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Redundancy modulation of nuclear DNA sequences in *Dasypyrum villosum*

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Abstract In order to assess fluid domains in the genome of *Dasypyrum villosum*, Feulgen/DNA cytophotometric determinations and molecular and cytological DNA-DNA hybridization experiments were carried out in resting embryos and developing seedlings from yellow and brown caryopses belonging to different populations. The cytophotometric data showed that the basic amount of nuclear DNA is, on average, 12% higher in 2-day-old seedlings from yellow caryopses as compared to those from brown caryopses. It increases in each individual during seed germination, to a higher extent in seedlings from yellow caryopses than in those from brown caryopses. DNA content also differs up to 13% between plants within a caryopsis-colour group and up to 40% between populations. Dot-blot hybridization of a 396-bp *D. villosum*-specific DNA repeat to genomic DNA extracted from embryos in dry seeds, or from seedlings belonging to single progenies of plants from different populations, confirmed the cytophotometric results. The redundancy in the genome of sequences hybridizing to the 396-bp element differs significantly both between populations and between plant progenies within a population. During seed germination these sequences are the more amplified the less they are redundant in the genome of resting embryos, and amplification occurs to a significantly-greater extent in seedlings from yellow caryopses than in those from brown caryopses. ^{3}H-labelled 396-bp sequences hybridize at or near the telomeres of most chromsome pairs though only to the shorter of the two subtelocentric pairs. The hybridization level is higher in seedlings from yellow caryopses that in those from brown caryopses, and a linear correlation exists between the number of silver grains counted over the labelled regions of each chromosome pair in the two groups of seedlings. Possible control mechanisms of the observed changes in the nuclear genome, and the role of these changes in developmental regulation and environmental adaptation, are discussed.

Key words *Dasypyrum villosum* · Repeated DNA sequences · Rapid genomic changes · Developmental regulation · Environmental adaptation

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Introduction

On account of the widely-accepted concept of the absolute primacy of DNA over all biological phenomena and of its function as the basis of heredity, the view that the genome must be constant, not only within a single individual but also within a species, was initially postulated as a tenet in biology. However, it is now generally accepted that nuclear DNA is intrinsically plastic, due to its content of independent replicative units and their ability to selective replication. Extra synthesis, under-replication or loss from the nucleus of repeated DNA sequences can rapidly alter the basic size and organization of the genome, possibly more commonly in plants than in animals (reviewed for plants by Walbot and Cullis 1985; Cionini 1989; Bassi 1990; Cullis 1990; Nagl 1990).

Developmental transitions, physiological stimuli, and the response to stress may all give rise to such genomic changes, and it has been suggested that these changes play a regulatory role in development and

represent factors in environmental adaptation. However, the mechanisms by which rapid alterations in genome size and organization are produced and controlled, the developmental stages at which they can occur, and the structure and chromosomal organization of the DNA sequences involved are still poorly understood.

In relation to these problems we have carried out an investigation in the plant *Dasypyrum villosum*. This species was chosen for two reasons. First, as also occurs in other genera, caryopses differing in size and colour are produced even in one and the same ear. Some caryopses look yellow (yellow 246; cf. Seguy 1936), while others look brown (red 112; cf. Seguy 1936); these latter are shorter and thinner than the former. Yellow and brown caryopses differ in the energy and power of their germination (Stefani and Onnis 1984) and in viability (De Gara et al. 1991). Moreover, differing histone contents (Innocenti and Bitonti 1980) and mitotic cycle durations (Innocenti and Bitonti 1983) have been observed in the meristems of embryos germinating from the two kinds of caryopses. This knowledge allowed us to hypothesize that differences in genomic characters might also exist between embryos in the two types of caryopses. The second reason for our choice was the availability of a DNA clone carrying a sequence of about 380 bp which had already been isolated from the genome of *D. villosum* and was shown to be a tandemly-repeated, species-specific element having discrete localizations in six out of the seven chromosome pairs of this species. Individual plants showed differences in the length and abundance of stretches of this sequence after its hybridization to Southern blots of genomic DNA partially digested with the *Sph*I restriction endonuclease (De Pace et al. 1992). Hence, this DNA repeat seemed to be a suitable probe for studying possible modulations in the redundancy of nuclear DNA sequences.

