ISSN 1682-296X (Print)
ISSN 1682-2978 (Online)

Bio Technology

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Biotechnology 7 (3): 595-598, 2008
ISSN 1682-296X

Nuclear DNA Content of an Endemic Taxon from Turkey, *Trachelium jacquinii* (Sieber) Boiss. subsp. *dalgiciorum* N. Özhatay and Dane (Campanulaceae)

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Abstract: Nuclear DNA content of *Trachelium jacquinii* subsp. *dalgiciorum*, an endemic taxon, was analysed using flow cytometry. Plants were collected from a natural population. While some plants were prepared as herbaria material, others were placed in pots and incubated in growth chamber. Fresh leaves for flow cytometry analysis were chopped in $MgSO_4$ buffer (with dithiothreitol, propidium iodide and triton x-100) on ice in a Petri dish and stained with propidium iodide (with RNase-DNase free). Stained nuclei were analysed on an EPICS XL (Beckmann Coulter) model flow cytometer. The mean DNA content per plant was based on 10000 scanned nuclei. Nuclear DNA content (2C-value) of *Trachelium jacquinii* subsp. *dalgiciorum* was found as 3.44±0.14 pg (1C-value 1.72 pg). This study contributes present data on nuclear DNA content of angiosperm taxa.

Key words: Endemic, flow cytometry, nuclear DNA content, propidium iodide, *Trachelium jacquinii* subsp. *dalgiciorum*

INTRODUCTION

Trachelium L. has 3 species as *Trachelium caeruleum* L., *Trachelium asperuloides* Boiss. and Orph. and *Trachelium jacquinii* (Sieber) Boiss. distributed through the West Mediterranean, Southern Balkans and Aegean Islands in Europe (Tutin *et al*., 1976). *Trachelium jacquinii* was added to Turkish Flora in 1993 by Ozhatay *et al*. (1993). *T. jacquinii* is represented in Europe by 3 subspecies; subsp. *jacquinii,* subsp. *rumelianum* (Hampe) Tutin and subsp. *dalgiciorum* N. Özhatay and Dane (Tutin *et al*., 1976; Dane and Özhatay, 2001). *T. jacquinii* subsp. *jacquinii* distributes in Crete and Athos between altitudes of 1100-2200 m, *T. jacquinii* subsp. *rumelianum* grows in NC and NE Greece and Bulgaria up to 1500 m and *T. jacquinii* subsp. *dalgiciorum* has a distribution area limited in Edirne, Mecidiye on sea shore rocks up to 20 m (Tutin *et al*., 1976; Dane and Özhatay, 2001). According to red data book of Turkish plants, conservation status of *T. jacquinii* subsp. *dalgiciorum* is vulnerable (Ekim *et al*., 2000).

Chromosome numbers of *Trachelium* species are known as 2n = 32 for *T. caeruleum* subsp. *caeruleum*, 2n = 34 for *T. asperuloides*, 2n = 34 for *T. jacquinii* subsp. *jacquinii*, 2n = 32, 34 for *T. jacquinii* subsp. *rumelianum* and 2n = 34 for *T. jacquinii* subsp. *dalgiciorum* (Tutin *et al*., 1976; Dane and Özhatay, 2001). However, nuclear DNA contents of *Trachelium* taxa are unknown (Bennett and Leitch, 2005b).

Nuclear DNA content is estimated using techniques such as microspectrophotometry, microdensitometry, flow cytometry and image cytometry (Suda, 2004). Since it is easy, quick and reliable to apply, flow cytometry has been a widely used technique for estimating nuclear DNA content in recent years. It is frequently used in immunology, molecular biology, genetics, pharmacology, zoology, marine biology and botany (Suda, 2004). Analysis of nuclear DNA amount is the most widespread application of flow cytometry in modern plant biosystematics. DNA content of a diploid somatic nucleus is expressed as 2C-values. The term C-value refers to the DNA content of an unreplicated haploid chromosome complement. DNA C-values vary remarkably between angiosperm taxa (Bennett and Leitch, 1997).

DNA C-values have been estimated in plant species for over fifty years (Swift, 1950; Bennett and Leitch, 2005b), but, only 1.4% of those angiosperms are known (Dolezel and Bartos, 2005). Since 1976, Bennett and co-authors have published 7 reference lists of nuclear DNA amounts which together include C-values for approximately 4400 angiosperm species collected from about 465 sources (Bennett and Smith, 1976, 1991; Bennett *et al*., 1982, 2000; Bennett and Leitch, 1995, 1997,

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2005a). DNA C-value data of the global angiosperm flora have still massive gaps. There are various problems in the estimation of DNA C-values due to geographical distributions, plant life forms and requirement of high-costing equipment. Especially, estimation of nuclear DNA contents for endemic taxa growing in limited geographical regions is the major problem. This study provides contribution to Angiosperm DNA C-values database (Bennett and Leitch, 2005b).

