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Molecular Analysis of Intra- and Inter-specific Genetic Diversity in Two Species of *Townsendia leptotes* and *T. incana* (*Asteraceae*)

Masoud Sheidai [1*], Jeannete Whitton [2]
1- Department of Biology, Faculty of Science, Shahid Beheshti University, G.C
2- Department of Botany, University of British Columbia, Vancouver, Canada

Abstract

RAPD analysis was performed in twenty-two plants of *T. leptotes* and seventeen plants of *T. incana* to study the intra- and inter-specific genetic diversity and to see if RAPD markers may be of use in delimitation of the species. Hundreds of RAPD primers were used out of which forty produced bands. In total, 104 bands (loci) were identified in *T. incana* and 71 bands were identified in *T. leptotes*. In total, 33 polymorphic bands were observed in *T. incana*, out of which 21 were specific for a single plant while 22 polymorphic bands were observed in *T. leptotes* plants out of which 10 bands were specific in a single plant. *T. leptotes* showed a higher mean gene diversity compared to that of *T. incana* also supported by PCO and PCA ordinations. Statistical analyses showed genetic distinctness of the two species studied and revealed the possible use of RAPD markers in the *Townsendia* species delimitation.

keywords: RAPID, genetic diversity, *T. incana*, *Townsendia leptotes*

بررسی مولکولی تنوع ژنتیکی درون و بین گونه‌ای دو گونه *Townsendia leptotes* و *T. incana* از خانواده Asteraceae

مسعود شیدایی[1*]، جانت ویتن[2]
۱- دانشکده علوم زیستی، دانشگاه شهید بهشتی
۲- بخش بیولوژی، دانشگاه بریتیش کلمبیا، ونکوور، کانادا

چکیده

آنالیز مارکرهای RAPD در ۲۲ گیاه از گونه *T. leptotes* و ۱۷ گیاه از گونه *T. incana* به منظور بررسی تنوع ژنتیکی درون جمعیتی و میان جمعیتی و کاربرد این مارکرها در تفکیک گونه های *Townsendia* انجام گرفت. تعداد ۱۰۰ پرایمر RAPD استفاده شد که از این میان تعداد ۱۰۴ باند در گونه *T. incana* و ۷۱ باند در گونه *T. leptotes* شناسایی شدند. تعداد ۳۳ باند پلی مرف در گونه *T. incana* مشاهده شد که از این میان ۲۱ باند در تک گیاهان این گونه انحصاری بودند و ۲۱ باند در ۲ گیاه حضور داشتند. در گونه *T. leptotes* تعداد ۱۰ باند در تک گیاهان مطالعه شده انحصاری بودند. گونه *T. leptotes* میانگین تنوع ژنتیکی بالاتری را در مقایسه با گونه *T. incana* نشان داد که توسط نمودارهای PCO و PCA تایید شد. آنالیزهای آماری تفاوت های ژنتیکی این دو گونه و کاربرد مارکرهای RAPD را در تفکیک آنها نشان دادند.

کلید واژه ها: RAPD، تنوع ژنتیکی، *T. incana*، *Townsendia leptotes*.

* Corresponding author. *E-mail Address:* msheidai@yahoo.com

Introduction

Townsendia species of the family *Asteraceae* are annual, biennial or perennial herbs growing mainly in the Rocky Mountains of the United States, but the ranges of apomictic populations of three species extend into Canada (Beaman, 1957). The North American genus *Townsendia* comprises approximately 25-30 taxa that occur predominantly at mid to high elevations in the Rocky Mountains. Most *Townsendia* species have narrow distributions, some are restricted to specific edaphic conditions and diploid sexual and polyploid asexual populations occur in a number of species (Whitton *et al.*, 2001).

The Rocky Mountain genus *Townsendia* is an interesting system for documenting multiple origins of polyploidy (Thompson and Whitton, 2006) because, within this genus, autopolyploidy goes hand in hand with asexuality through apomixis (although apomicts are overwhelmingly polyploid, the corollary is not true for plants - most polyploid plants retain their capacity for sexuality). Cytogenetic and embryological investigations by Beaman (1957) discovered a tight association between polyploidy and apomixis. In polyploid individuals of *Townsendia,* unreduced female gametophytes are produced through meiotic abnormality in the megasporocyte (diplospory), followed by parthenogenetic development of the embryo that does not require pollen for embryo or endosperm initiation (i.e. nonpseudogamous parthenogenesis). Diploid populations are reported to be invariantly sexual. Sexual diploid and apomictic polyploid plants are nearly identical morphologically, but can be distinguished on the basis of differences in pollen size and viability. Sexual diploids have small pollen grains, while apomictic polyploids have large pollen grains and a high percentage of inviable pollen (Beaman, 1957).