In this paper, we report on the results of cytophotometric determinations and of molecular and cytological hybridization experiments which show the existence of highly-fluid domains in the genome of *D. villosum*. We also offer some suggestions on the way in which these quantitative changes in nuclear DNA are produced and controlled.

Materials and methods

Plant material

Seeds (caryopses) were collected from Italian populations of *D. villosum* at the following locations (region and district code are indicated in brackets): Bomarzo (Latium; VT), Bullicame (Latium; VT), Campobasso (Molise; CB), Mottola (Apulia, TA), Pachino (Sicily; SR), Pisa (Tuscany; PI), Viterbo (Latium; VT). Seeds were germinated in Petri dishes on wet paper at room temperature in the dark. Material to be used for DNA cytophotometry was fixed in ethanol-acetic acid 3:1 (v/v) or in 10% neutral formalin. The roots of some plantlets, to be squashed for in-situ hybridizations, were treated with an 0.2% aqueous solution of colchicine at room temperature and fixed in ethanol-acetic acid. For DNA extraction, seeds of individual plants grown in the greenhouse were used as sources of embryos or seedlings. These plants were obtained by germinating seeds from half-sib families (caryopses from single open-pollinated plants) collected from each population.

DNA cytophotometry

Fixed root or shoot apices of embryos in dry seeds, or of seedlings at different developmental stages, were treated with a 5% aqueous solution of pectinase (Sigma) for 1 h at 37 °C and squashed under a coverslip in a drop of 45% acetic acid. The coverslips were removed by the solid-CO_2 method and the preparations were Feulgen-stained after different hydrolysis durations in N HCl at 60 °C: 8 min for those made with material fixed in ethanol-acetic acid, and 20 min for those made with formalin-fixed material. After staining, the slides were subjected to three 10-min washes in SO_2 water prior to dehydration and mounting in DPX (BDH). Since simultaneous processing was not possible due to the large number of preparations to be analyzed, squashes made with the root tips of a single plantlet of *Vicia faba* were concurrently stained for each group of slides and used as standards in order to make the results comparable. All notable differences in Feulgen/DNA absorption between preparations observed using the above method of comparison were further checked by analyzing repeat preparations that were all processed together. Feulgen/DNA absorptions in individual cell nuclei were measured at a wavelength of 550 nm, using a Leitz MPV 3 microscope photometer equipped with a mirror scanner and a HP 85 computer. Relative Feulgen/DNA units were converted into picograms of DNA by assuming a 4C DNA content of 53.31 pg (Bennett and Smith 1976) in the *V. faba* plant used as a standard. By this method, the C-value (the DNA content of an unreplicated haploid nucleus) of each individual was calculated.

DNA extraction

For DNA preparation, five embryos from dry seeds, 2–3 2-day-old seedlings, one 10-day-old seedling, or about 0.5 g of fresh leaf tissues from adult plants, were pulverized with a pestle and mortar in the presence of liquid nitrogen and homogenized in 5 ml of a pH 8 buffer containing 0.1 M Tris, 0.005 M EDTA, 0.5 M NaCl and 0.01 M mercaptoethanol. DNA extraction and purification were performed as described by Della Porta et al. (1983) and the isolated DNA was suspended in TE buffer (0.01 M Tris-HCl pH 8 plus 0.001 M EDTA pH 8), and then stored at 4 °C.

Probe preparation and sequencing

A highly-repeated DNA sequence of about 380 bp, previously isolated from a *D. villosum* genomic library (De Pace et al. 1992), was subcloned in the *Sph*I region of the multiple cloning site of pT7/T3α18 vectors (p380 plasmid) and sequenced using the dideoxy method of Sanger et al. (1977) as modified by Chen and Seeburg (1985) for double-stranded plasmid DNA. Computer analysis of the sequence data was carried out using the Pustell program (IBI).

