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Polymorphism at rDNA loci in barley and its relation with climatic variables

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Abstract The variation in length of the intergenic spacer (IGS) region of the ribosomal DNA repeat unit was examined in 63 accessions of wild barley, *Hordeum spontaneum*, and seven accessions of cultivated barley, *Hordeum vulgare*. The accessions of wild barley were collected from ecologically diverse climatic and edaphic microsites in Israel, and the barley cultivars were those grown in India. Sixteen spacer-length variants (slvs) observed in the present study presumably belonged to two known rDNA loci (*Rrn1* and *Rrn2*). Each accession had one or more variants, which together represented the rDNA phenotype. The rDNA phenotypes of wild barley accessions were widely diverse and differed substantially from those of cultivated barley. The slv phenotypes and the corresponding alleles were shown to be largely correlated with different climatic, edaphic and ecogeographical microsites and niches (the "Evolution Canyon" at Lower Nahal Oren, Mount Carmel; and Tabigha, Eastern Upper Galilee Mountains), so that a particular rDNA phenotype of an accession could be used to predict the climate and soil to which the accession belonged. This sharp microsite ecogeographic variation in ribosomal DNA appears adaptive in nature, and is presumably driven by climatic and edaphic natural selection.

Keywords rDNA · Spacer-length variants · Adaptation · Barley · Ecogeographic variation · Natural selection

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Introduction

Eukaryotic ribosomal RNA genes (known as ribosomal DNA or rDNA), that encode for 18 S, 5.8 S and 26 S ribosomal RNAs (rRNAs), are found as parts of repeat units that are arranged as tandem arrays, located at the chromosomal sites known as nucleolar organizing regions (NORs) (Long and Dawid 1980; Jorgensen and Cluster 1988; Clegg 1989; Dvorak 1989). Each repeat unit consists of a highly conserved coding region (for 18 S, 5.8 S and 26 S rRNAs) and a variable intergenic spacer (IGS) non-coding region. The latter is, however, believed to contain some regulatory sequences for modulating the transcription of adjacent rRNA genes. IGS itself consists of a non-transcribed spacer (NTS) region flanked by external transcribed spacers (ETS) at its two ends. In the coding region also, on either side of 5.8 S rRNA gene are found internal transcribed spacers (ITS), described as ITS1 and ITS2.

The intergenic spacer (IGS) region contains motifs referred to as subrepeats that range from 100 to 300 bp in length in plants. In barley, these subrepeats are 115-bp long. The length of subrepeats, within most individual species, does not differ by more than a few base pairs and their sequences show a high degree of similarity (e.g. Appels and Dvorak 1982; Arnheim et al. 1982). However, the number of subrepeats within a rDNA repeat is often quite variable, not only at different NOR loci within individual species but sometimes also at the same NOR locus in different individuals of the same species. This variation in the number of subrepeats alters the length of the whole IGS region, leading to the occurrence of spacer-length variants ("slvs"). These variants for the length of IGS region at a locus are described as alleles and can be identified by restriction enzyme digestion coupled with Southern hybridization (Saghai-Maroof et al. 1984, 1990; Balyan et al. 1996; Gupta 1996). The variation in the number of these subrepeats in an IGS has been attributed to unequal crossing-over (Rogers and Bendich 1987).

In barley, there are two pairs of satellited chromosomes (6 or 6H and 7 or 5H), each carrying one rDNA locus (*Rrn1* on chromosome 6H and *Rrn2* on chromosome 5H) that is associated with the corresponding NOR (Saghai-Maroof et al. 1984; Brown et al. 1999). The number of slvs in a genotype is known to vary and ranges from 1 to 4 in different *Hordeum* species (Gupta 1996), and from 1 to 3 in different genotypes of *Hordeum spontaneum* and *Hordeum vulgare* (Balyan et al. 1996). So far, in barley 21 distinct slvs of the IGS region have been identified (15 slvs by Saghai-Maroof et al. 1984; 5 slvs by Zhang et al. 1990; 1 slv by Zhang et al. 1994). The shortest slv (4,280 bp in length) is designated as a spacer-length variant 097 (slv 097) and the longest slv (6,695 bp in length) is designated as 118. Nineteen (100–118) of the 21 slvs (097, 100–118, 108a) form a series, with the two closest slvs differing by a single subrepeat that is 115 bp in length. The length of the shortest slv 097 differs by three subrepeats (345 bp) from its closest slv 100, and the two extreme slvs differ by 2,415 bp, which is equal to 21 subrepeats. The length of an exceptional slv, designated as 108a, is intermediate between 107 and 108, which differ by 115 bp.

