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Research Article

Nuclear DNA Contents of Some Endemic *Hedysarum* L. Species

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Abstract: The nuclear DNA contents and nuclear areas of some endemic *Hedysarum* L. species were investigated. A significant variation in the DNA content of *Hedysarum* species was established. The 2C nuclear DNA amount in the *Hedysarum* species (x = 8) ranged from approximately 4 to 6 picograms. It was found that the variation in the 2C nuclear DNA content was not related to chromosome numbers. However, there was a very high positive linear relationship between nuclear DNA content and nuclear area.

Key Words: *Hedysarum,* Nuclear DNA content, Nuclear area, Microspectrophotometry.

Bazı Endemik *Hedysarum* L. Türlerinin Çekirdek DNA İçerikleri

Özet: Bu çalışmada, bazı endemik *Hedysarum* L. türlerinin çekirdek DNA içerikleri ve çekirdek alanları araştırıldı. *Hedysarum* türlerinin DNA içeriğinde önemli bir varyasyon saptandı. *Hedysarum* türlerinin (x = 8) 2C çekirdek DNA miktarı yaklaşık olarak 4–6 pikogram arasında değişmektedir. Çekirdek DNA içeriğindeki varyasyonun kromozom sayısıyla ilişkili olmadığı bulundu. Ancak, çekirdek DNA içeriği ile çekirdek alanı arasında pozitif linear bir ilişki gözlendi.

Anahtar Sözcükler: *Hedysarum,* Çekirdek DNA içeriği, Çekirdek alanı, Mikrospektrofotometre.

Introduction

Nuclear DNA contents of angiosperm species have been published in numerous papers (1–3). Researchers have shown that the DNA content per genome is usually constant and therefore is characteristic for each species (4, 5). However, considerable interspecific variation in DNA content per genome has been noted (6–8). Changes in nuclear DNA sequence occur in the divergence and evolution process of species (9–11).

Many organisms contain more nuclear DNA than is apparently required for genetic and regulatory function (12, 13). Much research has been carried out in order to clarify the source and function of extra DNA and it has been indicated that it is the repeated fraction of the genom that is largely responsible for rapid changes in the DNA content in plants (14–16).

Quantitative and qualitative composition of the nuclear DNA content can vary dynamically not only between tissues within a single organism but also within the same cell during the course of the development of the organism (17). Knowledge of the nuclear DNA variation is useful for cytotaxonomical and evolutionary studies.

The genus *Hedysarum* L. belongs to the family *Fabaceae (Leguminosae)* and comprises 21 species in Turkey, 11 of them endemic (18). The basic chromosome number in the genus is x = 8 (19). According to the literature, the cytological studies concerning *Hedysarum* species are seldom. The aim of the present work was to determine the nuclear DNA contents and nuclear areas of some endemic *Hedysarum* species.

Materials and Methods

The seeds of *Hedysarum* species were directly collected from wild populations in the Central and East Anatolian regions of Turkey (Table 1). The *Hedysarum* species used are endemic to Turkey, except for *Hedysarum varium* Willd.

The seeds were germinated on filter paper moistened with distilled water in petri dishes at 24°C. After three

days, primary root tips about 1–2 cm in length were excised and fixed in freshly made 3:1 absolute ethyl alcohol: glacial acetic acid (v/v) for 24 hours at 4° C. Root tips were washed in distilled water for 30 minutes. Thereafter, they were hydrolysed in 5N HCl for one hour and stained in Feulgen solution for one hour (Feulgen solution: 0.5 g basic fuchsin and 0.5 g potassium metabisulfite ($K_2S_2O_5$) were mixed in 15 ml of 0.15 N HCl, 85 ml distilled water was added and the mixture was shaken on a mechanical shaker 24 hours in darkness at 4° C. Thereafter, 0.5 g activated charcoal was added and filtered through Whatman No. 1 filter paper in a funnel). The stained root tips were washed in three changes of SO_2 water for 10 minutes each and dried briefly on absorbent paper. Darkly stained root tips were squashed in 45% acetic acid (20). Photometric measurement of 2C telophase nuclei were made, using a Reichert Zetopan microspectrophotometer, at a wavelength of 550 nm. On average, 35 2C telophase nuclei were measured in each of the three replicates in every species.

Measurements were converted into absolute amounts using *Allium cepa* L. as a standard (2C = 33.5 pg) (4). The areas of nuclei were determinated with an ocular micrometer, at least 30 nuclei per slide. In addition, the approximate values for nucleotide pairs for each species were calculated (21), by the equation $1pg \cong 9.13x10^8$ nucleotid pair.

The differences in the DNA content were tested by analysis of variance (ANOVA) and comparisons between means were performed with the Tukey test.