Dot-blot hybridization and calculation of sequence copy numbers

Replicated samples of 25 ng or 100 ng of genomic DNA were suspended in 10 µl of TE buffer and applied to a Zeta Probe (Biorad) filter using the Minifold I apparatus (Schleicher and Schuell). Also on the same filter, amounts of p380 plasmid equivalent to 2×10^4, 2×10^5, or 2×10^6 copies of the *D. villosum* insert were delivered (p380 dilution spots). The filter was heated at 80 °C in a vacuum oven for 2 h, prehybridized for 5 h using 0.2 ml/cm^2 of hybridization buffer (5 × SSC, 0.1% N-lauroylsarcosine, 0.02% SDS, 1% blocking re-

agent) and then hybridized for 10 h using 0.025 ml/cm^2 of hybridization buffer containing 30 ng of freshly-denatured p380 DNA which was labelled with digoxigenin-11-dUTP using a Boehringer labeling kit. After hybridization, filters were washed for 2 × 5 min at room temperature with 2 × SSC containing 0.1% SDS, and 2 × 15 min at 65 °C with 0.1 × SSC containing 0.1% SDS. Immunological detection of digoxigenin haptens in DNA-DNA hybrids was performed with a DigDNA detection kit (Boehringer). Densitometric scanning of the coloured spots was carried out using an LKB 2202 Ultro Scan laser densitometer. The amounts of absorbance of the incident 590 nm laser beam were then measured by an LKB 2210-062 potentiometric recorder and the height of the plotted absorbance peaks was used to evaluate the relative colour development, and therefore the relative amounts of hybridization of the spotted DNA to the labelled probe. The linear regression equation relating the natural logarithm of the copy number of the *D. villosum* insert in the dilution spots and the natural logarithm of the corresponding densitometric readings was used to calibrate the relationship between the copy number of the sequence probed in the samples of *D. villosum* genomic DNA and the height of the densitometric peaks.

In-situ hybridization

Tips of colchicine-treated and fixed roots were squashed after treatment with pectinase as already described (Cionini et al. 1985), in such a way as to have separate squashes of apices from yellow or brown caryopses on each slide. Cytological hybridization was performed according to MacGregor and Mizuno (1976). Chromosomal DNA was denaturated in 0.07 N NaOH for 2 min at room temperature and p380 DNA, which had been labelled by nick-translation (Rigby et al. 1977) to a specific activity of 3.5 × 10^6 cpm/μg, was hybridized at a concentration of the *D. villosum* insert of 1 μg/ml. After incubation, unbound labelled DNA was removed by stringent washings (Hennen et al. 1975) and the preparations were covered with Ilford L_4 emulsion. After exposure times ranging from 1 week to 2 months, the slides were developed and stained with Giemsa (Merck).

Results

Cytophotometry

All the cytophotometric data given below were obtained from material fixed in ethanol-acetic acid. Comparable results were obtained when formalin-fixed material was used. The mean Feulgen absorptions of early prophases in the root and shoot meristems of 2-day-old seedlings from yellow or brown caryopses collected at Pisa are given in Table 1. Significant differences in the Feulgen/DNA absorptions never occur between shoot and root meristems of individual seedlings, as is evident from the standard errors of the means. By contrast, the mean Feulgen/DNA absorption is higher in seedlings from yellow caryopses as compared to those from brown caryopses (+12%, taking the value found in the former seedlings as a standard), and the Feulgen/DNA content differs up to 13% (taking the higher value as a standard) between individuals within each caryopsis-colour group. These differences are highly significant.