Each individual barley plant or accession studied so far, generally has two different slvs and such plants breed true for these two variants (barley is approximately 99% self-fertilized). Furthermore, in an individual barley plant, one of the two slvs is believed to belong to the group with nine smaller variants (slvs 097, 100 to 107) at the *Rrn2* locus, and the other variant is believed to belong to the other group with 12 longer variants (slvs 108a, 108 to 118) at the *Rrn1* locus (Allard et al. 1990; Saghai-Maroof et al. 1990). Earlier studies suggested that the two slvs in a plant are inherited as codominant alleles of the two unlinked Mendelian loci, *Rrn1* and *Rrn2* (Saghai-Maroof et al. 1984). In previous studies, it was also shown that, rarely, a single slv belonging to either of the two groups, two slvs belonging to the same group (slvs 097, 100–107 or 108a, 118–118), and three or four slvs belonging to two groups may be present (Saghai-Maroof et al. 1990). In the present study, such anomalies were found to be more frequent than hitherto reported. This makes it difficult to assign alleles to specific loci following earlier studies (Allard et al. 1990; Saghai-Maroof et al. 1990; Zhang et al. 1990). Therefore, the system followed in these earlier studies for assigning alleles to loci has been dispensed with in the present study.

Although, variation in ribosomal DNA repeat unit length is presumed to be largely free from selective constraints, several studies suggest that natural selection is the major force that directs differentiation. For instance, populations of wild tetraploid wheats (*Triticum dicoccoides*) in Israel have been shown to be geographically structured at the rDNA loci, so that the rDNA slv phenotype could be used to predict the ecological conditions to which a plant is adapted, or vice versa (Flavell et al. 1986). Dramatic changes in the frequencies of different rDNA alleles were also reported in the study of a barley population that was produced from a composite cross through 54 generations (Saghai-Maroof et al. 1984). Likewise, Saghai-Maroof et al. (1990) concluded that natural selection acting differentially on various rDNA alleles plays a major role in the development and maintenance of observed patterns of the molecular genetic organization of rDNA variability. Such observations suggest that perhaps natural selection may operate on rDNA differentiation, and that the latter may provide genotypes with variable ecological adaptations. In view of these earlier observations, the present study was undertaken: (1) to examine the level of rDNA diversity among 63 wild barley accessions from four different microgeographic climatic and edaphic habitats in Israel, and compare it with that among seven barley cultivars grown in India; and (2) to analyse whether rDNA diversity appears to be driven, at least partly, by natural selection.

Material and methods

Material

The material for this study consisted of 63 accessions of wild barley (*H. spontaneum*) studied extensively in Israel (Nevo et al. 1979a, b, 1981, 1983, 1986a, b; Owuor et al. 1997) and the near East (Nevo et al. 1986b), and seven accessions of barley (*H. vulgare*) cultivated in India. The seeds of wild barley accessions were collected in Israel from two diverse microsites; the seven barley cultivars were kindly supplied by the Directorate of Wheat Research (DWR), Karnal, India. The barley cultivars (cv) included three 6-rowed cvs (Azad, C 138 and RD 2508) recommended for cultivation under rainfed conditions, one 6-rowed cv (Jyoti) recommended for cultivation under irrigated conditions, two 2-rowed cvs (Alpha 93 and BCU 73) recommended for cultivation under irrigated conditions, and a solitary bold-seeded cv (Jagrati). All accessions were raised in a single row plot, 2.5-m long, at the Research Farm of Ch. Charan Singh University, Meerut, India. A single plant of each accession (including *H. vulgare* and *H. spontaneum*) was used for scoring rDNA spacer-length variants (slvs).

Ecological microsites in Israel

The material for 63 accessions of wild barley was collected from two different microsites in Israel. Thirty six (36) accessions were collected from "Evolution Canyon", near Western Mount Carmel, and the remaining 27 accessions were collected from 'Tabigha' in the Eastern Upper Galilee Mountains. While the former represents a 'microclimatic divergence model', the latter represents an 'edaphic divergence model'. The two microsites are separated by 53 km.

"Evolution Canyon": a microclimatic divergence model

The "Evolution Canyon" (so described due to the opportunities provided by this microsite for the study of evolution in action), is a small area of 7,000 m^2 located at Lower Nahal Oren, Mount Carmel, Israel (32°43´ N, 34°58´ E), and has two different abiotic conditions leading to dramatic biotic contrasts. These two abiotic conditions are represented by a North Facing Slope (NFS) and a South Facing Slope (SFS). The NFS and SFS are separated by 100 m at the bottom and 400 m at the top (Fig. 1 in Nevo 1995, 1997). The SFS obtains six-fold higher solar radiation than the NFS and, therefore, is much warmer, drier and variable, representing an African Savannah. The NFS is cooler and more humid, thus representing a temperate South European and Mediterranean dense-macquis live oak forest (Nevo 1995, 1997). In the present study, a

sample of 21 accessions of wild barley belonged to NFS and another sample of 15 accessions belonged to SFS, making a total of 36 accessions from the "Evolution Canyon".

'Tabigha': an edaphic divergence model

Tabigha is located north of the Sea of Galilee at the Mediterranean sea level. It has two soil types, 'red terra rossa' and 'brown basalt'; the former being drier with a shallower soil layer than the latter (Nevo et al. 1981, 1983, 1988). The 'terra rossa' also has many more base rocks, and is more hilly than the flat 'basalt' with a deeper soil layer. Of the 63 accessions of wild barley used in the present study, 27 were collected from Tabigha. Of these 27, 14 were collected from 'terra rossa' and 13 from 'basalt'.