Studies related to the genetic variation within as well as phylogenetic relationship of the *Townsendia* species have been performed by using molecular markers including isozymes and nuclear ribosomal spacers such as external transcribed spacer DNA (ETS), internal transcribed spacer DNA (ITS), as well as amplified fragment length polymorphism (AFLP) and enzymatic digestion of *trn*K1/*trn*K2 and *ndh*F1/*ndh*F14 chloroplast regions (Whitton *et al.*, 2001; Jennings and Whitton, 2003; Thompson and Ritland, 2006; Thompson and Whitton, 2006). The present study considers the use of Randomly Amplified Polymorphic DNA (RAPD) markers in revealing the intra- and inter-specific genetic variation of two species namely *T. incana* and *T. leptotes*. RAPD markers have been used for studying the genetic diversity in different plant species. These molecular markers provide the opportunity for the direct genetic study and comparison of the plant materials without any other influences (Bautista *et al.*, 2003; Weising *et al.*, 2005).

Materials and Methods

Plant Materials and DNA Extraction

Twenty-two plants of *T. leptotes* (Gray) Osterhout, and 17 plants of *T. incana* Nutt. were collected randomly from their natural habitats and their fresh as well as frozen leaves were used for DNA extraction. The DNA extraction was done by using a Plant Minikit (QIAGEN) according to the manufacturer's instructions.

One hundred RAPD primers obtained from the University of British Columbia were used. The PCR reaction mixture consisted of template DNA (10ng/µL), 1 x PCR buffer (10 mM Tris-HCJ pH 8.8, 250 mM KC12), 200 µM dNTPs, 1 µL 10-base random primers and 1 unit of Taq polymerase, in a total volume of 25 µl. DNA amplification was performed on a palm cycler GP-001 (Corbet, Australia). Template DNA was initially denatured at 94^0C for 3 mm, followed by 35 cycles of PCR amplification under the following parameters: denaturation for 1 mm at 92^0C, primer annealing for 1 mm at 36^0C and primer extension for 2 mm at 72^0C. A final incubation for 10 minutes at 72^0C was performed to ensure that the primer extension reaction proceeded to completion. The PCR amplified products were separated by electrophoresis on a 1.5% agarose gels

using 0.5 X TBE buffer (44.5 Mm Tris/Borate, 0.5 Mm EDTA, pH = 8). The gels were stained with ethidium bromide and visualized under UV light. RAPD markers were named by primer origin, followed with the primer number and the size of the amplified products in base pairs. One hundred random primers of University of British Columbia were used. The experiment was repeated three times and the reproducible bands were scored.

Statistical Analysis

For studying intra-specific genetic diversity, RAPD bands (loci) were coded as binary (0=absent, 1 =present) and used for determination of their Nei and Lie (1979) as well as Rogers and Tanimoto distances (1960) among the plant specimens collected from each species. The genetic distances were then double centered and used in the Eigen command of NTSYS, based on which principal coordinate analysis (PCO) was performed (Chatfield and Collins, 1995). In order to identify the most variable RAPD loci in each species, principal components analysis (PCA) was performed.

In order to determine the diversity of genes, the RAPD bands (loci) obtained were treated as a separate locus having two alleles and the Nei gene diversity (1987) was determined by using POPGENE program Ver.1.32 (1997).

In order to study the molecular distinctiveness of the two *Townsendia* species studied, UPGMA clustering as well as PCA and PCO ordination were performed (Ingrouille, 1986). UPGMA (unweighted paired group with arithmetic average) and NJ (neighbor joining) trees were obtained from the genetic distance determined. NTSYS software Ver. 2.2 (1988), SPSS ver. 9. (1998) and DARwin5 (2008) were used for statistical analyses.