MATERIALS AND METHODS

Plants were collected from a natural population in Edirne, Kesan, Mecidiye (European Turkey) (Fig. 1). The locality of plants was determined using a GPS (Garmin eTrex Vista). Some plants were prepared as herbaria materials and voucher specimens were deposited in EDTU Herbarium (Trakya University, Edirne, Turkey). Others were transferred into pots containing sterilised sand and they were placed in a growth chamber at 27°C with a 16/8 h photoperiod. Young leaves of growing plants were prepared for flow cytometry according to Tuna *et al.* (2001). As internal standard diploid *Hordeum vulgare* L. cv. Hitchcock (2n = 2x = 14 and 2C-value 10.68 pg) was used.

Fresh leaf fragments of *T. jacquinii* subsp. *dalgiciorum* and *H. vulgare* were chopped with a razor blade on ice in a petri dish containing 1 mL of solution A [24 mL $MgSO_4$ buffer (ice-cold); 25 mg dithiothreitol; 500 µL propidium iodide stock (5 mg PI in 1 mL distilled H_2O); 625 µL Triton X-100 stock (1 g Triton X-100 in d H_2O)]. Then, suspension was filtered through a 30 µm nylon mesh and centrifuged at 13000 rpm for 2 min. The supernatant was removed and the pellet was homogenized in 500 µL of solution B [7.5 mL solution A; 17.5 µL RNase (DNase free)]. The suspension was incubated at 37°C for 20 min in oven before flow cytometric analysis.

The prepared materials were analysed in Trakya University, Faculty of Medicine on an EPICS XL model flow cytometer (Beckman Coulter). Analyses were repeated three times for each specimen. The mean DNA content per plant was based on 10000 scanned nuclei. The formula used for converting fluorescence values to DNA content was sample nuclear DNA content = [(sample G_1 peak mean)/(standard G_1 peak mean)] × standard 2C DNA content (pg DNA) (Dolezel and Bartos, 2005).

RESULTS AND DISCUSSION

Trachelium jacquinii subsp. *dalgiciorum* was collected from a rather limited region. The location of plants was determined using a GPS. Their location coordinates are 40°36' N and 26°32' E.

The applied cytometric procedure was very suitable for the material that is analyzed in this study. During analysis of samples, no technical problems were encountered. The 2C-value of *T. jacquinii* subsp. *dalgiciorum* was found as 3.44±0.14 pg (1C-value 1.72 pg) (Fig. 2). The species has a small genome. The average Coefficients of Variation (CV) of the 2C nuclei population were always less than 3%. It is known that there exist intraspecific variations in DNA C-values in angiosperms. But in our present study, the variations in 2C-values of *T. jacquinii* subsp. *dalgiciorum* were found to be statistically insignificant (SD: 0.14).

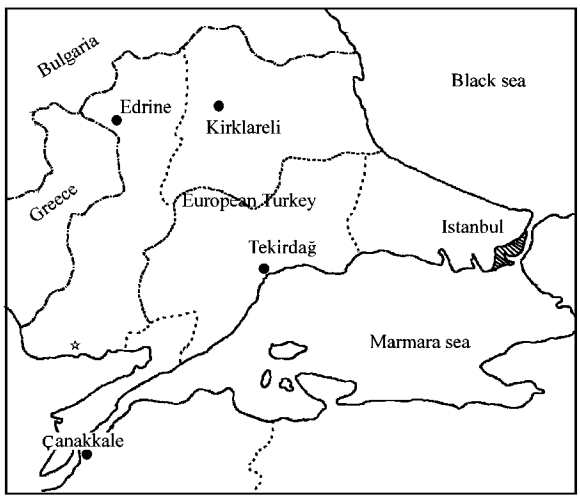

Fig. 1: The location of *Trachelium jacquinii* subsp. *dalgiciorum*

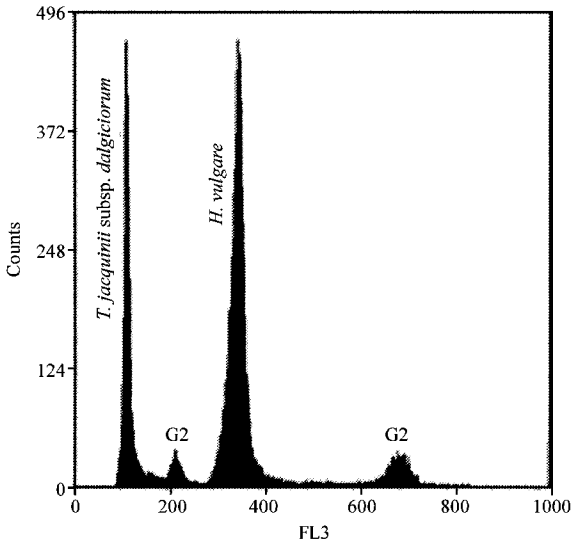

Fig. 2: The flow cytometric histogram of *Trachelium jacquinii* subsp. *dalgiciorum*

