Chromosomal Analysis in Cultured Cells of Barley (*Hordeum vulgare* L.): Structural Alterations in Chromosomes

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Chromosomal instability of callus and cell suspension cultures have often been reported (D'Amato 1977). Chromosomal instability in cells grown *in vitro* not only includes numerical variations, but also comprises changes in chromosome structure and basic karyotype (Sunderland 1977). Numerical changes have been examined most often (Evans and Reed 1981). A detailed assessment of chromosome rearrangement or other structural changes requires sophisticated karyotype analysis or chromosomal banding technique (Evans and Reed 1981). Unfortunately detailed analysis of chromosomal rearrangement has been completed only in *Crepis capillaris* (Scristan 1971). Despite of several attempts, we were not able to standardize a reproducible banding technique for barley chromosomes. Regenerability of a callus is often ascribed to numerical and structural changes in the chromosomes (Muir 1965, Murashige and Nakano 1965, Torrey 1967). Chromosomal interchanges have been described to be a basis for obtaining somaclonal variation (Pijnaker and Ferweda 1987, Krap and Bright 1985). In barley, variability in callus culture has been observed to be expressed within the regenerants (Orton 1980). Recently we have reported the chromosome number variation in cultured cells of barley (Mohanty 1990). In the present communication we report the structural aberrations of the chromosomes of different ploidy levels obtained from cultured cell karyotypes of barley.

Materials and methods

The root tips obtained from germinating barley seeds (served as control) and 150 days old mesocotyl-explant derived calluses of barley (Mohanty 1987, Mohanty 1990) were pre-treated with α -bromonaphthalene for 3 hr, washed several times with distilled water and fixed with aceto-ethanol (1:3) for 8 hr. The fixed materials were then hydrolysed in 1N HCl for 8 min at 58°C and stained with aceto-orcein. Randomly selected small portions of the stained callus were squashed and mounted in 45% acetic acid. For karyotype analysis, 14 well spread metaphase cells were randomly selected from temporary slide preparations. The karyotypes containing folded or twisted form of chromosomes were not taken into consideration. Idiograms and subsequent analyses were performed from the camera lucida drawings of individual karyotypes drawn at a magnification of 1500 X. Karyomorphology of metaphase chromosomes in cultured cells were analysed considering the following parameters: (i) absolute length of the individual chromosomes in micron (s), (ii) arm index (r) and form percent (F%) value for determining the nature of primary construction in the chromosomes (Krikorian *et al.* 1983), (iii) relative length of the chromosome complement (Krikorian *et al.* 1983), and (iv) disparity index (DI) of chromosomes in a karyotype was calculated as follows:

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$$DI = \frac{\text{Longest chromosome} - \text{Shortest chromosome}}{\text{Longest chromosome} + \text{Shortest chromosome}} \times 100$$

Results and discussion

Structurally altered chromosomes were readily observed in the karyotypes of the cultured cells in the form of dicentric and acrocentric chromosomes. Occurrence of chromosomal

Fig. 1. A-F.

Fig. 1. G-I.

Fig. 1. J-O.

Fig. 1. A-O, Idiograms of callus cells chromosomes of *Hordeum vulgare* L. A, Idiogram of a normal diploid root tip cell ($2n=14$). B-F, Idiograms of diploid callus cells karyotypes ($2n=14$) showing changes in basic karyotype nature due to structural alteration in chromosomes. G, an aberrant tetraploid callus cell ($2n=28$). H-N, Idiograms of aneuploid callus cell with aberrant chromosomes ($2n=36$, $2n=32$, $2n=26$, $2n=25$, $2n=18$ and $2n=8$). N and O callus cells idiograms showing $2n=5$ chromosomes each, but their karyotypes varied due to different degrees of structural aberration in chromosomes.

fragments in cultured metaphase cells also indicated structural alterations in chromosomes through deletion. In order to quantify all the minor and major structural changes, randomly selected 14 well spread metaphase plates selected from 5 callus samples were drawn. Idiograms were constructed (Fig. 1 A-O). The chromosomes were classified into eight groups and there were four types in each group. The eight groups were A, B, C, D, AS, BS, CS and DS. The four types were A_1 , A_2 , A_3 , A_4 for A group, B_1 , B_2 , B_3 and B_4 for B group and so on. Comparative analysis of the fourteen cultured karyotypes is depicted in Table 1. Analysis of these cultured karyotypes was performed with reference to a control karyotype, obtained from the root tip squashes of the same cultivar. All the 14 cultured karyotypes with control classified to different groups and types described supra, are presented in Table 1. It is evident that number of satellite chromosomes in cultured metaphase cells varied from minimum 1 to maximum 5. For most of the cultured karyotypes, disparity index (DI) was higher compared to control karyotype (Table 1). Structurally altered chromosomes were frequently encountered under the cultural conditions irrespective of the ploidy status of the karyotypes (Tables 1 and 2). These altered chromosomes formed different types or groups depending upon the degree of aberration (s). The average frequency (%) of normal and structurally altered chromosomes in diploid, tetraploid and aneuploid callus cells is presented in Table 2. Analysis of diploid karyotypes revealed both major and minor structural changes in the chromosomes.