DNA extraction and purification

Total cellular DNA was isolated from 1-month old, field-grown individual plants using the modified CTAB method of Saghai-Maroof et al. (1984). The isolated DNAs were further purified by RNaseA treatment and phenol:chloroform:isoamyl alcohol following Sambrook et al. (1989). The quality and quantity of DNA samples were checked on agarose gels after staining with ethidium bromide.

Restriction enzyme digestion, Southern blotting, hybridization and autoradiography

Purified DNA samples (8 µg) from each of the 70 genotypes were digested overnight, separately with 40 units of *Sac*I at 37°C according to the manufacturer's instructions (Amersham, UK). The digested DNAs were fractionated by electrophoresis on a 1% agarose gel overnight at a constant voltage of 40 V and were transferred to a positively charged nylon membrane (Hybond N+) in a 0.4 M NaOH buffer at room temperature (Amersham, UK). The filters were pre-hybridized at 65°C for 5 h in pre-hybridization buffer [5×SSPE, 5×Denhardt's solution, 0.5% (w/v) SDS] containing 0.5 mg/ml of sonicated, denatured salmon-sperm DNA. The pre-hybridized filters were hybridized at 65°C for 16 h with a ^{32}P-labelled pTa71 DNA probe consisting of a clone of one wheat rDNA repeat unit (Gerlach and Bedbrook 1979). The hybridized filters were washed sequentially in 2×SSC, 1×SSC and 0.5×SSC with 0.5% SDS at 65°C for 15 min, and then visualized using autoradiography.

Detection and designation of slvs

The autoradiograms revealed the lengths of ribosomal DNA repeat units and those of the corresponding intergenic spacer (IGS) regions (see Fig. 1). Lengths of the DNA fragments in each band (slv phenotypes) were calibrated by fitting a curve derived from the migration distance traversed by the specific DNA molecular-weight marker (λ DNA/*Hin*dIII), using the software SEQAID (Rhoads and Roufa 1989). The rDNA slvs were designated following Saghai-Maroof et al. (1990), and Zhang et al. (1990), but were not assigned to individual rDNA loci (*Rrn1*, *Rrn2*).

Statistical analyses

Chi-square tests for the independence of attributes were conducted using the SAS package programme (SAS 1996), following Lewontin and Felsenstein (1965). These chi-square tests were used for testing the significance of differences in the distribution and composition of slv phenotypes in: (1) accessions belonging to the two microsites, and (2) accessions belonging to two locations within each microsite.

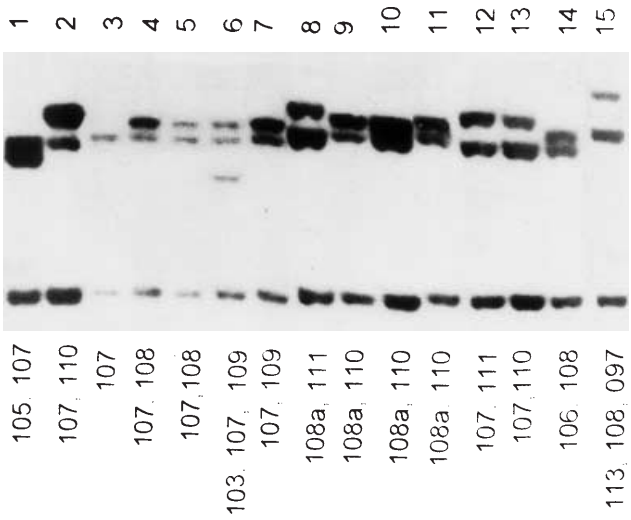

Fig. 1 An autoradiogram showing a variety of spacer length variant (slv) phenotypes in 15 accessions (1–15 at the top) of barley; at the bottom, slv phenotypes are represented by allelic compositions (for details see text)

Results

In the different accessions of wild and cultivated barley used in the present study, length of the IGS region differed and variants were assigned to known allelic variants without assigning them to the two known NOR loci (*Rrn1* and *Rrn2*) of barley (Fig. 1). Different lengths of the spacer region (IGS) are known as spacer length variants (slvs), and 1–3 variants were present in an individual. A combination of these 1–3 variants in an accession is described as slv phenotype of that particular accession. The data on the frequencies of different phenotypes are summarized in Tables 1 and 2 and those on the frequencies of individual alleles are summarized in Tables 3 and 4.