Results and Discussion

Out of 100 RAPD primers used, 40 primers produced bands. In total, 104 bands (loci) were identified in *T. incana* and 71 bands in *T. leptotes* (Figures 1 and 2). The RAPD bands varied in molecular weight from 0.6 kb to 1.4 kb in *T. incana* and from 0.4 kb to 2.0 kb in *T. leptotes*. In total 33 polymorphic bands were observed in *T. incana*, out of which 21 bands were specific for a single plant while 12 bands occurred only in two plants. The mean Nei gene diversity was in 0.834 *T. incana*.

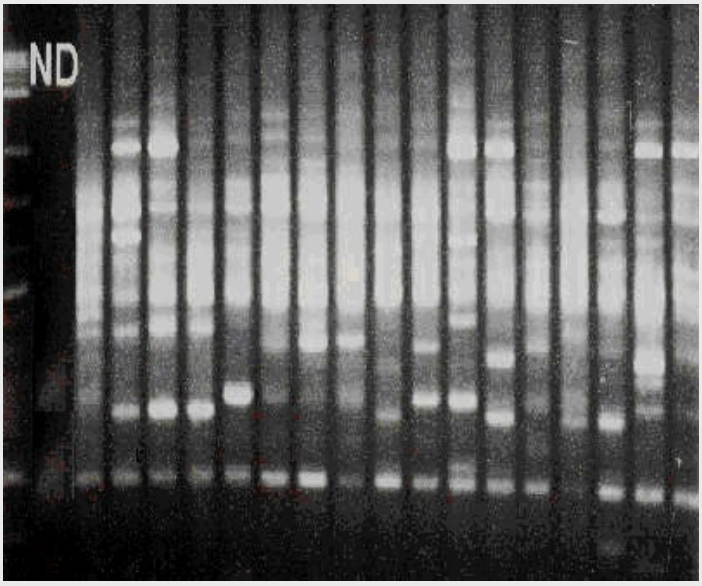

Figure 1. RAPD profile of *T. incana* by primer No. 38.
The columns from left to write: Molecular ladder, No DNA, *T. incana* plants.

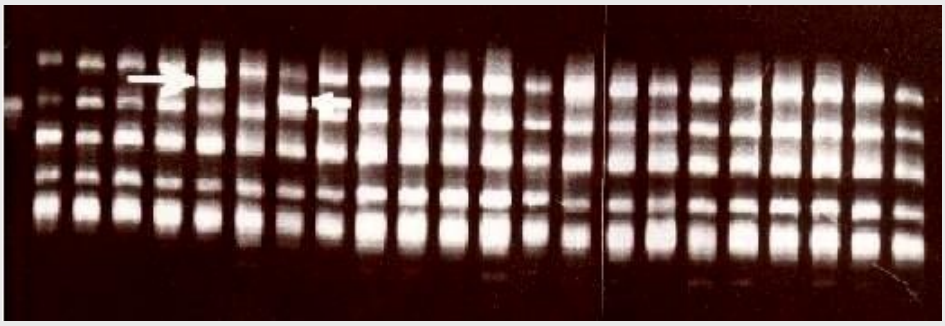

Figure 2. RAPD profile of *T. leptotes* by primer No. 45.
The columns from left to write: No DNA, *T. leptotes* plants (arrows indicate specific bands).

The results of PCO and PCA ordination of *T. incana* plants produced similar results (Figure 3). The plants numbered 1, 5, 7, 8 and 9 are genetically different from the other plants and are placed far from the others.

Twenty-two polymorphic bands were observed in *T. leptotes* plants studied, out of which 10 bands were specific. The mean Nei gene diversity was 0.169 (SD=±0.17) in *T. leptotes*, much higher than the same value observed in *T. incana* (0.083, SD = ±0.02) Therefore a higher genetic diversity is present in *T. leptotes*.

The results for PCO and PCA ordination of *T. leptotes* plants produced similar results (Figure 4). The plants numbered 2, 14, 20, 21 and 22 are genetically different from the other plants and are placed far from the others.

The genetic distinctiveness of the two species studied is shown in Figure 5. Different clustering methods were performed that grouped the representative plants of *T. leptotes* and *T. incana* into two distinct separate clusters, also supported by PCO ordination. RAPD markers may therefore be of use in species delimitation in the genus *Townsendia*.