Results and Discussion

The somatic chromosome numbers of the six *Hedysarum* species were determined to be 2n=16. The nuclear DNA amounts and nuclear areas of the species are presented in Table 2. From this table, we observed that there is a variation in the nuclear DNA amount, ranging from 4.65 pg in *H. pycnostachyum* Hedge & Hub.-Mor. to 6.75 pg in *H. rotundifolium* Boiss. & Noë; and also in the nuclear area, ranging from 48.40 in *H. varium* to 70.27 in *H. rotundifolium.* Although there were no differences found in the chromosome numbers (2n=16) among the *Hedysarum* species, there were significant differences in the 2C nuclear DNA contents. Analysis of variance did not show statistically significant differences in the nuclear DNA amount of *H. aucheri* Boiss. (5.65 pg), *H. pestalozzae* Boiss. (5.52 pg) or *H. nitidum* Willd. (5.42 pg). In the same way, no differences were found in the nuclear DNA amount between *H. varium* (4.92 pg) and *H. pycnostachyum* (4.65 pg). Table 2 also shows approximate values for nucleotide pairs of the *Hedysarum* species. It was found that the nucleotid pairs were highest in *H. rotundifolium* and lowest in *H. pycnostachyum.*

The correlation and regression analysis between the nuclear DNA contents and the nuclear areas are shown in Figure 1. A very high positive correlation between the nuclear area was observed. A similar finding was also reported in *Vicia* L. (15).

It has been suggested that the quantitative changes in the nuclear DNA of diploid *Vicia* species (22) and the genus *Lathyrus* L. (11, 14, 23) are achieved by changes in the amounts of both repetitive and non–repetitive DNA sequences. The variation in the 4C nuclear DNA content within *Secale* L. is largely due to the amount of heterochromatin located at or close to the telomeres (6). DNA–DNA hybridization showed that six species of *Vicia* contain a fraction of DNA with nucleotide sequence of varying degrees of repetition (13). As a result, we could say that quantitative changes in the nuclear DNA contents of *Hedysarum* species could be attributed to differences in both repetitive DNA sequence and amounts of heterochromatin. Although we were not able to examine the analysis of repeated DNA sequences, we strongly believe that further studies in the field are necessary in order to provide more detailed insight on the quantitative DNA variation in *Hedysarum* species.

Table 1. Localities of six *Hedysarum* species.

Species[x]	Locality and altitude
H. varium	B6 Sivas: Sivas to Hafik, Seyfebeli, 1300 m
H. pestalozzae	B6 Sivas: Sivas to Hafik, Seyfebeli, 1300 m
H. nitidum	B7 Erzincan: Refaiye to Imranlı, 1600 m
H. aucheri	B7 Malatya: Malatya to Hekimhan, 1100 m
H. rotundifolium	B7 Malatya: Malatya to Arapkir, 750 m
H. pycnostachyum	B7 Malatya: Kale, Bridge of Kömürhan, 800 m

x: Species were arranged in order of evolutionary sequence, simplest first.

Table 2. The 2C nuclear DNA amounts and nuclear areas of six *Hedysarum* species.

Species	Chromosome number (2n)	DNA amount (pg) Mean±S.E*	Mean nuclear area (arbitrary units)	Average DNA per chromosome	Nucleotid pair
H. varium	16	4.92 ± 0.10 a	48.40	0.61	4.49 x 10^9
H. pestalozzae	16	5.52 ± 0.06 b	57.45	0.69	5.04 x 10^9
H. nitidum	16	5.42 ± 0.01 b	56.41	0.68	4.95 x 10^9
H. aucheri	16	5.65 ± 0.10 b	58.81	0.71	5.16 x 10^9
H. rotundifolium	16	6.75 ± 0.03 c	70.27	0.84	6.16 x 10^9
H. pycnostachyum	16	4.65 ± 0.15 a	51.21	0.58	4.24 x 10^9

* Means with the same letters do not significantly differ in their nuclear DNA content at 0.05 level.
S.E.: Standard Error.
pg: picogram.

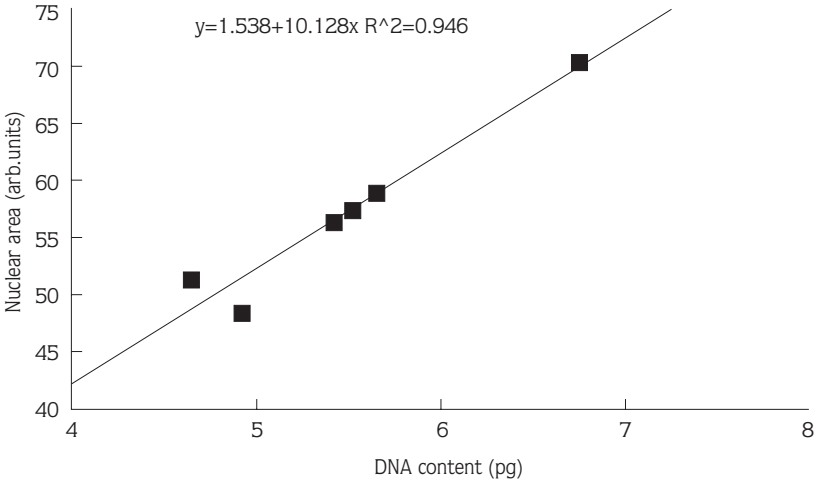

Figure 1. Relationship between the nuclear DNA content and nuclear area.

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