The data presented in Table 1 suggest that the slv phenotypes present at one microsite are generally not present at the other microsite. Likewise, within the same microsite, the phenotypes present in one climatic or edaphic condition are generally not present in the other climatic or edaphic condition. Only a few exceptions were found (Table 1). For instance, out of the 30 slv phenotypes scored, seven phenotypes (about 23%) were exceptional, from which each was found in at least one accession from each of the two microsites, "Evolution Canyon" and Tabigha. These included phenotypes II, IV, XV, XVIII, XX, XXIII and XXVIII, of which five phenotypes namely, IV, XV, XX, XXIII and XXVIII, were found at the more humid NFS of "Evolution Canyon" and also at the more humid basalt of Tabigha. Of these five phenotypes, phenotype XXVIII is the predominant phenotype of the NFS. The slv phenotype XVIII was represented by one solitary accession each at the NFS of "Evolution Canyon" and at the terra rossa of Tabigha. Phenotype IV was found not only at both the slopes of

Table 1 Distribution of 30 slv phenotypes in 63 accessions from two different microsites each from "Evolution Canyon" and Tabigha

Phenotype number	Phenotype	Number of accession (n) and relative frequency (f)								Phenotypes common at		
		"Evolution Canyon"				Tabigha				Microsites	Niches	
		NFS		SFS		Terra rossa		Basalt			Slopes	Soils
		n	f	n	f	n	f	n	f			
I	102	–	–	–	–	–	–	1	0.08	–	–	–
II	106	–	–	3	0.20	–	–	1	0.08	+	–	–
III	107	–	–	3	0.20	–	–	–	–	–	–	–
IV	108	3	0.14	1	0.07	–	–	1	0.08	+	+	–
V	108a	1	0.05	–	–	–	–	–	–	–	–	–
VI	109	1	0.05	–	–	–	–	–	–	–	–	–
VII	110	–	–	–	–	2	0.14	2	0.15	–	–	+
VIII	107, 105	–	–	2	0.13	–	–	–	–	–	–	–
IX	108, 106	–	–	–	–	1	0.07	1	0.08	–	–	+
X	108, 107	–	–	–	–	2	0.14	–	–	–	–	–
XI	109, 104	–	–	1	0.07	–	–	–	–	–	–	–
XII	109, 105	1	0.05	–	–	–	–	–	–	–	–	–
XIII	109, 107	–	–	–	–	1	0.07	–	–	–	–	–
XIV	110, 105	–	–	4	0.27	–	–	–	–	–	–	–
XV	110, 107	1	0.05	–	–	–	–	1	0.08	+	–	–
XVI	110, 108a	–	–	–	–	4	0.29	–	–	–	–	–
XVII	111, 105	–	–	–	–	–	–	1	0.08	–	–	–
XVIII	111, 107	1	0.05	–	–	1	0.07	–	–	+	–	–
XIX	111, 108a	–	–	–	–	1	0.07	–	–	–	–	–
XX	112, 108a	1	0.05	–	–	–	–	2	0.15	+	–	–
XXI	114, 108	1	0.05	–	–	–	–	–	–	–	–	–
XXII	113, 108a	1	0.05	–	–	–	–	–	–	–	–	–
XXIII	114, 109	1	0.05	–	–	–	–	1	0.08	+	–	–
XXIV	115, 109	4	0.19	–	–	–	–	–	–	–	–	–
XXV	109, 104, 097	–	–	1	0.07	–	–	–	–	–	–	–
XXVI	109, 105, 103	1	0.05	–	–	–	–	–	–	–	–	–
XXVII	109, 107, 103	–	–	–	–	1	0.07	–	–	–	–	–
XXVIII	113, 108, 097	4	0.19	–	–	–	–	1	0.08	+	–	–
XXIX	114, 109, 104	–	–	–	–	–	–	1	0.08	–	–	–
XXX	114, 110, 107	–	–	–	–	1	0.07	–	–	–	–	–

+ indicates common phenotypes (genotypes) at microsites and niches

"Evolution Canyon", but also at the basalt soil type at Tabigha. Phenotypes VII and IX were available at both the soils of Tabigha, and phenotype II was found on SFS at "Evolution Canyon" and on basalt at Tabigha (Table 1).

It may also be noticed from the data presented in Table 1 that the slv phenotypes XXIV, XXVIII and IV were most frequent among the accessions from the NFS and the phenotypes XIV, III and II were most-frequent among accessions from the SFS of "Evolution Canyon". Similarly, among accessions from Tabigha, phenotype XVI was most-frequent in the terra rossa soil, and the phenotypes VII and XX were most-frequent in the basalt soil type. slv 097 was observed only in phenotypes with three slvs, although in barley cultivars it is also found in a phenotype with two slvs (Table 2).

A comparison of Tables 1 and 2 suggests that none of the six slv phenotypes observed in barley cultivars from India was available in any of the 63 accessions of wild barley from Israel. All the six slv phenotypes in barley cultivars are unique to this study, and five of the six phenotypes carry slv 097.