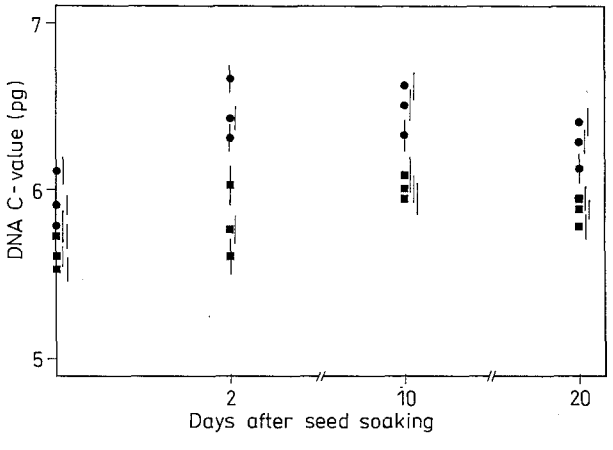
Figure 1 shows the results of cytophotometric determinations carried out on the root meristem of embryos in dry seeds or of seedlings at different days after seed soaking. These data indicate that significantly-different Feulgen/DNA absorptions can be measured in the resting embroys contained in different dry seeds. However, a clear-cut distinction between the two groups differing in caryopsis colour cannot be made at this developmental stage. During seed germination the Feulgen/DNA contents increase in all seedlings, but to a higher extent in those from yellow caryopses than in those from brown caryopses. Thus, as predicted from the data given in Table 1, the DNA C-values in the two groups of seedlings appear to be clearly separated at 2 days after seed soaking. At 10 days after seed soaking, the DNA C-values of seedlings from brown caryopses have increase still further while those of seedlings from yellow caryopses remain unchanged. Therefore, the differences between the two groups of seedlings are lowered, even though a distinction between the two groups is still possible. At 20 days after seed soaking, the differences between seedlings from yellow or brown caryopses tend

Table 1 Mean Feulgen absorptions (arbitrary units) of early prophases in the shoot and root meristems of seedlings from yellow and brown caryopses of plants grown at Pisa. Thirty prophases were measured in each meristem 2 days after seed soaking

Seedling no.	Seedling from			
	Yellow caryopses		Brown caryopses	
	Root	Shoot	Root	Shoot
1	2048.1 ± 34.6	2043.9 ± 20.5	1977.3 ± 37.6	1980.5 ± 28.8
2	2140.1 ± 53.5	2179.2 ± 61.5	1899.1 ± 37.2	1915.1 ± 29.4
3	2369.7 ± 42.1	2318.0 ± 47.3	1837.7 ± 36.6	1867.3 ± 23.1

Source of variation	F	*P*
Caryopsis colour	98.43	< 0.0001
Seedlings from yellow caryopses	16.07	< 0.0001
Seedlings from brown caryopses	7.44	0.0010

Fig. 1 DNA C-values of embryos in dry seeds or of seedlings at different developmental stages from yellow (●) or brown (■) caryopses collected from the population at Pisa. The values were calculated on the basis of the Feulgen absorptions of DNA presynthetic nuclei ($G_1 = 2C$) in the root apices of resting embryos and of early prophases (= 4C) in the root meristems of developing seedlings. Each point is the mean of 20 determinations. Fiduciary limits at $P \leq 0.01$



to decrease further, and all Feulgen/DNA absorptions are lowered towards those found in resting embryos.

The data given in Fig. 2 show that Feulgen/DNA contents may also differ significantly when different populations of *D. villosum* are compared. Taking the most variant values, the mean Feulgen/DNA absorptions in the resting embryos of yellow caryopses from the population at Pisa (89.2 arbitrary units) reach only 60.03% of the values found in the resting embryos contained in caryopses collected at Viterbo (148.6 arbitrary units).

Dot-blot hybridizations

The genomic DNA extracted from resting embryos or from seedlings belonging to single progenies of plants from different populations was dot-blot hybridized to the DNA repeat whose nucleotide sequence is shown in Fig. 3. This DNA element is a *Sph*I-*Sph*I sequence which is enriched in A + T base pairs (40% G + C), displaying some rather long stretches in which no G + C occurs. It contains the *Sac*I site already found by De Pace et al. (1992) and, though 396 bp long, clearly belongs to the family of *D. villosum*-specific DNA sequences already mentioned in the Introduction.

No quantitative difference was observed in the hybridization of the 396-bp element to the DNA extracted separately from roots or shoots collected from 10-day-old seedlings (data not shown). The data given in Fig. 4 show that, paralleling the variation observed for the DNA C-value (Fig. 1), the copy number of DNA sequences hybridizing to the 396-bp repeat increases significantly in the genome of embryos in yellow caryopses soon after seed soaking and continues increasing up to

