mean 1.82 mm. *Trifolium turkemena* (non Medicagoid) has aculate –reticulate-verrucate testa ornamentation, elliptic hilum shape, uniform homogenity cell size, creased testa texture, brown color ,allantoid –oblong outline , lengh mean 2 mm and width mean 0.63 mm.

Melilotus officinallis has aculate –tuberculate – verrucate testa ornamentation, elliptic hilum shape, uniform homogenity cell size, smooth testa texture, dark brown color, ovoid outline, lengh mean 2.25 mm and width mean 1.52mm.

From Khandani & al. (2016b) can be concluded that phylogenetic tree consists of two main clades (A&B). Clade A includs *Bucerates* and *Lunatae* sections , therefore they are monophyletic taxa. They are also separated from non Medicagoid species and are close to *Medicago radiata* L. than non Medicagoid *Trigonella* species. Other clade includes all non Medicagoid species i. e. *Trigonella coerulescens* (M. B.) Halacsy, *T. elliptica* Boiss, *T. calliceras* Fisch., *T. spruneriana* Boiss., *T. trurkmena* M. Popov and *Melilotus* species.

According to Khandani & al. (2016a), morphological seed analysis observations confirmed the same results based on quantitative and qualitative seed characters (Figs. 1 & 2). Their work introduced phenogram with UPGMA method based on eight traits with highest phenetic correlation (r>o.60) and showed two main clusters. One of them comprises non Medicagoid *Trigonella*, *Medicago radiata* and *Melilotus officinalis* as sister group and the other includes majority of Medicagoid *Trigonella* species forming a separate group. These results are in agreement with previous studies based on seed morphological and molecular data (Tia, 2004; Ceter & al., 2012; Turki & al.2013; Bena, 2001; Dangi & al. 2016; Downie & al. 1998 and Steele & al. 2010).

In this study, the relation between *Medicagoid Trigonella* species that were shown in both MP and UPGMA analyses (Khandani & al. 2016b) confirmed the works by previous authors (Salimpour & al. 2013). In addition, our work proved close phylogenetic relationship between Medicagoid *Trigonella* species and *Medicago* genus, based on both morphological traits and molecular data. Also, this result is in agreement with some studies on the phylogeny of section *Bucerates*.

Our work shows that both nucleotide sequence data from the internal transcribed spacer (ITS) and seed morphological characters confirm Medicagoid *Trigonella* species separation from other non Medicagiod *Trigonella* species, which was suggested by Small (1987), based on floral characters. These results agree with the treatment in Flora Iranica (Rechinger 1984) which separated majority of Medicagoid *Trigonella* species in *Bucerates* section, such as two close subclusters based on seed morphology (Khandai & al. 2016a) and one cluster based on ITS sequences data (Khandai & al. 2016 b).

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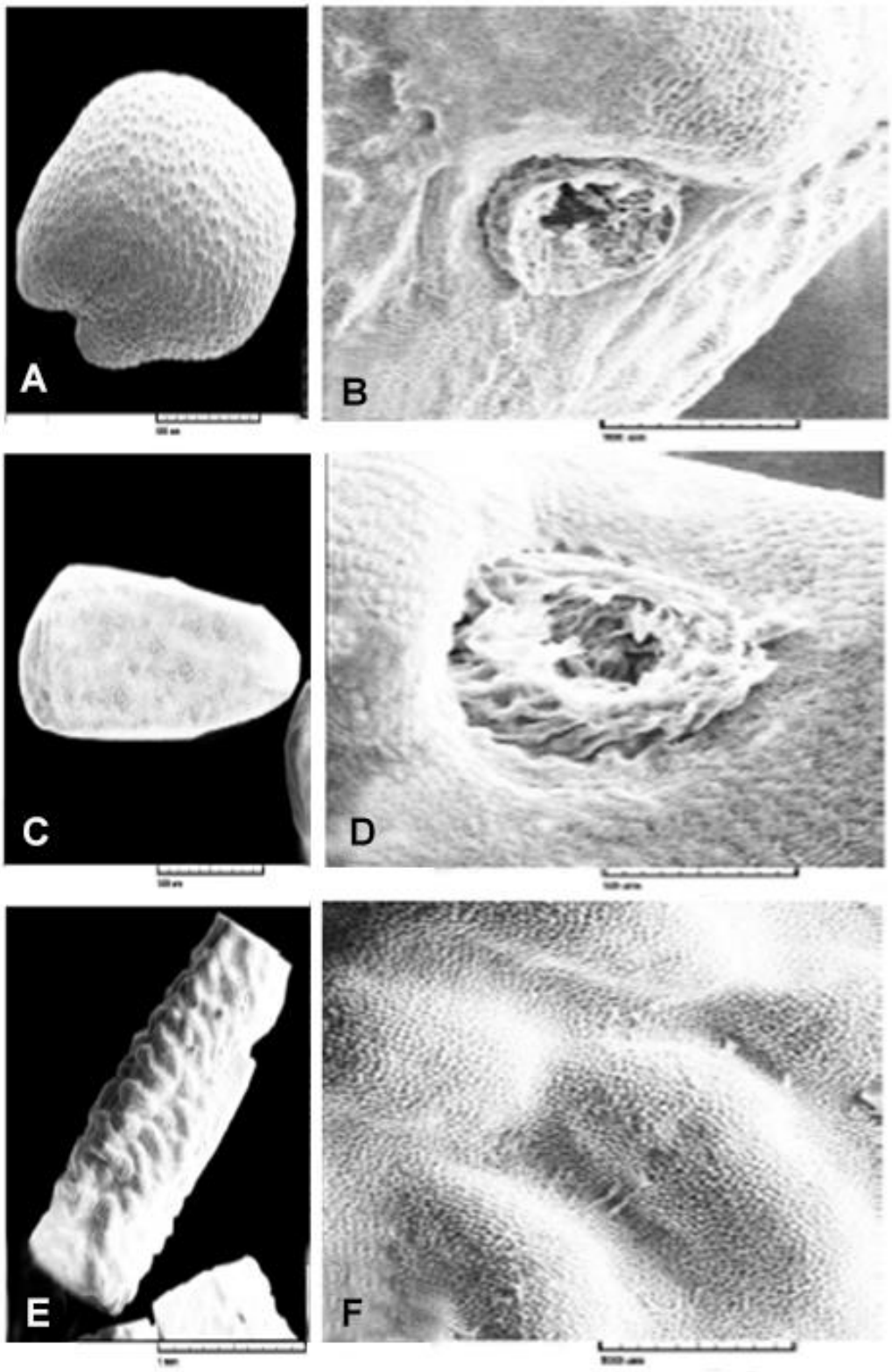

