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Karyotype and seed protein profile analysis of diploid and tetraploid *Hordeum bulbosum* L.

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With 3 Figures (1 Plate)

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Summary

Symeonidis L. A., Moustakas M. B. & Coucoli H. D. 1985. Karyotype and seed protein profile analysis of diploid and tetraploid *Hordeum bulbosum* L. — Phyton (Austria) 25 (1): 31—38, with 3 figures. — English with German summary.

Two native greek diploid and tetraploid cytotypes of *Hordeum bulbosum* L. (*Poaceae*) morphologically similar, were collected from two separate localities and studied by combining karyotype analysis and seed protein profiles obtained by isoelectric focusing. The karyotype of the diploid cytotype (2n = 14) was fully established and it was found a symmetrical one. In the tetraploid cytotype, six chromosome pairs, recognized as markers, were found to present in double presence three corresponding pairs of the diploid complement. The protein profiles received from both cytotypes of *H. bulbosum* were found to possess the same 22 pI bands, only some bands are different in intensity. The data corroborate the view that *H. bulbosam* 4x is a case of typical autotetraploidy and they are discussed and interpreted on the basis of gene dosage effect.

Zusammenfassung

Symeonidis L. A., Moustakas M. B. & Coucoli H. D. 1985. Analyse der Karyotypen und der Samenprotein-Profile von diploidem und tetraploidem *Hordeum bulbosum* L. — Phyton (Austria) 25 (1): 31—38, mit 3 Abbildungen. — Englisch mit deutscher Zusammenfassung.

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Die morphologisch sehr ähnlichen diploiden und tetraploiden Cytotypen von *Hordeum bulbosum* L. (*Poaceae*) wurden an Hand je einer Population aus Griechenland auf Karyotyp und Samenprotein-Profil (erhalten mittels isoelektrischer Fokussierung) untersucht. Der diploide Karyotyp (2n = 14), der einen recht symmetrischen Chromosomensatz aufweist, ist als Idiogramm dargestellt. Die „Marker-Chromosomen" (drei Paare des diploiden Satzes) wurden im tetraploiden Karyotyp in doppelter Anzahl wiedergefunden. Die Proteinprofile beider Cytotypen besitzen die selben 22 pI-Banden, bei einigen Banden traten Unterschiede in der Stärke auf. Die Ergebnisse unterstützen die Ansicht, daß in *Hordeum bulbosum* 4x ein typischer Fall von Autotetraploidie vorliegt, sie werden auf der Basis des Gen-Dosis-Effektes diskutiert.

Introduction

Among the cytologically distinct known taxa of the genus *Hordeum*, about 40 in number according to Bothmer & al. 1981, *H. bulbosum* L. has been recognized as one of the two distinct allogamous species of the genus, possessing a sporophytic incompatibility system (Lundqvist 1962, Bothmer 1979).

This particular species is known to contain two distinguished cytotypes, one diploid and one tetraploid, the latter being more widespread. More concretely, *H. bulbosum* is a tall perennial species, distinguished by its bulb formation and found chiefly in the Mediterranean region, the Balkans and Asia Minor (Vosa 1976). The two cytotypes co-exist in Greece, since the diploids occur in the Mediterranean area up to W — E Greece, whereas the tetraploids can be found from Central Greece eastwards (Jörgensen 1982).

Because of their most prominent presence and distribution tetraploids of *H. bulbosum* have been firstly studied and reported in connection with investigations of somatic chromosome numbers (Ghimpu 1932, Kuckuck 1934, Chin 1941).

On the parallel the tetraploid cytotype has been largely used in hybridization experiments with *H. vulgare* L., (Kuckuck 1934, Konzak & al. 1951, Rajhathy & al. 1964). More recently, Jensen 1977 developed "the bulbosum method" and applied it in intergeneric crosses for the production of polyhaploids in the *Triticeae*, whereas Shigenobu & Sakamoto 1981 managed to obtain successful intergeneric hybrids with *Agropyron*.

Diploid *H. bulbosum* (2n = 14) had not been reported as an object of cytological work but in 1959 by Morrison. Since then, a number of workers utilized it both in cytological and hybridization experiments (Bowden 1965, Sadasivaiah & Kasha 1973, Vosa 1976, Jörgensen 1982).