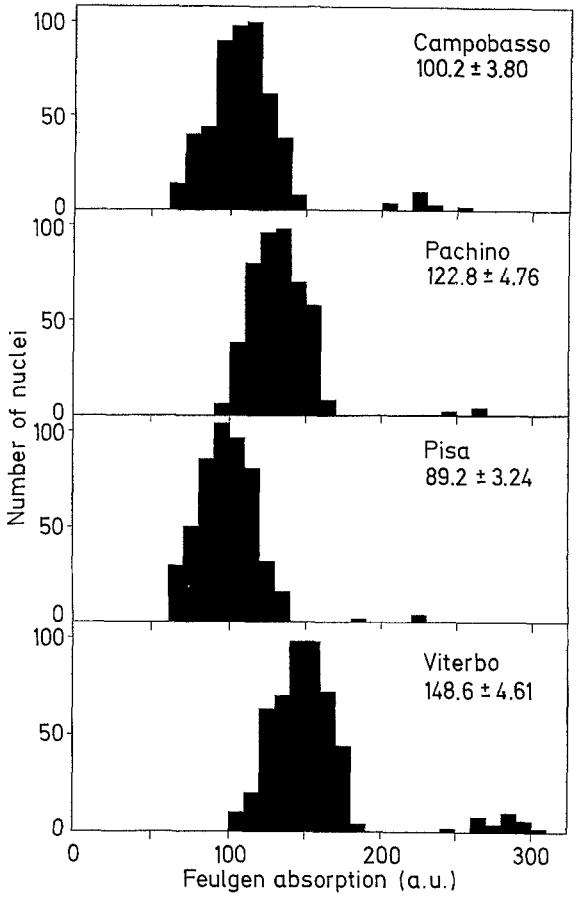


Fig. 2 Feulgen absorptions of nuclei in the root apex of embryos in dry yellow caryopses collected at different locations. One-hundred nuclei in each of five embryos per population were measured. The mean absorption values (± SE) of DNA presynthetic (G_1) nuclei in each population are shown

Fig. 3 Nucleotide sequence of the *D. villosum* insert in a p380 clone

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                10        20        30        40        50        60
                *         *         *         *         *         *
↑CTCGTTGTTGTTGTTGTCGATGTTGTAGTAAAGTCTTCGAGATCATGATTATTAGAG
SphI

                70        80        90       100       110       120
                *         *         *         *         *         *
TCCTATCATCTCAATTCCCCGATACCTTTCCAACACCTACGAAATTACTCAAACGGAGCT↑
                                                            SacI

               130       140       150       160       170       180
                *         *         *         *         *         *
CGTTATGACTCATGTTGATAGTCTCATTCAATCACATCTATATGGTGAATTTTGTCTTTT

               190       200       210       220       230       240
                *         *         *         *         *         *
CTCTGTTCACCGATTCTTCCCTAATTGTGAGCCTAACACCATGTGACCCAATTCTATCGT

               250       260       270       280       290       300
                *         *         *         *         *         *
CAAGGTGCATATTTCTACAGACCATACGGATGAGGTTGTCCCATATTTTTTTGAGCTGGG

               310       320       330       340       350       360
                *         *         *         *         *         *
AAGTGCAAATTGTTCCTTTTTTTCAATCCTTCGGATCCATTTTTTTGAGGGAACTAAACG

               370       380       390
                *         *         *
GCCCTGTGTTTTGGCGCTAAGAATTTGCGCCGCATG↑
                                    SphI
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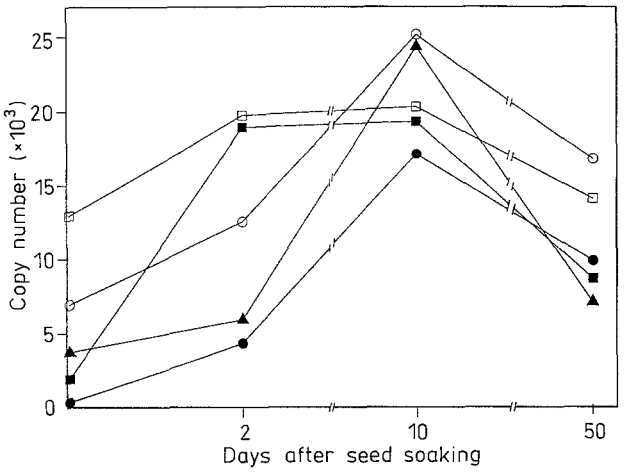