Fig. 1. SEM microphotographs of *Trigonella* seeds: A-B, *Trigonella coerlescens*; C-D, *T. orthoceras*; E-F *T. persica.*

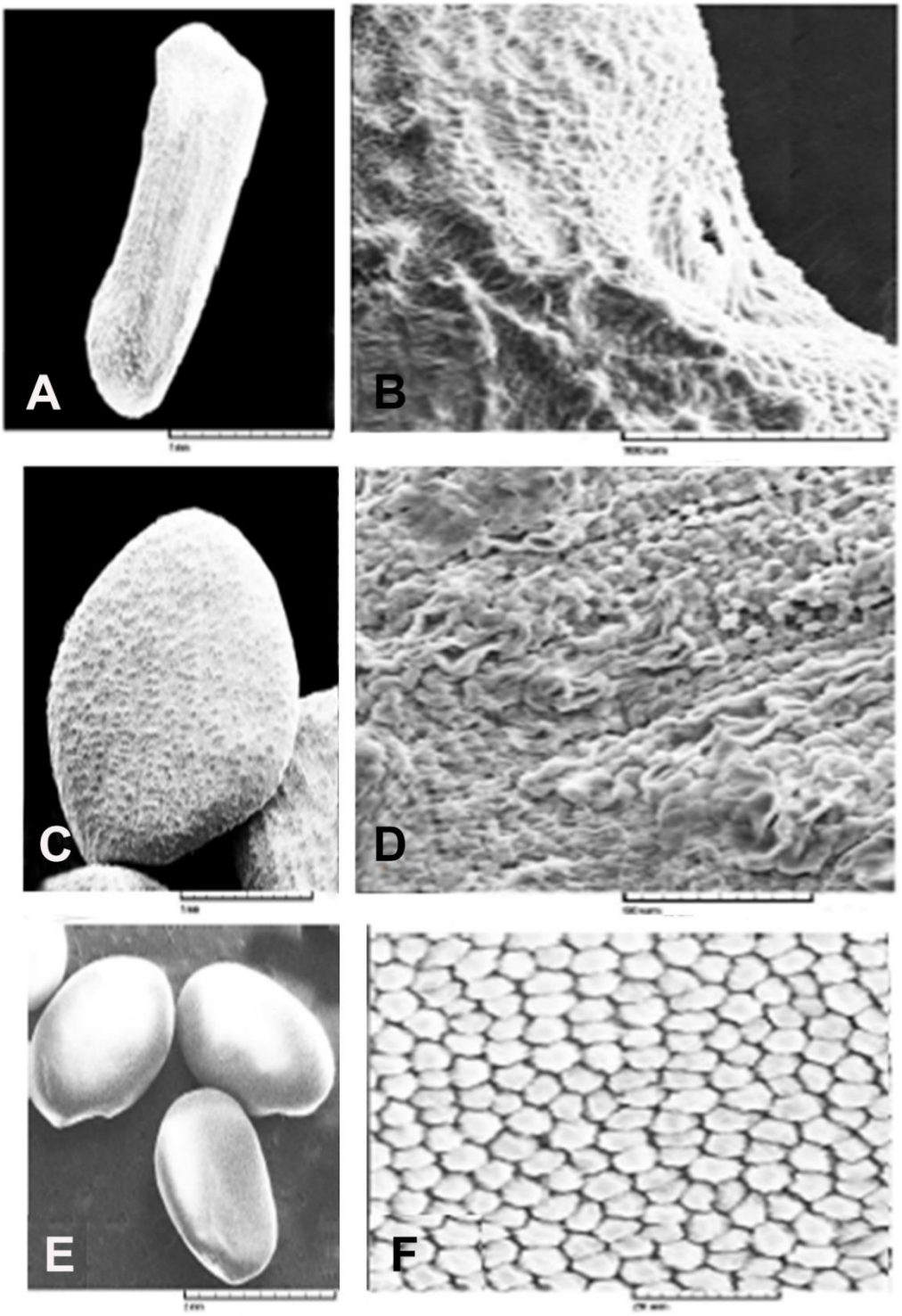

Fig. 2. SEM microphotographs of *Trigonella* and *Melilotus*. A-B, *Trigonella turkemena*; C-D, *T. calliceras*; E-F, *Melilotus officinalis*.