The purpose of the present work is to check the cytotype constitution and ploidy relationships of *H. bulbosum* plants collected in the

field, and to document the results by using the corresponding seed protein profiles obtained by isoelctric focusing. The additive properties of the seed protein profiles have been effectively utilized in studies aiming at elucidating the nature of ploidy levels in plants LADIZINSKY & HYMOWITZ 1979).

Materials and Methods

Plants recognized as almost similar morphologically, were collected in two Greek localities, namely, in Ioannina (Epiros) and Trikala (Thessaly), representing the diploid and tetraploid *H. bulbosum* populations respectively.

The usual Feulgen technique of staining and squashing was applied for the cytological observations and the microscopic method of handling and measuring the slides was performed as described in an earlier paper (COUCOLI & SYMEONIDIS 1980).

Water soluble proteins were extracted from bulked seeds (collected randomly from about thirty plants of each population) by grinding mature seeds with cold (4° C) distilled water over an ice bath. The resulting mixture was centrifuged at 10.000 g for 15 min. and the supernatant was lyophilized. Electrophoresis was carried out using the isoelectric focusing method (MOUSTAKAS & al. 1983). A polyacrylamide gel containing 2.2% w/v carrier ampholite with pH range 4.0—6.5 was used.

Results and Discussion

1. Karyotypes

Diploid *H. bulbosum* (2 n = 14)

The established karyotype appeared as a symmetrical one with chromosomes varying in length between 5 and 7 μm approximately (Table 1). The chromosome set contains two distinct chromosome pairs (Fig. 1 and 2), one SAT pair (No 6 in the idiogram, Fig. 1) and one pair of rather long, prominently heterobrachial chromosomes (No 3) with almost subterminal centromeres (arm ratio a. r. = 0,47—0,52). All the other members are either metacentric or submetacentric without conspicuous features. This symmetry makes their identification rather difficult and perhaps not worthwhile for preparing full idiograms of individual chromosomes.

However, according to the data of the present work, as presented in Table 1 and illustrated in the corresponding idiogram (Fig. 1) a good step towards identifying particular members of the genome has been accomplished due to the following points. Chromosome No 2 can be easily distinguished from No 1 (not significantly differing in chromosome size)

Table 1

Length of chromosomes in absolute mean values (μm) and relative lengths measured in 10 plates of the diploid *H. bulbosum*

Chrom. pair	Total Absolute Length	Short Arm Length	Long Arm Length	Relative Length	Arm Ratio	Centromere Class
1	7.14±0.48	3.05	4.09	16.51	0.74	SM
2	6.74±0.39	3.11	3.63	15.58	0.85	M
3	6.46±0.60	2.10	4.36	14.94	0.48	ST
4	6.20±0.42	2.76	3.44	14.33	0.80	M
5	5.69±0.32	2.49	3.20	13.16	0.78	M
6	5.63±0.38	2.52 (1.54+0.98*)	3.11	13.02	0.81	M
7	5.39±0.35	2.32	3.07	12.46	0.75	SM
Genome Length	43.25±2.94	—	—	—	—	—

* Satellite length

by being constantly and strictly metacentric. The shortest member of the genome (No 7) seems to be fairly submetacentric approximating the lowest threshold of SM class (a. r. = 0.75). Only two members (No 4 and No 5) remain not discernible from each other being both metacentric and overlapping in size.

In general, the chromosomes of this particular species were found to have large absolute chromosome size, comparable to that of *H. vulgare* species (Coucoli & al. 1981). The variation in size between the

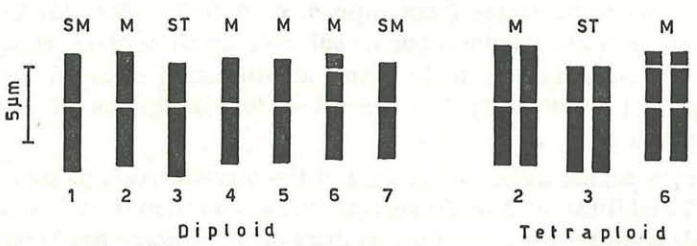

Fig. 1. Idiogramm of the two cytotypes of *Hordeum bulbosum*. Diploid karyotype and chromosome markers of the tetraploid

different members of the complement appeared rather low, i. e. the shortest chromosome is equal to 75.5% the length of the longest one (5,39 μm/7,14 μm).

