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TETRAPLOID POPULATIONS OF BULBOUS BARLEY (*HORDEUM BULBOSUM* L.)

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Hordeum bulbosum L., according to our results, occurs as tetraploid (2n = 4x = 28) along the Adriatic coast, in Serbia and Macedonia (Fig. 1).

A comparison of Feulgen karyotypes (Fig. 2) and the C-banding pattern of all the populations examined (Table 1) shows a very small variation. It is most likely that there is a close relationship among them.

Introduction

Perennial barley (*Hordeum bulbosum* L.) with a bulbous stem is a submediterranean floral element (Domac 1967, Cincović and Kojić 1976). A chromosome investigation of bulbous barley was carried out very early (Kuckuck 1933), but it only dealt with chromosome counts 2n = 28. Later investigation considered diploids (2n = 14) as well as the behaviour of chromosomes in meiosis, first without indication of localities (Berg 1936) and next with a list of localities and habitats of all the individuals investigated (Lein 1948, Katznelson and Zohary 1967).

Interest in the distribution and evolution of karyotypes of *H. bulbosum* has increased in the last thirty years, for practical reasons of modern breeding technique application. »The Bulbosum Method« (Konzaki et al. 1951, Jensen 1977) gives an opportunity for the production of a relatively large number of homozygous diploids of agricultural barley (*H. vulgare* L.). This method necessarily involves the crossing of cultivated barley with the wild perennial *H. bulbosum*.

According to the literature data (Lein 1948, Katznelson and Zohary 1967) a *H. bulbosum* species occurs in two chromosomal types:

a diploid ($2n = 2x = 14$) and a tetraploid ($2n = 4x = 28$). Diploids and tetraploids are morphologically relatively similar, they only differ in size and are geographically differently distributed. Diploids occur in the western and central parts of the European and African Mediterranean region. Their geographical discontinuity seems to depend on ecological requirements. Although diploid bulbosum does not seem to be a common plant, its affiliation to a specific phytocenological association has not been stated. The main geographical centre of tetraploids is the Middle East. To the west of their centre they occupy parts of Bulgaria, Yugoslavia and Greece, whereas to the north they enter Central Asia. Tetraploid forms are in general much more continuously distributed than the diploid ones.

As both chromosome types appear together only in the region of Albania (Lein 1948) and Greece (Katznelson and Zohary 1967), the assumption was that a similar situation could be found in parts of South Macedonia.

The aim of the investigation was to find out the level and character of polyploidy, as well as to gather information about the distribution and ecological affinity of the *H. bulbosum* populations in Yugoslavia.

Material and Methods

All the livinig plants examined were grown at the Botanical Garden of the Zagreb University Department of Botany, from plants collected in the fields (Table 1). The plants showed a very high adaptability to new climatic conditions.

Voucher specimens of these plants are kept in a herbarium. Taxonomic remarks and conclusions are partly based on this material and partly on the herbarium material of the Zagreb University Department of Botany, Professor I. Trinajstić's (Faculty of Forestry, University of Zagreb) and dr. J. Matevejeva's (Skopje) herbariums.

Root-tips were pretreated (0.05% aqueous colchicine for 3 h at room temperature or 1-bromonaphthalene at a temperature of about 4° C for about 22h), and were subsequently fixed in 3 : 1 ethanol/acetic acid overnight.

Table 1. Source and chromosome number of *Hordeum bulbosum* populations examined.

Locality	Habitat	Collector	2n
Sv. Katarina, island near Rovinj (Istria)	path's edge	V. Bosiljevac	28
Premantura (Istria)	path's edge	J. Topić	28
Zadar	path's edge	E. Kletečki	28
Solin	cultivated field's edge	I. Trinajstić	28
Solin	abandoned field	V. Bosiljevac	28
Canyon of the Crni Drim (Macedonia)	cultivated field's edge	I. Trinajstić	28
Prespansko jezero lake in Macedonia	abandoned field	Lj. Grupče	28

One part of the material was stained with Feulgen after hydrolisis in 1 M HCl at 60° C and squashed in the usual way.