Table 1: Nuclear DNA contents and chromosome numbers of Campanulaceae members

Species	Chromosome numbers (2n)	1C (pg)
Brighamia insignis	2n = 28 (Lammers, 1993)	1.05 (Bennett and Leitch, 2005b)
Trachelium jacquinii subsp. *dalgiciorum*	2n = 34 (Dane and Özhatay, 2001)	1.72 (Present study)
Campanula rotundifolia	2n = 4x = 68 (Bennett and Leitch, 2005b)	2.65 (Bennett and Leitch, 2005b)
Canarina canariensis	2n = 4x = 34 (Suda *et al*., 2003)	2.85 (Suda *et al*., 2003)
Lobelia dortmanna	2n = 24 (Bennett and Leitch, 2005b)	6.38 (Bennett and Leitch, 2005b)

The chromosome number of *T.* subsp. *dalgiciorum* is 2n = 34 (Dane and Özhatay, 2001). Since these chromosomes are very small, their morphologies could not be determined clearly (Dane and Özhatay, 2001). *Trachelium* is closely related to *Campanula* genus. *Campanula rotundifolia* has 2.65 pg 1 C-value (Bennett and Leitch, 2005b). The diploid chromosome number of *C. rotundifolia* is 2n = 34 (x = 17) (Tutin *et al*., 1976). But this species also has tetraploid populations (2n = 4x = 68) (Mraz, 2005). C-values are only known for four taxa in Campanulaceae. *Brighamia insignis*, another Campanulaceae member has 1.05 pg 1C-value (Bennet and Leitch, 2005b). Besides, *Canarina canariensis* and *Lobelia dortmanna* have 2.85 and 6.38 pg 1C-values, respectively (Bennett and Leitch, 2005b; Suda *et al*., 2003). 1C-values and chromosome numbers in some taxa of Campanulaceae were shown in Table 1. The data were presented by combining current literature knowledge and the results in this study.

The DNA 1C-values of angiosperms vary about 1000-fold ranging from 0.16 pg (*Arabidopsis thaliana* (L.) Heynh.) to 127.4 pg (*Fritillaria assyriaca* Baker.) and are characteristics of each taxon (Bennett *et al*., 2000; Bennett and Leitch, 2005a, b). 1C-values of Campanulaceae range from 1.05 to 6.38 pg (*B. insignis* and *L. dortmanna*, respectively) (Bennett and Leitch, 2005b). The difference among 1C-values of these species is approximately 6-fold. The average of 1C-values was found as 2.93 pg and the standard deviation as 1.84. For the evaluation of nuclear DNA amounts of Campanulaceae more data are needed concerning 1C-values of this family.

According to Murray *et al*. (1992), genus *Pratia* belongs to Lobeliaceae. On the other hand it was also included in Campanulaceae by Lammers (1993). In this study, *Pratia* genus was not taken into account in present evaluation, because it was not included in Campanulaeceae in Angiosperm DNA C-values database (Bennett and Leitch, 2005b). *Pratia* is represented in Angiosperm DNA C-values database with six species and their 1C-values range from 2.53 to 9.60 pg (Bennett and Leitch, 2005b).

Since sample preparation and analysis for this method are both easier and faster the use of flow cytometry for estimation of DNA content is a popular method. It is also suitable for large amounts of samples analyzed for determination of ploidy and polysomaty, cell cycle analysis and estimated of DNA amount. With this method the massive gap of angiosperm DNA amount data can be easily filled. It should be especially considered for determination of nuclear DNA amounts of rare and endemic plants growing in limited geographical regions. Similar investigations should be encouraged to contribute to future molecular systematic studies of angiosperm taxa and to improve representation of the global angiosperm flora.

ACKNOWLEDGMENTS

This study was supported by the Scientific Research Fund of Trakya University (Project No. TUBAP-763). We would like to thank in advance to The Scientific Research Fund of Trakya University for financial support. We thank the staff at Hematology Laboratory (Department of Hematology, Faculty of Medicine, Trakya University) for their help in performing the flow cytometric analyses and Volkan Aksoy for carefully correcting the English of manuscript.

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