By comparing the present data with the information derived from MORRISON's 1959 drawing on diploid material of Italian origin, yet without measurements and quantitative analysis, we can reassure the similarity of the chromosome morphology but the SAT pair looks different, being more heterobrachial in MORRISON's paper, i. e. a typical SM member. The same holds for the SAT pair of the diploid *H. bulbosum* (origin from Italy and Caucasus) in the C-banded karyotype presented by VOSA 1976. Instead, in our material the satellited chromosome was recognized as typically metacentric. The different origin of the materials should be a reasonable excuse for the observed changes since SAT chromosomes in the *Triticeae* are well known to manifest morphological variation of shape and indices among different populations or varieties of one species (HENEEN 1977, COUCOLI & al. 1981). The presence of typical SM satellited chromosomes occurs more frequently among the wild *Hordeum* species studied so far (RAJHATHY & al. 1964, VOSA 1976, COUCOLI & SYMEONIDIS 1980).

Tetraploid *H. bulbosum* (2n = 28)

Six chromosome pairs have been identified and recognized as three groups of four members each expressing a high degree of similarity (Table 2). In other words, the crucial markers are represented by the quadruple presence of the following chromosome types: 1) four SAT chromosomes similar morphologically in length and arm ratios (Fig. 1 and 2). The two pairs seem to be identical to the SAT pair of the diploid *H. bulbosum*. 2) two long pairs, strictly metacentric in centromere class

Table 2

Length of chromosome markers in absolute mean values (μm), measured in 7 plates of the tetraploid *H. bulbosum*

Group of 4 chrom. each	Total Absolute Length	Short Arm Length	Long Arm Length	Arm ratio	Centromere Class
2	6.96±0.63	3.32	3.64	0.91	M
3	6.17±0.95	2.01	4.16	0.48	ST
6	5.71±0.39	2.61 (1.64+0.97*)	3.10	0.84	M

* SAT length

(a. r. = 0.91) and very similar with pair No 2 of the diploid. 3) a group of four members, represented by median in size, almost subterminal chromosomes (a. r. = 0.48), identical to chromosome pair No 3 of the diploid.

The comparsion between the chromosome markers of the tetraploid and the corresponding pairs of the diploid shows obviously that at least on the basis of chromosome morphology the situation of *H. bulbosum* 4x is a typical case of autotetraploidy as it was previously assumed on the basis of multivalent formation at meiosis (Berg 1936, Lein 1948, Katznelson & Zohary 1967). The results are in agreement with Morrison's 1959 statement that tetraploid *H. bulbosum* is an example in *Hordeum* where no change has occured in the morphology of the chromosomes. However, Shigenobu & Sakamato 1981 using a certain accesion of tetraploid *H. bulbosum* (HB-7) to study the degree of autosynthesis in multivalents, doubted the unquestionably accepted view of autopolyploidy.

2. Seed protein profiles

The isoelectric focusing patterns of seed soluble proteins, which were extracted from diploid and tetraploid plants of *H. bulbosum* L. were found to possess the same pI bands (Fig. 3). The characteristic protein profile was recognized to be composed of 22 bands. No qualitative protein differences were noticed and the observed variation concerned actually the intensities of some particular bands among which bands numbered 3, 4, 5 and 13 manifested the most conspicuous intensity differences.

According to the different band intensity relationships between the diploids and tetraploids we classified the protein bands of the profiles into three types, using partially the way applied by Nakai 1977.

Type I. The same pI bands with the same intensities, in the two levels of ploidy (the majority of the bands);

Type II. The same pI bands with increased amount of protein in the tetraploid (bands 3, 4, 5 and 7);

Type III. The same pI bands with increased amount of protein in the diploid (bands 13 and 22).

In parallelizing the protein profiles of the two populations, differing in ploidy level, the decisive role of gene dosage functioning towards maintaining, increasing or decreasing the amount of protein, is evident. More concretely, in the case of type I bands, the double dose of genes responsible for the water soluble proteins did not affect the amount of their products. The situation does occur between a diploid and its autotetraploid, as mentioned by Nakai 1977 in the protein profile of *Astragalus*.