Fig. 4 Copy number of sequences related to the 396-bp repeat in the haploid genome of resting embryos or in seedlings at different days after seed soaking. Yellow caryopses from the progeny of single plants belonging to the populations at Bomarzo (●); Bullicame (▲); Mottola, half-sib 5 (○); Mottola, half-sib 6 (□); Viterbo (■) were used. Each point is the mean of the values calculated from the results of dot-blot hybridizations which were repeated six times, three for each of two different DNA extractions. Standard errors never exceed 8% of the means

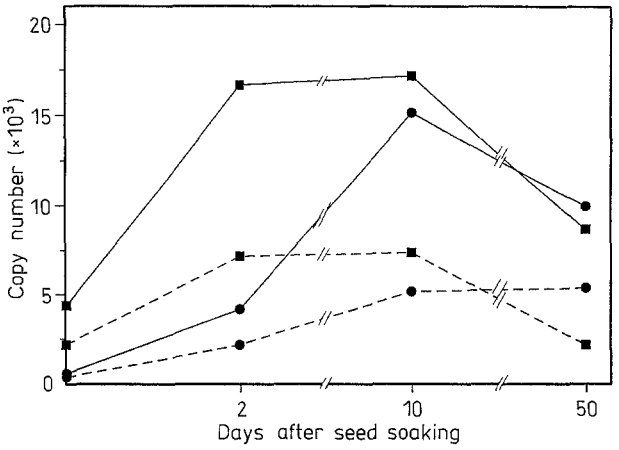
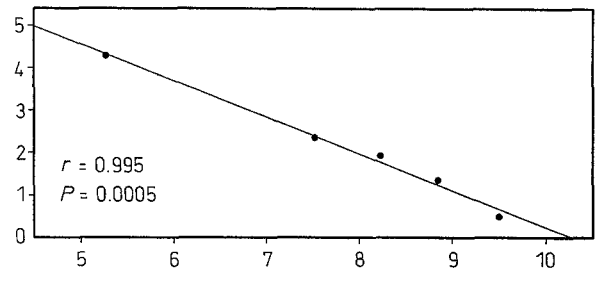


Fig. 6 Copy number of sequences related to the 396-bp repeat in the haploid genome of resting embryos or in seedlings at different days after seed soaking from yellow (——) or brown (– – –) caryopses. The progenies of two plants belonging to the populations at Bomarzo (●) and Viterbo (■) were used. Each point is the mean of the values calculated from the results of dot-blot hybridizations which were repeated six times, three for each of two different DNA extractions. Standard errors never exceed 8% of the means

10 days. Then, with further plant development, the redundancy of these sequences decreases towards the values found in resting embryos. Even though accompanied by certain temporal shifts, this trend is similar in all five populations studied. Sequence redundancy differs significantly between populations and between plant progenies within a population (compare the two progenies of plants from Mottola). These differences are more pronounced in resting embryos than after seed germination. This latter observation finds support in the highly significant ($P = 0.0005$) negative correlation ($r = -0.995$) shown in Fig. 5. This correlation indicates that the less redundant the DNA sequences in resting embryos, the more they are amplified with seed germination.

It can be seen from Fig. 6 that this DNA amplification follows temporal trends which are similar in embryos germinating from yellow or brown caryopses, but occurs to an extent which is significantly greater in the former.

Fig. 5 Correlation, in five populations, between the natural logarithm of the copy number of sequences related to the 396-bp repeat in the genome of seedlings at 10 days after seed soaking (on the abscissa) and the natural logarithm of the ratio between this copy number and that in the genome of resting embryos (on the ordinate). The data given in Fig. 4 was used



In-situ hybridization

The results obtained by hybridizing in situ ^{3}H-labelled 396-bp sequences to the chromosomes in the root meristems of 2-day-old seedlings from yellow (Fig. 7a) or brown (Fig. 7b) caryopses are shown in Fig. 7. In agreement with the results already obtained by De Pace et al. (1992), all the chromosome pairs except the shorter one of the two subtelocentric pairs are substantially labelled at or near the telomeres. Differences between Fig. 7a and b cannot be observed as far as the chromosomal locations of silver grain are concerned. However, a much higher hybridization level can be seen in the former figure. In agreement with the results of dot-blot hybridizations, this finding indicates that the copy number of sequences hybridizing to the 396-bp repeat is higher in seedlings from yellow caryopses than in those from brown caryopses.