The other part of the material was stained differentially with Giemsa C-banding technique by Noda and Kacha (1978) using 7 min hydrolysis at 60° C in 1 M HCl, 1% solution of pectinase at 37° C for 13 min, and a 35 seconds immersion in 0.07 M NaOH. Staining was in 60 × diluted Giemsa solution (Merck) in Sörenson phosphate buffer, pH 6.9 for about 40 min, after which slides were rapidly air dried prior to mounting in Euparal.

Well-defined Feulgen stained cells were drawn with a Zeiss drawing tube attachment and chromosomes were karyotyped.

Stages of meiosis were obtained by squashing fresh pollen mother cell in 2% aceto-orcein. Pollen stainability was counted after the staining in 1% aceto-carmine.

All the slides were made permanent by the use of liquid CO_2.

Results and Discussion

Bulbous barley *(H. bulbosum)* is not a common range grass in Yugoslavia contrary to the description by Katznelson and Zohary (1967). It is distributed along the Adriatic coast (Istra, some islands, partly in Dalmatia, Hercegovina and Montenegro), on the territory of Serbia (Timočka krajina, Stara Planina and Kosovo) and in large parts of eastern and southern Macedonia (Soška 1953, Katznelson and Zohary 1967, Cincović and Kojić 1976). Conclusions are also partly based on our field observation and on the different herbarium material (see Material and Methods).

Table 1 and Fig. 1 show that all the populations investigated contained only tetraploids with 2n = 4x = 28 chromosomes. With regard to their habitat, it seems that all the tetraploid populations colonize new ecotypes such as forest margins, edges of paths, and edges of cultivated and abandoned fields, which is in full accordance with the observations by early investigators (Katznelson and Zohary 1967).

The Feulgen karyotypes of tetraploid *H. bulbosum* were drawn (Fig. 2) and compared with C-banded karyotypes. About ten karyotypes from each population have been analysed. A comparison of karyotypes from all the populations has yielded very similar results to those of the comparison of karyotypes within a single population. Chromosomes are similar in morphology. They were ranged in seven groups of four chromosomes: meta- to submetacentrics (groups 1, 2 and 6), submetacentrics (groups, 3, 4 and 5) and submetacentrics with a secondary constriction each (group 7).

The most obvious feature of the C-banded karyotypes of tetraploid *H. bulbosum* was the presence of prominent centromeric bands. Secondary constrictions had a rather small amount of constitutive heterochromatin (Figs. 5 and 6).

Chromosome group 1 regularly contained a bigger chromosome, two very similar ones and a more or less different one (Fig. 2). Heteromorphy amongst chromosomes of group 1 was noticeable owing to different patterns of centromeric bands. Again, the biggest chromosome had a smaller centromeric band than the others (Fig. 6). Variation among chromosomes of all groups was noticed as well (Figs. 2—6). Some karyo-

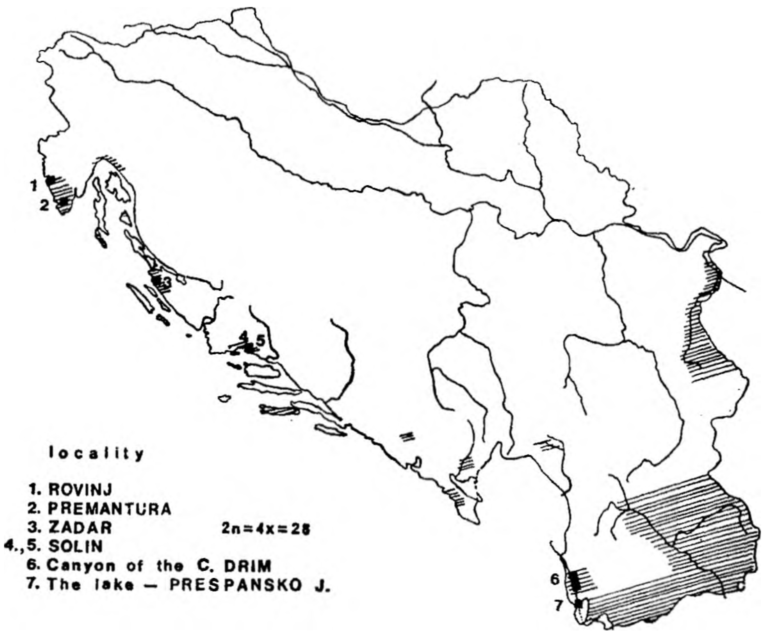


Fig. 1. Distribution of H. *bulbosum* in Yugoslavia and populations investigated (1—7).