Although, the callus cells showed same ploidy level ($2n=14$) as in the original plant (Fig. 1 A–F), the basic karyotype nature was changed due to structural rearrangement in chromosomes. An average of 44% structurally altered chromosomes were analysed from the cultured diploid karyotypes (Table 2). The frequency of aberrations within the chromosome groups of a specific karyotype also varied. In control root tip squashes ($2n=14$) the satellite group of chromosomes (BS) were contributed to 28.5% of the total chromosomes. However, in diploid callus cells ($2n=14$) only 7.1% satellite chromosomes were structurally similar to control, 4.2% satellite chromosomes were found with minor structural changes and rest were eliminated from the cells (Tables 2 and 3). These minor structural changes placed them in different new types within the satellite group (Table 2). Interestingly a new typical non-

Table 1. Comparative accounts of 14 cultured karyotypes of mesocotyl callus cells of *Hordeum vulgare*

Karyo type	Chromosome number (2n)	No. of satellite chromosomes	DI%	Karyotype formulae
Control (root tip)	14	4	14.28	$6B_1+2C_1+2C_2+4BS_1$
Culture karyotype (No.)				
1	14	4	21.73	$5C_1+1C_2+3D_1+1D_2+2CS_1+1CS_2+1DS_1$
2	14	3	33.33	$6B_1+1B_2+2C_2+1C_3+1D_3+1BS_2+1BS_4+1DS_1$
3	14	2	17.64	$8B_1+1B_2+1C_1+1C_2+1BS_2+2CS_2$
4	14	4	58.82	$5A_1+3B_1+1B_2+1C_1+2D_3+1AS_2+1BS_1$
5	14	4	20.93	$2A_1+3A_2+5B_1+3BS_1+1BS_2$
6	28	5	60.00	$5A_1+2A_2+8B_1+2C_2+2C_1+2C_3+1D_1+1D_2+2AS_1+1BS_1+2BS_2$
7	36	2	42.86	$7B_1+5B_2+1B_4+8C_1+7C_2+2C_3+1D_1+1D_2+2BS_2+1BS_3+1CS_4+1F$
8	32	1	46.15	$1B_1+3B_2+10C_1+3C_2+3C_3+2C_4+5D_1+2D_2+1D_3+1D_4+1BS_1$
9	26	5	31.03	$1A_2+9B_1+6C_1+2C_2+1C_3+1C_4+1D_2+1AS_2+3BS_1+1BS_4+2F$
10	25	2	47.83	$1B_1+11B_4+2C_1+4D_2+2D_3+3D_4+2DS_1+2F$
11	18	1	53.80	$6B_1+4C_1+6D_1+1D_3+1BS_1$
12	8	1	24.13	$3B_1+3C_1+1C_2+1CS_1+2F$
13	5	1	25.00	$3B_1+1C_1+1CS_1$
14	5	2	37.05	$1B_1+1C_3+1D_1+1BS_1+1BS_2$

A=Very long chromosomes (15–24 μm) without satellite; B=long chromosomes (10–14.99 μm) without satellite; C=Medium sized chromosomes (7–9.9 μm) without satellite; D=Short sized chromosomes (3–6.99 μm) with satellite; AS=chromosomes very long (15–24 μm) with satellite; BS=chromosomes long (10–14.99 μm) with satellite. Script 1=form percent, 40–50, 2=form percent, 30–39.99; 3=form percent 20–20.99, 4=form percent 10–19.99, F=acentric chromosomal fragment.

satellite group having very long, metacentric to nearly metacentric chromosomes (A), appeared considerable frequency (10%) in the cultured diploid karyotypes (Table 2). Origin of this group of chromosomes might be due to non-reciprocal translocation. It has been observed that plant regeneration in callus culture mostly selects cells with diploid chromosome number (Vasil 1983, Larkin and Scowcroft 1981). Variation within the regenerated plants (Somaclonal variation) is often reported (Larkin and Scowcroft 1981, Scowcroft 1984, McCoy and Phillips 1982). The possibility of minor genetic reshuffles in the form of minor structural changes in chromosomes, without affecting their original chromosome number as the origin of variants in regenerants can not be ruled out. Thus, the callus culture can be proposed as a potential source of genotypes and regenerants bearing structural changes in the chromosomes which

obviously involve genetic changes of the corresponding degrees and, this in turn might result in the generation of somaclonal variants.