Silver-grain counts indicated that the amount of DNA sequences hybridizing to the 396-bp repeat is not the same at different chromosomal locations. This can be seen in Fig. 8, where chromosome pairs are indicated according to the classification of Cremonini et al. (1993) who reported a *D. villosum* caryotype comparable to that found in the material studied by us. A linear correlation exists between the numbers of silver grains counted over corresponding chromosome regions in preparations made with roots from yellow or brown caryopses (Fig. 8). This suggests that the copy numbers of the DNA sequences in seedlings from yellow and brown caryopses are similarly correlated at each chromosomal location.

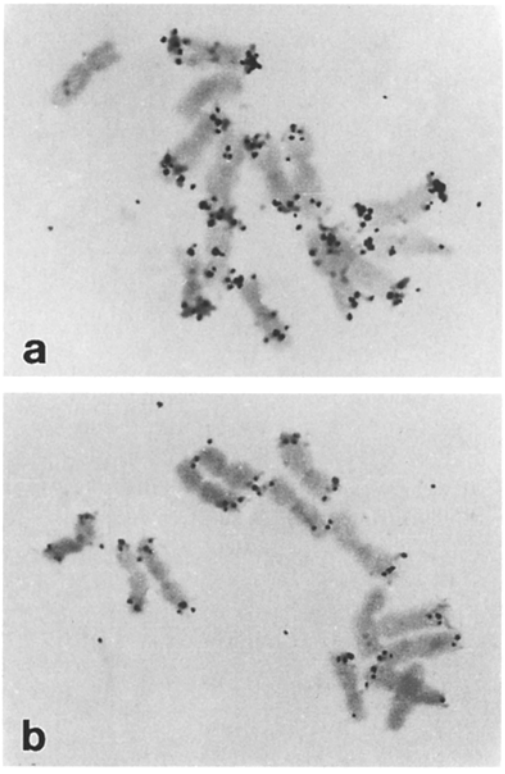


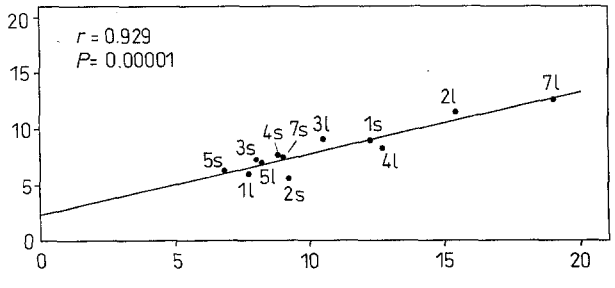
Fig 7a, b In-situ hybridization using ^{3}H-labelled 396-bp sequences. Metaphase plates in the root meristems of seedlings at 2 days after seed soaking from yellow (**a**) or brown (**b**) caryopses belonging to the progeny of a plant grown at Pisa. Giemsa × 3000

Discussion

Our findings are in line with evidence reported for other species which indicates that fluid domains exist in the nuclear DNA of plants (see Introduction). *D. villosum* can be added to the number of species within which rapid variations in genome size and organization have been detected (cf. Cavalini and Natali 1991). Indeed, the cytophotometric data shows that the basic amount of nuclear DNA may differ not only between plants within a population (Table 1 and Fig. 1) but also, on average, between populations (Fig. 2). Moreover, the genome size varies within each individual during embryo germination and to a different extent in seedlings from yellow or brown caryopses (Fig. 1). These results are supported by those obtained from molecular and cytological hybridization experiments, which indicate that redundancy variations of subtelomeric repeated DNA sequences are involved in these genomic changes (Figs. 4 and 6).