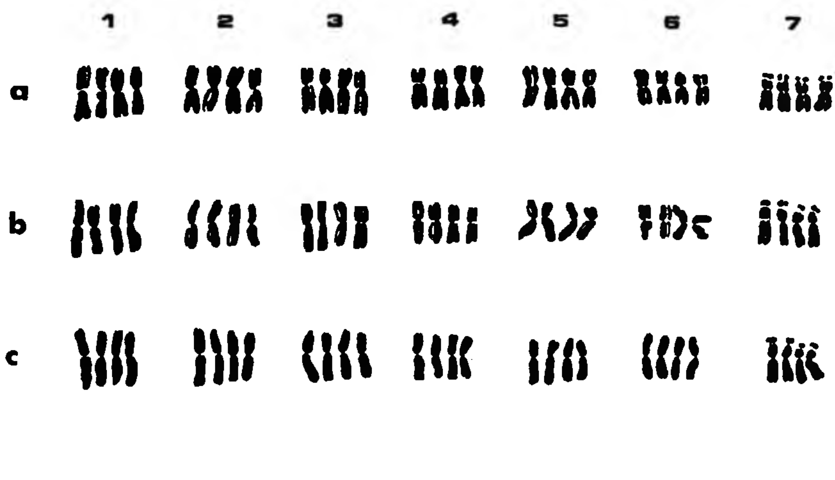
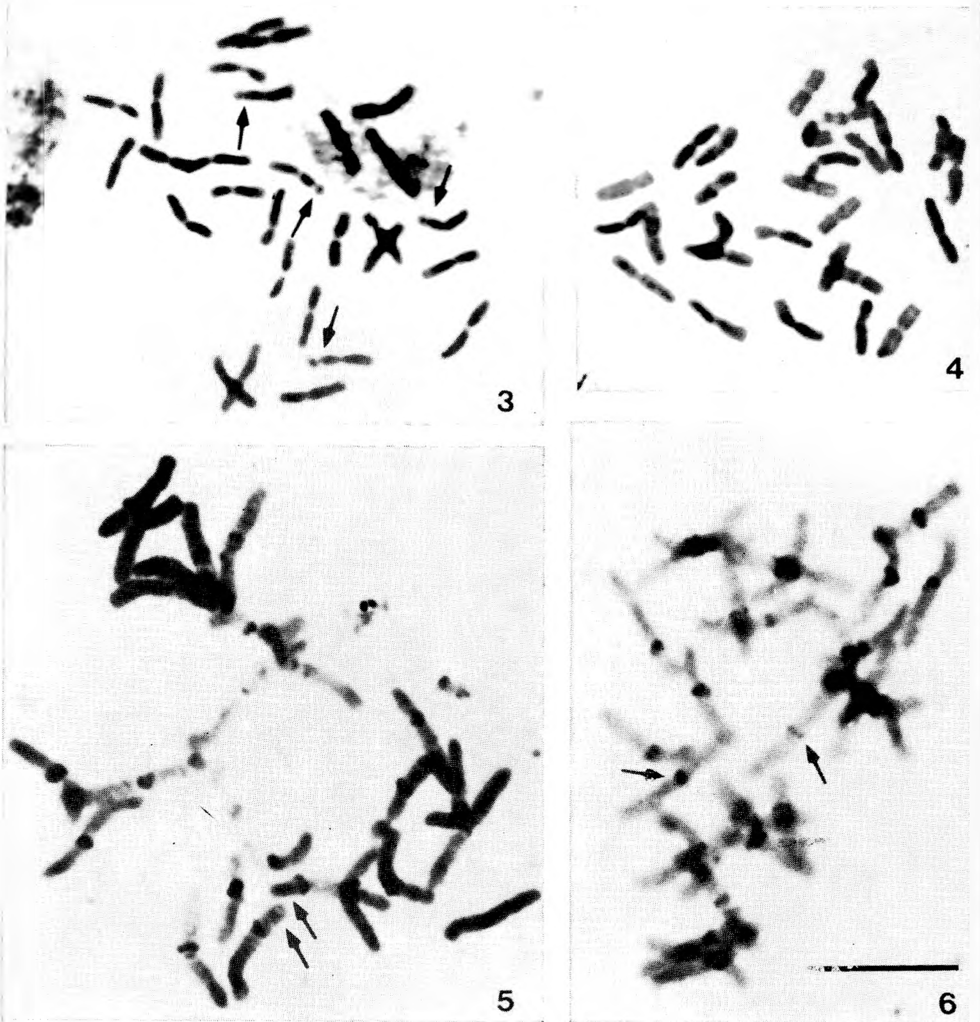
