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Intervarietal Nuclear DNA Variation in Barley (*Hordeum vulgare* L.)

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Nuclear DNA estimations made in ten varieties of barley (*Hordeum vulgare*) using feulgen cytospectrophotometry revealed significant intervarietal variation in nuclear DNA content with the 2C DNA values ranging from 11.25 to 13.78 pg. A positive relationship between DNA density and DNA content was observed, though it was not strictly linear. DNA density was higher in 4C nuclei compared with 2C nuclei. Using Duncan's multiple range test the varieties showing significant differences in 2C DNA content were identified and these were arranged into four different groups.

Key Words: Nuclear DNA content, DNA density, Intervarietal variation, Barley

Introduction

The last three decades have witnessed rapid strides in better understanding of the size, structure, organization and evolution of plant genome (Raina 1990). Extensive variation in DNA content between different species of higher plants is reported in contrast to earlier studies where DNA values within the species were shown to vary slightly. The theory of constancy of DNA content within the species was questioned when convincing examples of intraspecific variation became available and it is now generally accepted that such a phenomenon may no longer be regarded as exceptional.

Barley, an important cereal and a model crop for many research programmes, has been extensively used for genetical studies. However, no comprehensive studies on the DNA variation patterns in *Hordeum* have hitherto been carried out (Bennett & Smith 1971, Rao & Sharma 1987, Bothmer et al 1987). Therefore, the present study undertakes estimation of nuclear DNA contents in ten barley varieties and attempts to find out the extent of intervarietal nuclear DNA variation.

Materials and Methods

The material comprised ten barley varieties, obtained from the germplasm collections maintained at the Department of Agricultural Botany, Meerut University, Meerut, and a standard in the form of *Allium cepa*. Nuclear DNA content was estimated by feulgen cytospectrophotometry using a Vicker's M-85 Scanning Microdensitometer. The procedure of Teoh and Rees (1976) was followed with slight modifications for material processing, staining and slide preparations.

For each accession, ten 2C nuclei and equal number of 4C nuclei were scanned in each of the 2 replications. The results obtained in arbitrary relative absorption units were converted into absolute amounts using *Allium cepa* as standard (2C DNA = 33.5 pg). By measuring the optical density of both standard and the material under study, proportionality was used to find out the amount of nuclear DNA in the given material, as:

$$\frac{\textit{Amount of DNA in standard (pg)}}{\textit{Optical density of standard (arbitrary units)}} = \frac{\textit{Amount of DNA in the genotype (pg)}}{\textit{Optical density of genotype (arbitrary units)}}$$

Density of nuclear DNA was also computed in arbitrary units by dividing the DNA value with the display area of the nucleus scanned. Duncan's multiple range test was applied to compare 2C DNA of each variety with those of every other variety and to find out which differences are significant and which are not.

Results and Discussion

Significant variation in nuclear DNA amounts was observed among the ten barley varieties and the data are given in tables 1 and 2. Based on Duncan's multiple range test, the ten varieties presently studied could be divided into 4 different groups, each group exhibiting significant difference from the rest. The results are shown by bars in the column of 2C DNA values in Table 2. Our data support earlier reports on the existence of intraspecific DNA variation in crops like maize

Table 1 *Analysis of variance of 2C DNA content in ten barley varieties*

Source	d.f.	Sum squares	Mean squares	F	P
Replication	1	0.103	0.103	0.81	ns
Accessions	9	21.660	2.406	18.94	0.05
Error	9	1.144	0.127		

Table 2 *Nuclear DNA content and DNA density in ten barley varieties*

Sl. No.	Variety	2C DNA amount (pg)	Mean 2C value computed from 4C	DNA density	
				2C	4C
1	Dl-265	11.25*	11.30	0.692	0.845
2	DL-243	11.33	10.55	0.691	0.945
3	DL-89	11.40	11.33	0.967	1.026
4	K-192	11.90	11.60	0.671	0.912
5	K-244	12.24	12.45	0.661	0.893
6	K-125	13.02	12.40	0.901	0.963
7	DL-270	13.70	14.05	1.008	1.514
8	DL-36	13.70	14.00	0.754	0.977
9	K-273	13.74	14.05	0.806	0.889
10	K-234	13.78	14.05	0.662	0.955

*Varieties covered by the same vertical bar do not significantly differ in their 2C DNA contents