In order to compare the level of genetic diversity of the two species studied PCA ordination was conducted. The preliminary PCA analysis showed that the first four factors comprise about 80% of total variance. The plot of the representative plants from the species *T. leptotes* and *T. incana* showed the higher genetic diversity of the *T. leptotes* along with the PCA axes obtained. In all the ordination plots obtained, plants of *T. leptotes* showed much more dispersal along the axes of the plot than plants of *T. incana* (Figures 6 and 7).

Although the present study is a preliminary analysis of two *Townsendia* species, it clearly indicates the use of RAPD markers both in species delimitation as well as studying the genetic diversity in the genus *Townsendia*. It would be useful in future studies to correlate RAPD marker diversity with the sexual or asexual breeding system of *Townsendia* species to see if these markers may be of help in distinguishing the breeding system of the species. It is expected that sexual plants possess higher genetic diversity as compared with asexual plants.

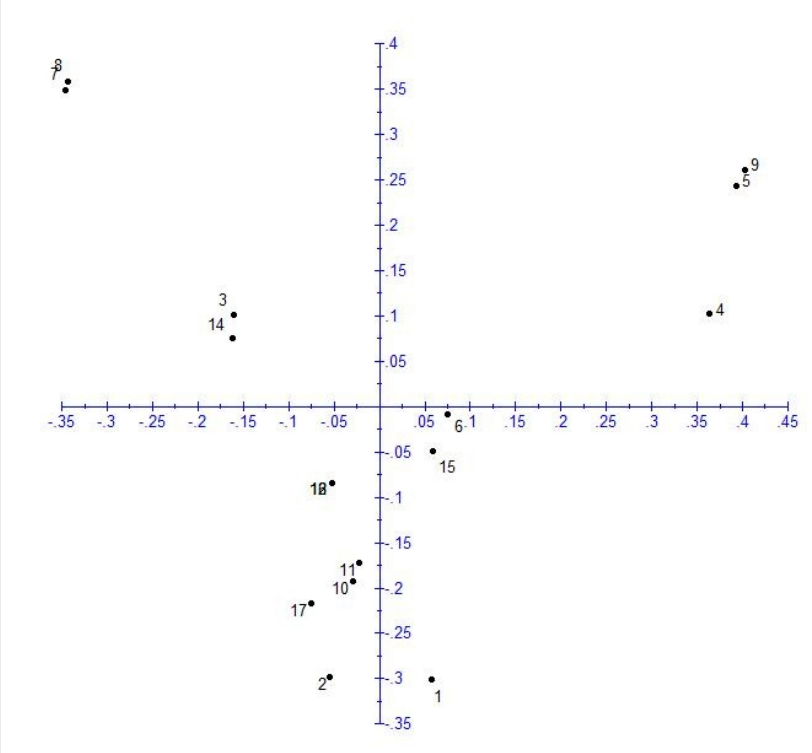

Figure 3. Ordination plot of *T. incana* plants based on the PCA axes 1 and 2.