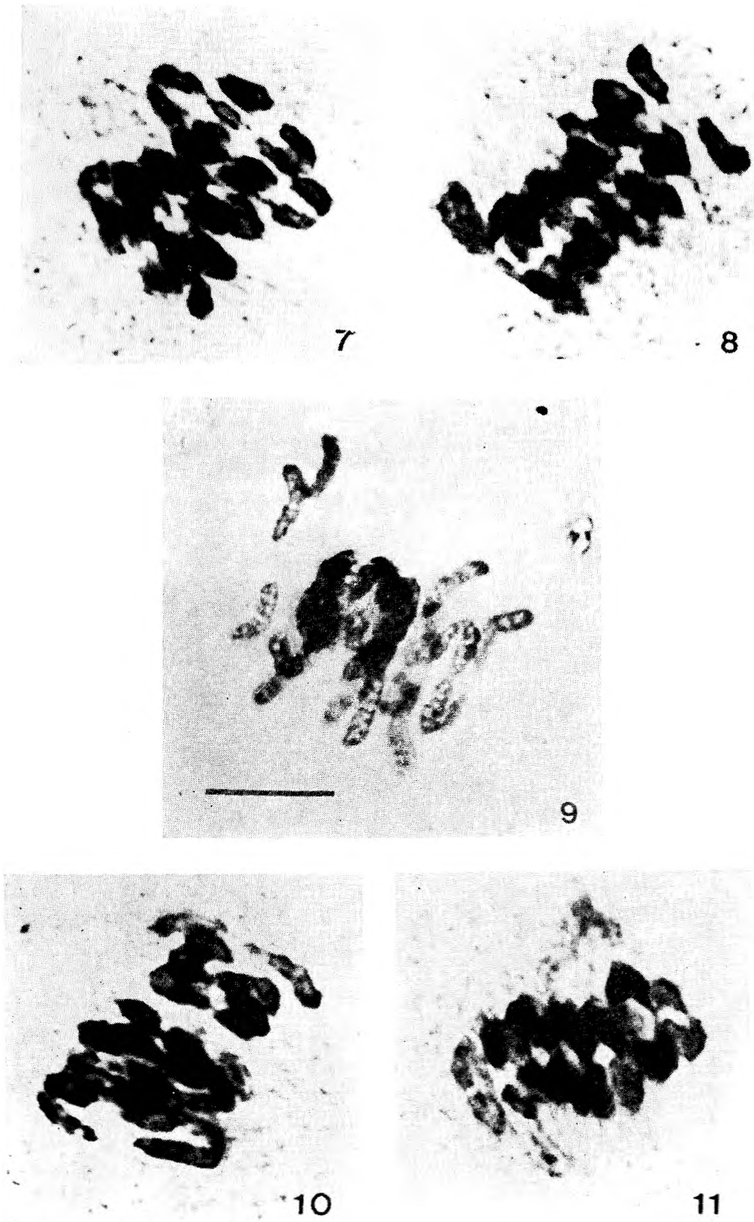