The chi-square tests were conducted to find out significant differences between: (1) the number of phenotypes observed at two microsites and those found at different climatic and edaphic niches on the same site, and between (2) the compositions of the more-frequently distributed phenotypes (present in at least three accessions) at two microsites ("Evolution Canyon" and Tabigha), and their corresponding climatic/edaphic niches. The chi-square tests for the number of phenotypes revealed a nonsignificant difference between the two microsites ($\chi^2_{(1)}$=0.71; p=0.791) as well as between the two edaphic niches of Tabigha ($\chi^2_{(1)}$=0.45; p=0.502), but revealed a

Table 2 Distribution of six novel slv phenotypes in seven barley cultivars from India

Phenotype	Genotype	n[a]	f[b]	Cultivars
112	112,112;112,112	1	0.14	Azad
097, 106, 112	097,106;112,112	1	0.14	Jyoti
097, 113	097,097;113,113	1	0.14	Jagrati
097, 106, 113	097,106;113,113	1	0.14	Alpha93
097, 109, 114	097,097;109,114	1	0.14	BCU73
097, 105, 115	097,105;115,115	2	0.29	C138, RD2508

[a] n=number of accessions
[b] f=relative frequency

Table 3 Frequencies of different rDNA alleles at "Evolution Canyon" and Tabigha microsites

rDNA alleles	"Evolution Canyon"			Tabigha		
	NFS *n* (f)	SFS *n* (f)	Total *n* (f)	Terra rossa *n* (f)	Basalt *n* (f)	Total *n* (f)
097	4 (4.76)	1 (1.67)	5 (3.47)	–	1 (1.92)	1 (0.92)
102	–	–	–	–	4 (7.69)	4 (3.70)
103	1 (1.19)	–	1 (0.69)	1 (1.79)	–	1 (0.92)
104	–	3 (5.00)	3 (2.08)	–	2 (3.85)	2 (1.85)
105	3 (3.57)	12 (20.0)	15 (10.41)	–	2 (3.85)	2 (1.85)
106	–	12 (20.0)	12 (8.33)	2 (3.57)	6 (11.54)	8 (7.40)
107	4 (4.76)	16 (26.67)	20 (13.88)	11 (19.64)	2 (3.85)	13 (12.03)
108a	8 (9.52)	–	8 (5.55)	10 (17.86)	4 (7.69)	14 (12.96)
108	18 (21.43)	4 (6.67)	22 (15.27)	6 (10.71)	7 (13.46)	13 (12.03)
109	18 (21.43)	4 (6.67)	22 (15.27)	4 (7.14)	3 (5.77)	7 (6.48)
110	2 (2.38)	8 (13.33)	10 (6.94)	17 (30.36)	10 (19.23)	27 (25.00)
111	2 (2.38)	–	2 (1.38)	4 (7.14)	2 (3.85)	6 (5.55)
112	2 (2.38)	–	2 (1.38)	–	4 (7.69)	4 (3.70)
113	10 (11.90)	–	10 (6.94)	–	2 (3.85)	2 (1.85)
114	4 (4.76)	–	4 (2.77)	1 (1.79)	3 (5.77)	4 (3.70)
115	8 (9.52)	–	8 (5.55)	–	–	–

n=number of accessions
f=relative frequency (%)

Table 4 Distribution of alleles in seven barley cultivars from India

rDNA alleles								
	097	105	106	109	112	113	114	115
n	8	2	2	1	6	4	1	4
f	28.57	7.14	7.14	3.57	21.43	14.29	3.57	14.29

n=number of accessions
f=relative frequency

nearly significant difference between the two slopes of "Evolution Canyon". Thus, the number of phenotypes was higher at the NFS than at the SFS (13 versus 7, respectively; $\chi^2_{(1)}$=3.80; p=0.051) of "Evolution Canyon". The chi-square test for the composition of phenotypes revealed significant differences between two microsites ($\chi^2_{(8)}$=22.9; p=0.003) as well as between the NFS and SFS ($\chi^2_{(5)}$=19.0; p=0.002) of "Evolution Canyon". Such a test on the composition of phenotypes was not possible between two edaphic niches of Tabigha, because inadequate repetitions were available. The above statistical tests suggest that although the number of phenotypes do not significantly differ, their compositions do differ between the two microsites. The two slopes of "Evolution Canyon", however, differ both for the number of phenotypes as well as for their composition. Thus, despite the small sample size examined, the above results and statistical tests suggest that regional and local differences in ribosomal DNA do occur. According to Lewontin and Felsenstein (1965) these chi-square tests are reliable even for the small sample sizes, as in the present study.

The results of the frequencies of individual alleles of rDNA loci are presented in Tables 3 and 4. It can be seen that out of the 16 alleles detected in 63 accessions of *H. spontaneum* only four alleles (107, 108, 109 and 110) were present in accessions belonging to both the ecological niches of each of the two microsites, "Evolution Canyon" and Tabigha (Table 3). In contrast to the above common alleles, a unique allele 102 was restricted to the accessions from the basalt of Tabigha only; similarly, allele 115 was unique in being present only on NFS of "Evolution Canyon". The remaining ten alleles were detected in accessions belonging to either one ecological niche each of the two microsites or to one ecological niche of one microsite and to both the ecological niches of the other microsite (Table 3). It may also be seen that alleles 108, 109, 107 and 105 are most frequent at "Evolution Canyon", and alleles 110, 108a, 108 and 107 were most frequent at Tabigha. When analyzed further for individual ecological niches, 108 and 109 alleles were most frequent at NFS and 107, 106 and 105 alleles were most frequent at the SFS of "Evolution Canyon". Similarly, alleles 110, 107 and 108a were most frequent at terra rossa and alleles 110, 108 and 106 were most frequent at the basalt of Tabigha (Table 3). In *H. vulgare* alleles 097 and 112 were most frequent (Table 4).