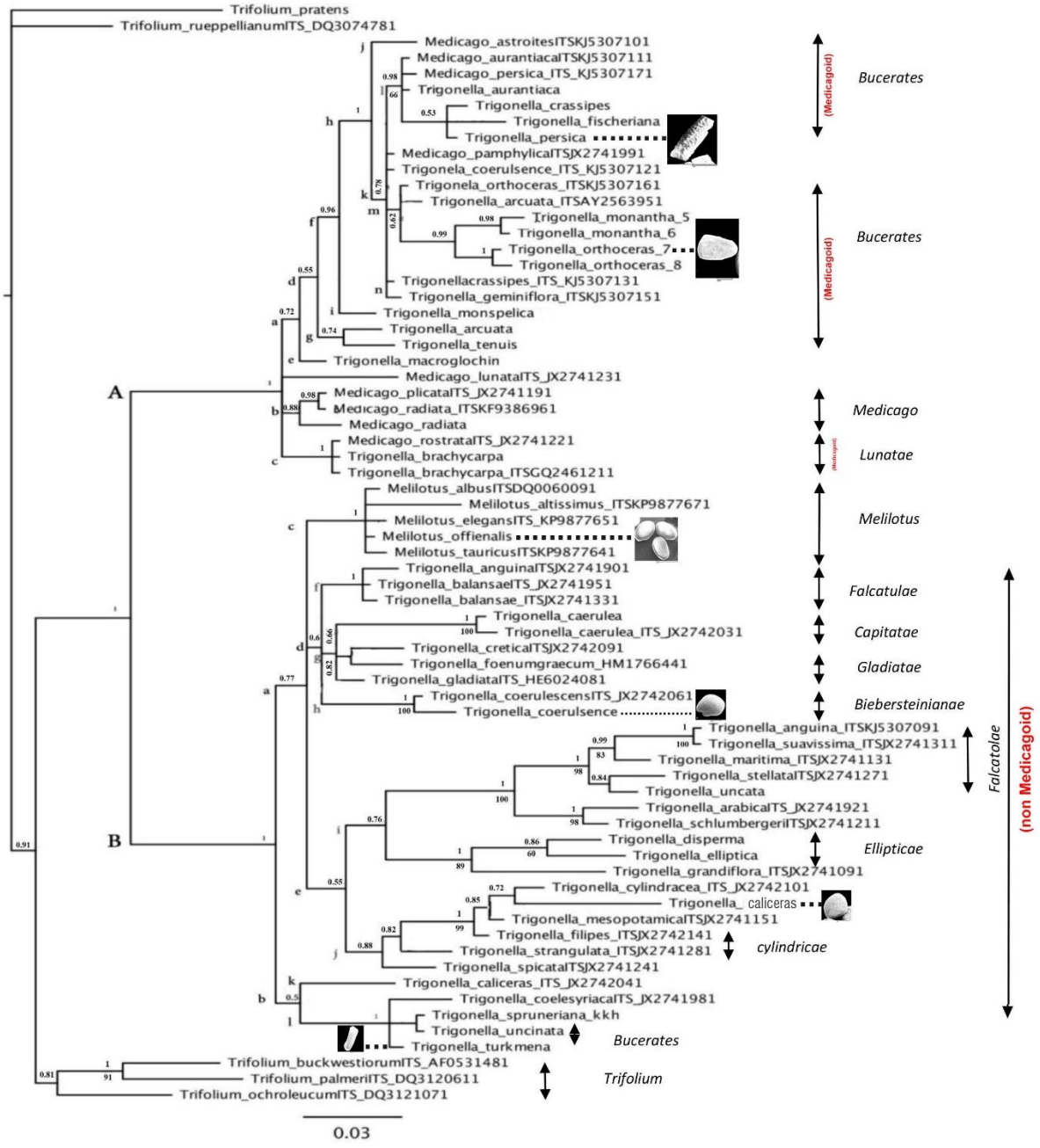

Fig. 3. Bayesian consensus phylogram tree. Numbers above branches are Bayesian posterior probabilities. Numbers below branches are bootstrap values) and SEM microphotography of *Trigonella* and *Melilotus***.**

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