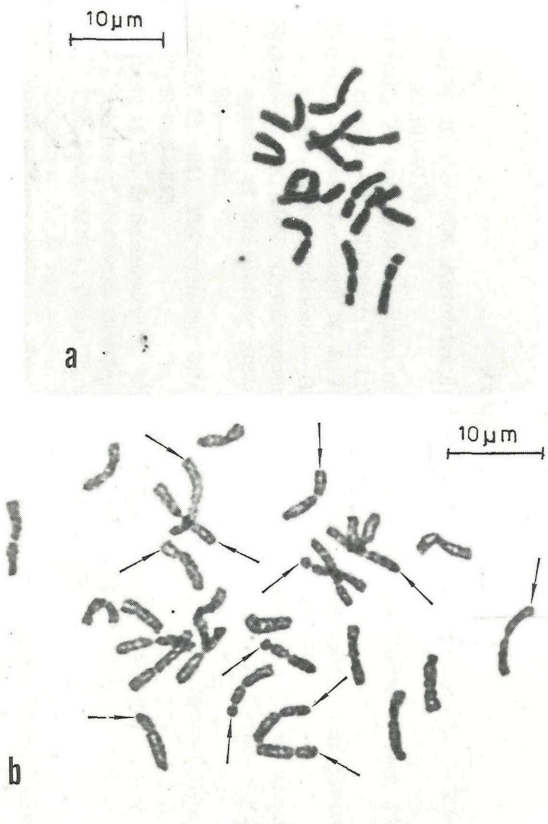

Fig. 2. Metaphase cells from root tips of the two *H. bulbosum* materials. a = diploid, b = tetraploid. Arrows indicate chromosome markers

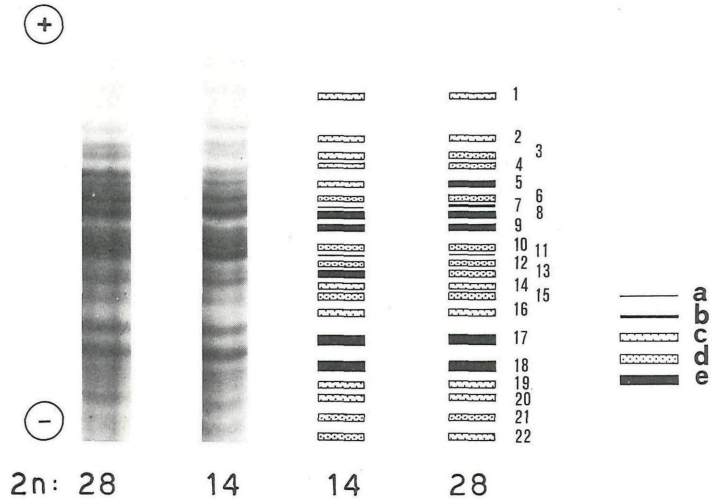

Fig. 3. Seed protein profiles of *H. bulbosum* (diploid and tetraploid). Photograph and corresponding schematic representation. On the right: Key to shading in order of increasing band intensities

The increased amount of protein found in the tetraploid (type II, four pI bands with higher intensity) and in the diploid (type III, two intense bands) represents another expression of the gene activity when it functions in double dose. Here no qualitative change of the products can be caused but an alteration of their quantity is certainly brought about. In other words, the particular bands are probably under control of quantitative gene systems (LADIZINSKY & HYMOWITZ 1979). NAKAI 1977 assumes that the increased amount of certain protein bands in some autotetraploids comparatively to their original diploids seems to be due to the change of ratio in structural and regulatory genes. He also suggests that the situation of increased amount of certain protein bands in the diploids comparatively to tetraploids (found in strains of *Brassica oleracea)* is dependent on an increased dose of regulatory genes being of primary importance in determining the protein amount of the affected bands.

The seed protein profiles studied in the particular greek populations of *H. bulbosum* (diploid and tetraploid) corroborate the data derived from morphological identity and chromosomal similarity of the two cytotypes. The data support the view that the tetraploid evolved as a typical autotetraploid, where the doubling of the chromosome number has not, or very slightly affected seed protein profiles (LADIZINSKY & JOHNSON 1972, NAKAI 1977). It should also be added that even autotetraploidy in certain instances can give a new property to an organism. There are already biochemical data from leaf esterases (JÖRGENSEN unpubl.) showing variant alleles in the tetraploid which could not so far be found in the diploid.

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