Discussion

A study of populations inhabiting microsites with ecological contrasts provides an opportunity for the study of forces driving evolution within a species at the molecular level. The present study on rDNA divergence between and within groups of barley accessions from "Evolution Canyon" and Tabigha microsites in Israel and a few barley cultivars from India, suggests adaptive radiation of the known ribosomal phenotypes and the allelic variants. Despite the limitation of the small sample size used in the present study, the analysis of data on slv phenotypes and slv alleles recorded during the present study does show divergence in slv phenotypes and slv alleles, not only between the populations of wild barley from two microsites but also between accessions from two

ecological niches of each microsite and between wild barley from Israel and cultivated barley from India.

Anomalous rDNA phenotypes

The 21 known alleles belonging to ribosomal DNA in barley are generally assigned to two groups of mutually exclusive alleles (each group having a continuous uninterrupted series) belonging to two loci (*Rrn1* with alleles 108a, 108–118, and *Rrn2* with alleles 097, 100–107), and the combination of two alleles at the two loci is treated as an individual's rDNA phenotype. However, in earlier studies, up to 17% individual barley plants examined were shown to deviate from this typical pattern of rDNA phenotypes (Saghai-Maroof et al. 1984, 1990; Allard et al. 1990; Zhang et al. 1990). In the present study, however, although as many as 34 (54%) accessions had rDNA phenotypes with 2 slvs, as expected, the typical pattern of rDNA phenotypes, as above, was available only in 16 (25.4%) of the 63 accessions of wild barley and in only one (5.9%) out of seven accessions of cultivated barley. These high frequencies of the so-called anomalous phenotypes recorded during the present study, in our opinion cannot be the result of errors in recording the data, but instead force us to question the system suggested in the above studies. Since occurrence of rDNA phenotype with a solitary slv variant in some accessions, both in earlier studies and in the present study, suggests the presence of even the same allele at both the loci, there is no reason why any two variants belonging to the same series can not be found at the two loci. The occurrence of complex loci each harbouring two slvs is also a possibility, particularly to explain phenotypes with three slvs, although three slvs may also result from the heterozygosity at one of the two loci. A higher frequency of anomalous phenotypes during the present study may also suggest some adaptive value of these anomalous phenotypes and, if so, in future studies these may be found to be more frequent at least at some locations.

Diversity in rDNA phenotypes

Regional inter-microsite divergence

The climate of Israel varies drastically southwards towards the Negev desert and eastward towards the Syrian and Jordanian deserts. The climate of Mount Carmel varies geographically from Mediterranean humid (600–700 mm annual rainfall) to the semi-arid marginal Mediterranean climate of eastern Galilee (400-mm annual rainfall) over a relatively short transect of 50–60 km. This affects the entire vegetation, which varies from the dense live oak macquis of *Quercus celliprinos* to the park forest of *Zizyphus spina-cristi*. The present study suggests that this eastward climatic transect of increasing aridity from "Evolution Canyon" to the Tabigha microsite is also reflected molecular-genetically at the level of rDNA polymorphism. It may be seen that, although the number of phenotypes at two microsites (19 at Evolution Canyon and 18 at Tabigha) does not differ, the composition of phenotypes at the two sites does differ significantly. Unfortunately, due to the small sample size, data for only 7 of the 30 phenoypes could be used for statistical tests on the composition of phenotypes. However, the data on the distribution of the remaining 23 phenotypes (not included in statistical tests) also gives useful information, as evident in the case of 4 of these 23 phenotypes (VII, VIII, IX and X) each having more than one accessions, but still restricted to one of the two microsites. Further, when the whole data set is examined, one may notice that only 7 of the 30 rDNA slv phenotypes are shared between the two sites separated by 53 km; all other 23 phenotypes (=77%) are different between the two microsites. Intriguingly, five of the above seven rDNA spacer phenotypes (approximately 17% of the total) are shared between the humid NFS at "Evolution Canyon" and the humid basalt soil (more humid than the terra rossa) at Tabigha. This suggests that even for those phenotypes, which are present at both microsites, a relationship with the climatic variable is sometimes apparent.