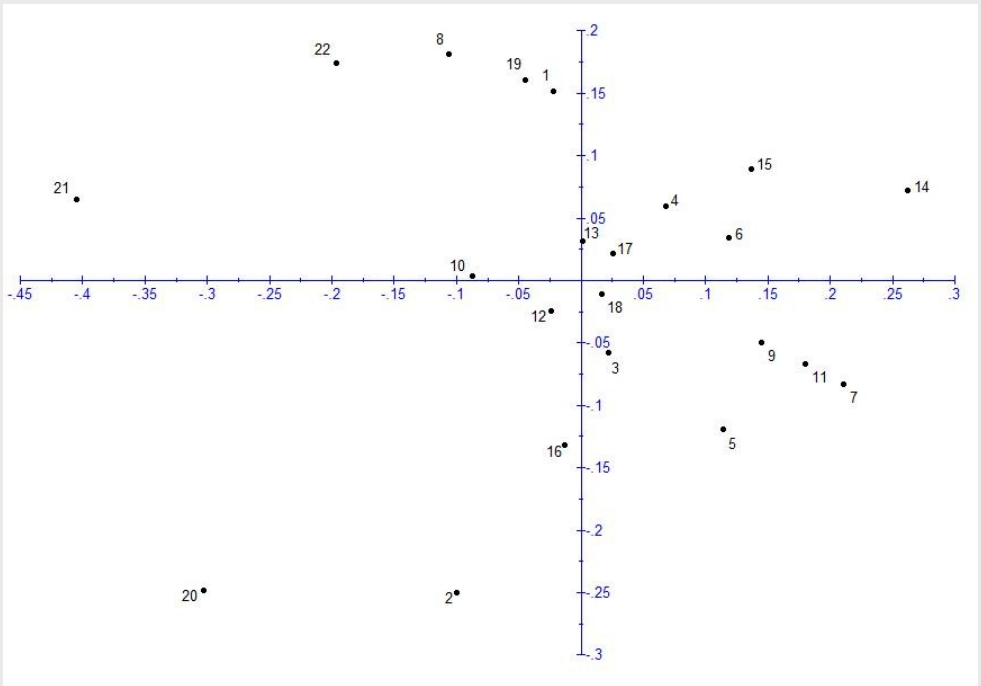

Figure 4. Ordination plot of *T. leptotes* plants based on the PCA axes 1 and 2.

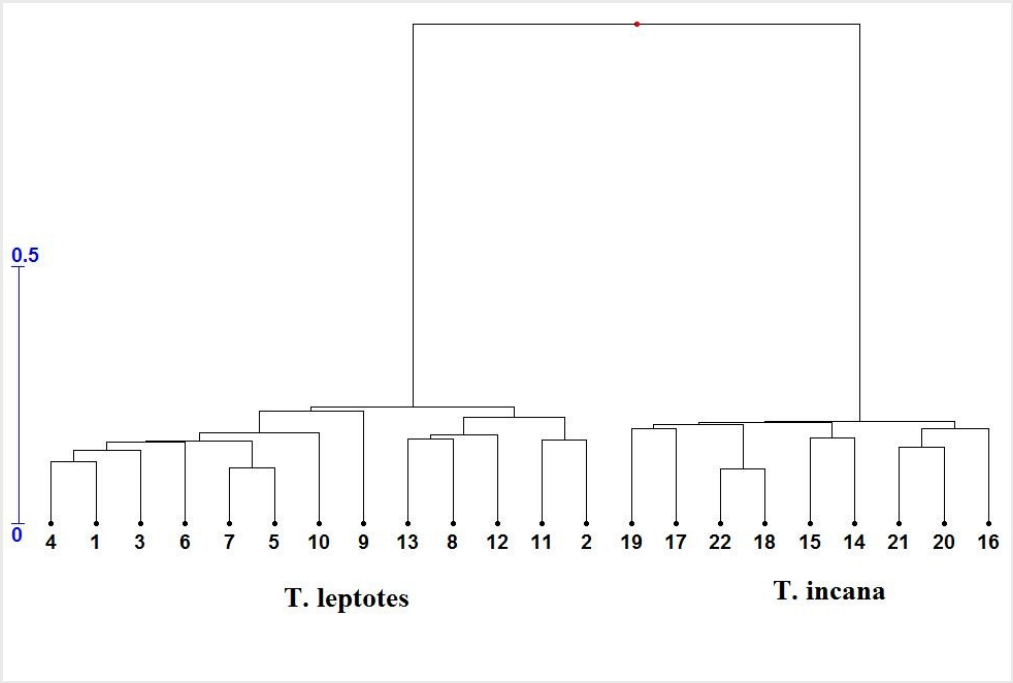

Figure 5. UPGMA dendrogram of *T. incana* and *T. leptotes* plants.