According to our results, redundancy modulation of repeated sequences in the nuclear DNA during the germination of the *D. villosum* seeds appears to be the rule. Other findings obtained in graminaceous species suggest that seed germination may be a step in development during which fluid DNA-domains are particularly prone to variation. Modifications in the redundancy of ribosomal cistrons and other DNA sequences have already been reported in the resting DNA during the early germination of wheat embryos (Chen and Osborne 1970). Moreover, synthesis of DNA sequences not related to either reduplication or repair has been found to occur in germinating cereal seeds (Bucholl and Buchowicz 1992, and references therein). In *Festuca arundinacea*, alterations in the basic amount of nuclear DNA which are induced by changes in temperature can occur only during seed germination (Ceccarelli and Cionini 1993). Amplification of DNA sequences in early developmental stages, followed by their elimination from the nuclear genome during plant development, has been shown to occur in rice (Kikuki et al. 1987; Zheng et al. 1987). Thus, variations in the redundancy of DNA sequences might be factors playing a role in developmental regulation. Our data show that the extent of amplification during seed germination of DNA sequences hybridizing to the 396-bp repeat is inversely related to the amount of these sequences in the genome of resting embryos (Fig. 5). This suggests that certain optimal redundancy levels of given DNA sequences are parts of regulatory mechanisms acting during specific developmental steps in *D. villosum* plants.

Fig. 8 Correlation between the mean number of silver grains counted, after in-situ hybridization with ^{3}H-labelled 396-bp sequences, over corresponding chromosome regions in preparations made with the root meristem of seedlings from yellow (on the abscissa) or brown (on the ordinate) caryopses. The preparations analyzed were those from which the microphotographs in Fig. 7 were taken. Numerals indicate chromsosme pairs (according to Cremonini et al. 1993); *l* and *s* indicate the long or short chromosome arm, respectively



The finding that the amounts of amplified DNA sequences hybridizing to the 396-bp repeat in seedlings from yellow or brown caryopses are similarly correlated at each telomeric or subtelomeric region of six chromosome pairs (Fig. 8) may be taken as an indication that the extra synthesis of these DNA elements is under one and the same control even if it occurs at different chromosomal locations. It is worth noting that the DNA sequences which are amplified during seed germination are also involved in the diversification of the genome size between individual plants of a population and between populations which can be observed at other developmental stages (Figs. 4 and 6). It is possible that sequence amplification during seed germination and the creation of more stable genomic diversity within the species may be related events. Indeed, amplified DNA sequences, which are per se unstable, may acquire

stability by becoming inserted into the chromosomal DNA (Nagl 1990). The differences in genome size between populations and between plants within a population may be hypothesized as being produced by quantitative differences in this insertion process.

The 396-bp repeat is species-specific to *D. villosum* (De Pace et al. 1990, 1992). As such, it may be in rapid evolutionary change both in structure and redundancy, as has been suggested for DNA sequences of this kind in other plant species (Dvořák 1983). *Secale cereale* shows strong homology in both morphological and biochemical characters with *D. villosum*, thus suggesting a common ancestry (Baum 1983). A 68.5% nucleotide-sequence homology exists between about half of a repeated sequence found in the telomeric heterochromatin of rye cv Imperial (Appels et al. 1981) and the 396-bp repeat of *D. villosum*. However, in spite of this sequence homology, no hybridization signal was detected after dot-blot hybridization of the 396-bp repeat to the DNA of other rye accessions (De Pace et al. 1990, 1992). This indicates that sequences making up a repetitive fraction of the genome of rye cv Imperial are not present, or are present in a very low copy number, in the genome of other rye plants. Therefore, the redundancy of these sequences has evolved differently in different rye accessions. Similar differences between rye accessions have also been suggested by cytogenetic studies (Martin and Hesemann 1988).

Quantitative changes in the nuclear DNA of the kind we have observed in *D. villosum* can, possibly through nucleotypic effects (Bennett 1987), affect several phenotypic characters at the cellular and organismal level (Cavallini et al. 1993; Ceccarelli et al. 1993; Natali et al. 1993). Therefore, the differences in genome size which are produced during seed germination may contribute to explaining the diversity of certain cellular characters in embryos germinating from yellow or brown caryopses and why the two kinds of caryopses differ in behaviour at germination and during aging (see Introduction). This developmental variability within a population may be of importance in buffering the effects of changing environmental conditions. The differences in the basic amount of nuclear DNA which are established in different populations might then play a role in the adaptation to peculiar, lasting characters of the environment (Ceccarelli et al. 1992). Alternatively, they may be a long-term effect of genetic drift involving the redundancy of repeated DNA sequences.

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