Three previous studies on regional collections of wild cereals are available, two involving wild barley (Saghai-Maroof et al. 1990; Chalmers et al. 1992) and one involving wild emmer wheat (Flavell et al. 1986). These three studies involved relatively large sizes sampled across the ecological spectra of these progenitors of cultivated barley and bread wheat. Populations used in these studies represented areas from north to central and northern Negev desert, i.e. primarily across a transect of increasing aridity from the humid Mediterranean region through the semi-xeric (steppic) and xeric region of the northern Negev desert. In these three studies, as in the present study, the phenotypic and allelic frequencies for ribosomal RNA loci were shown to differ widely in different habitats in correlation with environmental and allozymic variation.

In two other independent studies on subterranean mole rats, geographic variation in spacer rDNA polymorphism was also observed; in one study it correlated with the environment, involving the same gradient of increasing aridity in Israel (Suzuki et al. 1987), while in the other, covering a much bigger area (30-times larger) in Turkey (Suzuki et al. 1996), it suggested a major role of natural selection in rDNA spacer differentiation.

The above earlier studies, along with the present study conducted on ecogeographic rDNA polymorphism, provide evidence that natural selection acts differentially on various rDNA alleles or their combinations in rDNA phenotypes. Natural selection thus plays a major role in the development and maintenance of observed intermicrosite variation in the patterns of molecular and genetic organization of the rDNA polymorphism. Linkage disequilibrium and the pleiotropic effect of other unlinked loci, involved in rDNA transcription and ribosome assembly, may be offered as other possible explanations.

Local intra-microsite divergence

In samples from each of the two microsites examined during the present study, intra-site divergence was also observed in ribosomal DNA, as evident from significant differences in the composition of phenotypes between NFS and SFS. Taken together, only 1 out of 19 rDNA phenotypes (=5%) was common (phenotype IV) between the opposite slopes at "Evolution Canyon" and 2 out of 18 rDNA phenotypes (=11%) were common (phenotypes VII and IX) between the two soil types of Tabigha. Despite the limitation of small sample size used in the present study, such a level of genetic divergence among ribosomal DNA phenotypes (and alleles) in wild barley across very short ecological distances is striking and intriguing and cannot be explained on the basis of mere chance. The wild barley has been growing at "Evolution Canyon" and Tabigha microsites for at least several million years, thus giving sufficient time for appreciable gene migration to take place, despite the restricted seed dispersal (Nevo et al. 1979b). It is therefore logical to conclude on the basis of the above results, that when the unique combinations of alleles in rDNA phenotypes exhibiting contrasting patterns of variation (intense local divergence as described here) had occurred in the past, diversifying natural selection (leading to divergence) was the most probable evolutionary driving force, which should have operated through either microclimatic differential stress ("Evolution Canyon") or via edaphic differences (Tabigha).

Allelic diversity at rDNA loci

The results of the present study also suggest that individual alleles have adaptive properties. In the present study at "Evolution Canyon", the most-frequent alleles are 108 (18.1%), 109 (15.3%) and 107 (13.9%), while at Tabigha, the most-frequent alleles are 110 (25.0%), 108a (12.9%), 107 or 108 (each 12%). Among these most-frequent alleles, 108, 109 and 107 were also found to be frequent in an earlier study (Saghai-Maroof et al. 1990) where the most-frequent alleles were 107 and 112. This may suggest that 107 and 108, that are frequent at both the microsites, provide a wide range of adaptability, while the other frequent alleles provide adaptability to specific microsites. Differences were also noticed between the two ecological niches at the same microsite. For instance, at "Evolution Canyon", 108 (26.2%) and 109 (21.4%) were most frequent at NFS, and alleles 107 (26.7%) and 105 or 106 (each 20.0%) were most frequent at SFS. Similarly, at Tabigha, 110 (30.6%) and 107 (19.6%) were most frequent at terra rosa, and 110 (25.0%), 108a (12.9%) and 107 or 108 (each 12.0%) were most frequent at basalt. This does suggest that the different alleles are not randomly distributed, and that some alleles are more frequent at one ecological niche, and the others are more frequent at the other niches. Saghai-Maroof et al. (1990) also found that the most-frequent alleles, 107 and 112, occurred more frequently under low humid conditions, and the remaining alleles were found to be more frequent under high humid conditions. In the present study, while allele 107 does seem to occur more frequently under low-humid conditions (SFS, terra rosa), 112 is completely absent on these two low humid niches, and occurs at a very low frequency in the other two ecological niches. Therefore, our results, though confirming the selective advantage which allele 107 offers in low humid conditions, differ from the above earlier results with respect to allele 112 and suggest instead that this allele (112) may be more important for cultivated barley, rather than wild barley (see later).