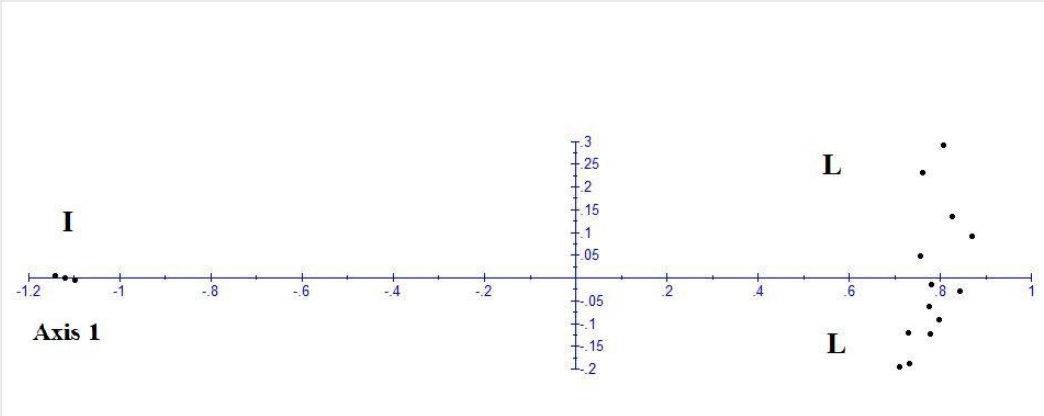

Figure 6. Ordination plot of *T. incana* and *T. leptotes* plants based on the PCA axes 1 and 2.

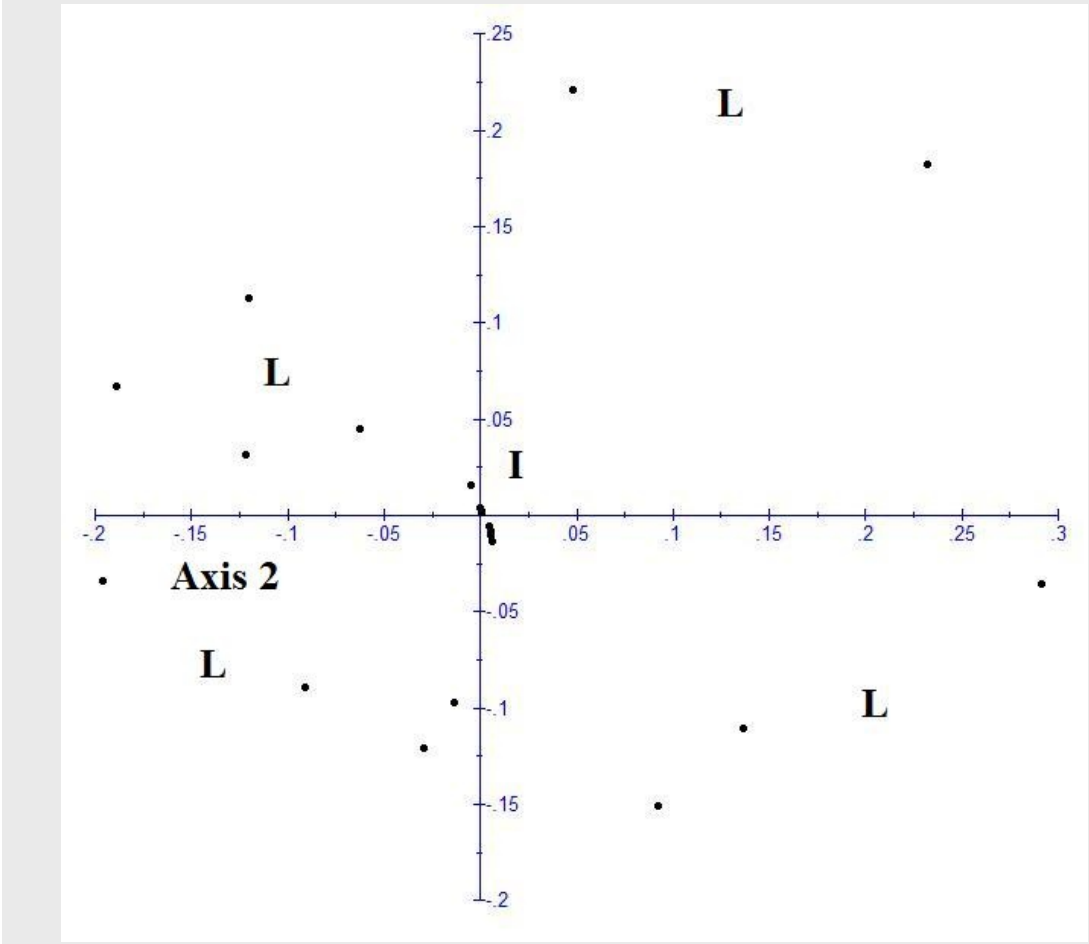

Figure 7. Ordination plot of *T. incana* and *T. leptotes* plants based on the PCA axes 2 and 3.

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