(Laurie & Bennett 1985), flax, *Microseris douglasii* (Price et al. 1981), *Capsicum annuum* (Mukerjee & Sharma 1990a), *Cajanus cajan* (Mukerjee and Sharma, 1990b), etc. Barley is one of the oldest cultivated crops and has been subjected to man's selection and breeding over a long period. Adaptation of cultivated barley to a very wide range of ecological and climatic conditions might have resulted in selected changes in DNA content to accumulate, leading to intervarietal nuclear DNA variation. Several earlier studies on patterns of variation in DNA content among closely related species and within species including *Microseris douglasii, M. bigilovii* and *Zea mays* also strongly suggested that DNA amount is of adaptive significance and subject to natural selection (Price 1988). However, Bennett & Smith (1971) and Rao & Sharma (1987) did not observe any significant intraspecific variation for nuclear DNA content in *Hordeum*.

Numerous mechanisms have been proposed to explain origins of variation in DNA content such as unequal crossing over, transposition, saltatory amplification and errors of DNA replications (c.f. Price 1988). Structural changes involving deletion and/or duplication can lead to variations in nuclear DNA content. In barley, Singh (1990) while studying the somatic karyotypes observed significant intervarietal differences in total chromatin length and volume, which were attributed to structural changes and loss or gain of heterochromatin material. S.N. Gupta (Personal communication) also oberved that variation in nuclear DNA content was closely associated with karyotype variation in pea genotypes. These suggest that the intervarietal nuclear DNA variation recorded in the present study might be due to structural changes.

DNA density exhibited a positive relationship with DNA content, though, the relationship was not found to be strictly linear (table 2). Similar observation was made by earlier workers (Rees & Jones 1972, Gupta 1976). DNA density was higher in 4C nuclei compared with 2C nuclei (table 2). This can be expected if an increase in DNA quantity is accompanied with DNA condensation.

The 4C nuclei should have double the DNA recorded in 2C nuclei since they belong to the same tissue of the plant and the 2C values computed from 4C values should be equal to 2C DNA values estimated directly. In contrast to this general expectation, the estimated 2C DNA values were observed to be slightly higher or lower than those computed from 4C (table 2), indicating thereby that the observed 2C values are relative over-estimates in the former and under-estimates in the latter. Both types of situations were reported earlier. It may not be possible to reach any definite conclusion for these differences, although experimental error factor may not be ruled out.

The results of the present study establish the existence of intervarietal nuclear DNA variation in barley (*Hordeum vulgare*) also and suggest that structural changes may possibly be associated with this.

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References

Bennett M D and Smith J B 1971 The 4C DNA content of several *Hordeum* genotypes; *Can. J. Genet. Cytol.* **13** 607-611

Bothmer R Von, Jorgensen R B and Linde-Leursen I 1987 Natural variation, phylogeny and genetic resources in *Hordeum; Barley Genetics* V 23-33

Gupta P K 1976 Nuclear area and nuclear drymass in thirteen species of *Crotalaria* (Angiospermae-Leguminosae); *Chromosoma* **54** 155-164

Laurie D A and Bennett M D 1985 Nuclear DNA content in the genera *Zea* and *Sorghum*. Intergeneric, interspecific and intraspecific variation; *Heridity* **55** 307-313

Mukerjee S and Sharma A K 1990a Intraspecific variation in nuclear DNA in *Capsicum annuum* L.; *Proc. Indian Acad Sci.* **100** 1-6

—— —— and Sharma A K 1990b Cytophotometric estimation of nuclear DNA in root and shoot meristems of *Cajanus cajan; Proc. Natl. Acad. Sci. India* **60(b)** 43-46

Price H J 1988 Nuclear DNA content variation within Angiosperm species; *Evolutionary Trends in Plants* **2** 53-60

Price H J, Chambers K L and Bachmann K 1981b Geographic and ecological distribution of genomic DNA content variation in *Microseris douglasii* (Astraceae); *Botanical Gazette* **142** 415-426

Raina S N 1990 Genome organization and evolution in the genus *Vicia*; In *Biological Approaches and Evolutionary Trends in Plants* pp 1-18 (London: Academic Press)

Rao V L K and Sharma A K 1987 Amount of DNA and genotypic differences in *Hordeum; Cytoligia* **53** 546-551

Rees H and Jones R N 1972 The origin of wide species variation in nuclear DNA amount; *Int. Rev. Cytol.* **32** 53-92

Singh V P 1990 Somatic karyotype analysis in barley (*Hordeum vulgare* L.); M.Sc. Thesis, Meerut University, Meerut

Teoh S B and Rees H 1976 Nuclear DNA amounts in populations of *Picea* and *Pinus* species; *Heredity* **36** 123-137