Selection at other loci may cause rDNA polymorphism

Natural selection, while affecting the observed rDNA phenotypes involving the *Rrn*1 and *Rrn*2 loci, may also act on other genomic DNA sequences. For instance, both in wild barley and wild emmer wheat populations from small areas of Tabigha in Israel, random amplified polymorphic DNA (RAPD) analysis was used to demonstrate genetic differentiation at many loci, driven by ecological factors. In such a study conducted on barley, the differentiation was shown to be driven by edaphic factors (Owuor et al. 1999), while in the study conducted on wild emmer wheat, it was shown to be driven by climatic micro-niches involving sunny and shady areas (Li et al. 1999). Allelic variation at microsatellite (SSR) loci was also examined in different natural populations of wild emmer wheat (Li et al. 2000a, b). This study revealed variation in allelic diversity that was influenced by different genetic and ecological factors. The phenomenon was observed not only between the populations collected from different microsites, but also between those collected from different habitats from the same microsite. These studies suggest that natural selection, driven by climatic and edaphic factors, can operate both on coding and non-coding sequences, including rDNA, SSR loci and RAPD loci, and also loci coding for a variety of products including isozymes and storage proteins.

Two important studies conducted on barley, *H. vulgare* (Saghai-Maroof et al. 1984; Allard et al. 1990) also suggested that slv phenotypes could be selected due to selection at other loci. In one of these studies, in a population derived from a composite cross and monitored over 54 generations, Saghai-Maroof et al. (1984) observed that some of the specific classes of slvs that were originally present at a low frequency became dominant in later generations and vice versa. The second study, conducted by Allard et al. (1990), suggested the possibility of selection acting directly on the sequence variability in the transcription units associated with the slvs in rDNA. In yet another study, Powell et al. (1991) demonstrated that in barley a particular rDNA locus was associated with genes for traits that will influence reproductive advantage in polymorphic populations, so that the observed frequencies of different slvs could be the result of selection exercised on these associated loci.

Wild barley in Israel

H. spontaneum in Israel displays a highly subdivided "archipelago" population structure, particularly in the central and southern xeric regions of the country (Nevo et al. 1979a, b). Notably, at the levels of both protein (allozymes) and genomic DNA (RAPDs, SSRs, AFLPs and rDNA spacer length), wild barley displays a non-random adaptive genetic landscape involving loci, populations, and habitats at both macro- and micro-geographic sites such as "Evolution Canyon" (Nevo 1997; Owuor et al. 1997) and Tabigha (Nevo et al. 1981, 1986b, 1988; Owuor et al. 1999; Li et al. 2000a, b). In all these studies, aridity stress and niche-width have been the best predictors of genetic diversity. The higher the stress and the wider the niche, the higher is the genetic diversity, at both protein and DNA levels. In the present study, we found high rDNA IGS polymorphism in *H. spontaneum*, at two microsites, to be relatively higher on the more humid NFS at "Evolution Canyon" (narrower niche than the SFS) and on basalt at Tabigha (wider niche than that of the terra rossa). Considerable rDNA IGS variability was also earlier observed both within and between *H. spontaneum* populations, and much of the variation between populations was correlated with a combination of ecological and climatic variables (Table 6 in Chalmers et al. 1992). A general question that arises from these studies is, how does selection generate and maintain the extensive IGS heterogeneity between and within populations? As discussed earlier, there are at least two possibilities: selection is operating directly upon the rDNA or, alternatively, the rRNA loci are linked to genes for adaptively important traits. Only future studies will resolve this question.

Cultivated barley from India vs wild barley from Israel

The ecological and environmental conditions of the habitats of wild barley *H. spontaneum* accessions sampled from Israel and those of the seven *H. vulgare* cultivars grown in irrigated and rainfed agricultural conditions in India are substantially different. This difference parallels the occurrence of six divergent and unique slv phenotypes in cultivated barley (Table 2). Although the sample was too small for calculating the frequencies of the six phenotypes, there are two interesting features that deserve attention: (1) five out of the six slv phenotypes carry a unique slv 097, which is present at very low frequency in wild barley accessions sampled from Israel, and (2) the allele 112, which had the lowest frequency in wild barley accessions, had the second highest (after 097) frequency in cultivated barley in the present study. In an earlier study of a much larger sample of 92 cultivars, the phenotype (104, 112) and the allele 112 were also most frequently followed by phenotype (107, 112) and the allele 104 (Saghai-Maroof et al. 1990). Another interesting feature, observed in the present study, as well as in the above earlier study, is the occurrence in cultivated barley, of an anomalous genotype (112, 112, 112, 112) with a solitary allele 112, that was completely absent in wild barley. These results are not surprising since in a breeding programme the selection is exercised for genotypes possessing a high and stable yield associated with adaptability for regions of their cultivation. This also explains why results of the present study, while resembling those from earlier studies on cultivated barley in some respects (phenotype 112) differ in other respects. For instance, the allele 097, which was found to be the most frequent among cultivated barley, actually belongs to the *Rrn2* locus, the variation at which has already been shown to be associated with variation for yield, 1,000-grain weight and water sensitivity (Powell et al. 1991). Therefore, the unique rDNA alleles and phenotypes in cultivated-barley genotypes may be contributing towards the superior adaptability to the agricultural field environment. The slv 097 in Indian barley cultivars may be of particular interest in this connection and requires more detailed studies since, in an earlier study, the most-frequent alleles recorded in *H. vulgare* were 104 (0.65) and 112 (0.98) (Saghai-Maroof et al. 1990).

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