Fig. 2. Feulgen karyotypes of different *H. bulbosum* populations (2n = 4x = 28). Chromosome groups are numbered from 1 to 7 for clarity: Bar: 10 µm.



Figs. 3—6. Mitotic chromosomes of tetraploid *H. bulbosum* (2n = 4x = 28). Figs. 3 and 4: Feulgen karyotypes, satellited chromosomes are arrowed. Figs. 5 and 6: Giemsa C-banded chromosomes, 5 showing heteromorphic pair of satellited chromosomes (arrows), and 6 showing heteromorphic pair of metacentrics (arrows). Bar: 10 μm.



Figs. 7—11. Meiotic chromosomes of *H. bulbosum* (2n = 28), showing different types of quadrivalent formation, which are predominating; also trivalents, bivalents, univalents and a multivalent association of 6. Bar: 10 µm.

types were heteromorphic for the short arm of one satellite chromosome (Fig. 2c). It has been noticed that satellite chromosomes are often involved in the formation of an anaphase bridge. This behaviour could explain the heteromorphy of the chromosome group 7. Similar but more obvious instability of a heteromorphic SAT-chromosome has been described in tetraploid *Ranunculus ficaria* L. (Marchant and Brighton 1971). Heteromorphy of one satellite chromosome was also noticable because of the presence of a bigger satellited band (Fig. 5). Some heteromorphy occurred in other groups as well, where 1 or 2 chromosomes usually bore intercalary bands while others had none.

At metaphase I of meiosis, quadrivalents were frequently 2 to 5 per cell in combination with bivalents, trivalents and univalents (Figs. 7—11). This behaviour of chromosome pairing is of the general autotetraploid type, the same as described by Berg (1938) and Chin (1941). In addition, they formed higher multivalent associations of 5 to 6 chromosomes in a substantial number of cells, showing that they were heterozygous for some interchanges. The occurrence of anaphase bridges in meiosis I and II suggests the possibility of an inversion in one or several chromosomes. Intercalary bands have been noticed in some chromosomes, but we have not been able to get enough C-band karyotypes for the detection of inversions.

Pollen stainability of tetraploid barley was very high, over 90%, as high at the pollen fertility described by Lein (1948).

Comparison of the Feulgen karyotypes and banding pattern analysis allows the hypothesis that the following mechanisms have been involved in the evolution of the recent tetraploid form of *H. bulbosum*: autotetraploidisation, partial alotetraploidisation and the still existing structural changes. Predominant vegetative reproduction, the existence of a high pollen fertility (over 90%) and easy adaption to different ecological factors prove that tetraploid *H. bulbosum* is a species which »invades« new ecotypes.

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SAŽETAK

TETRAPLOIDNE POPULACIJE VRSTE *HORDEUM BULBOSUM* L. U JUGOSLAVIJI

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Višegodišnji ječam (*Hordeum bulbosum* L.) s lukovičasto zadebljalom stabljikom predstavlja submediteranski florni element. U Jugoslaviji dolazi u primorskom području, teritoriju istočne Srbije i na velikom području istočne i južne Makedonije.

Primjerci svih ispitivanih populacija predstavljaju tetraploide ($2n = 4x = 28$). Kariološka ispitivanja obuhvatila su morfološku i strukturnu analizu somatskih kromosoma te parenje kromosoma u mejozi. Usporedbom kariotipova nisu zamijećene bitne razlike između populacija kao ni unutar pojedine populacije. Kromosomi su slične morfologije svrstani u sedam grupa (po četiri u svakoj). Prema položaju centromera klasificirani su kao metasubmetacentrični (tri grupe), submetacentrični (tri grupe) i submetacentrični sa sekundarnom konstrikcijom (jedna grupa). Analiza distribucije konstitutivnog heterokromatina podudara se s morfološkom analizom. U mejozi kromosomi se sparuju uglavnom u kvadrivalente, rjeđe trivalente, bivalente i univalente, a javljaju se i veće konfiguracije od kvadrivalenata.

Usporedba rezultata kariološke analize dopušta pretpostavku da su u evoluciji današnjeg tetraploidnog oblika vrste *Hordeum bulbosum* vjerojatno bili uključeni mehanizmi autotetraploidizacije, djelomične alotetraploidizacije te strukturnih promjena prisutnih i danas. Snažno vegetativno razmnožavanje uz postojanje visoke fertilnosti polena (preko 90%) te lako prilagođivanje različitim ekološkim faktorima ukazuju na to da je tetraploid *Hordeum bulbosum* vrsta koja »osvaja« nove ekotipove.

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