In tetraploid callus cell, 'A' group of chromosomes nonsatellited very long, meta-centric to nearly metacentric chromosomes appeared in considerable frequency ($A+A=25\%$) (Table

Table 2. Frequency (%) of normal (N) and structurally altered (SA) chromosomes in diploid, tetraploid and aneuploid callus cells of *H. vulgare*

Chromosome types	Control karyo type	Cultured cell karyotypes					
		Diploid cells		Tetraploid cell		Aneuploid cells	
		%N	%SA	%N	%SA	%N	%SA
A ₁	—	—	10.0	—	17.9	—	—
A ₂	—	—	4.38	—	7.1	—	0.6
B ₁	42.9	31.4	1.4	28.6	—	20.0	—
B ₂	—	—	1.4	—	7.1	—	5.2
B ₄	—	—	—	—	7.1	—	7.7
C ₁	14.3	10.0	—	—	—	21.9	—
C ₂	14.3	5.7	—	—	—	8.4	—
C ₃	—	—	1.4	—	7.1	—	4.5
C ₄	—	—	—	—	—	—	1.9
D ₁	—	—	4.3	—	3.6	—	8.4
D ₂	—	—	1.4	—	3.5	—	5.2
D ₃	—	—	4.3	—	—	—	2.6
D ₄	—	—	—	—	—	—	2.6
AS ₁	—	—	—	—	7.1	—	—
AS ₂	—	—	1.4	—	—	—	0.6
BS ₁	28.5	7.1	—	3.6	—	3.9	—
BS ₂	—	—	1.4	—	7.1	—	1.9
BS ₃	—	—	1.4	—	—	—	0.6
BS ₄	—	—	1.4	—	—	—	0.6
CS ₁	—	—	2.9	—	—	—	1.3
CS ₂	—	—	4.3	—	—	—	—
CS ₃	—	—	—	—	—	—	—
CS ₄	—	—	—	—	—	—	0.6
DS ₁	—	—	2.9	—	—	—	1.3
Total (%)	100	54.2	44.2	32.2	67.7	54.2	45.6

Table 3. Frequency (%) of different chromosome groups in cultured cell karyotypes of *Hordeum vulgare* L.

Chromosome groups	Frequency (%) of different chromosomes groups							
	A	B	C	D	AS	BS	CS	DS
a) Cultured cells	7.1	34.8	28.1	15.0	1.6	8.7	3.2	1.6
b) Control (root tips)	—	42.9	28.6	—	—	28.6	—	—
Theoretical difference*	7.1	-8.1	-0.5	15.0	1.6	-19.9	3.2	1.6

* Theoretical difference = b-a

(Loss or gain of chromosomes of different groups by cultured cell karyotypes)

2). In tetraploid callus cells, the normal satellited chromosomes were reduced to 3.6%, however, 7.4% satellited chromosomes were observed with minor structural alterations (Table 2). Total of 68% structurally altered chromosomes were recorded from tetraploid callus cells.

Thus comparing to diploid, the frequency of structurally altered chromosomes was higher in tetraploid callus cells. Similar trend was observed in callus cells of *Crepis capillaries* (Sacristan 1971). Hsu (1881) has also noted similar observation in animal cell cultures and reasoned it to be due to greater mitotic disturbances in polyploidized cells. Aneuploid cells of the culture also carried around 46% of structurally altered chromosomes in them (Table 2).

From the analysis of 14 cultured karyotypes, an estimate of gross deviation of chromosome structure from original groups are depicted in Table 3. In callus cell population, 'C' group of chromosomes (medium sized (length 7–9.9 μm) non satellited) observed to have maximum survival chances in culture condition, whereas, 'B' group had lost 8% of the original group of chromosomes. Around 20% of the BS group (long, satellited chromosomes, length 10–14.99 μm) chromosomes were eliminated from the cultured cells, compared to 28.6% present originally in control. However 4.8% were observed in structurally altered form. A new group "A" (very long, 15–24 μm length, non satellited) chromosomes appeared in considerable frequency (7%) in cultured cells. Other structurally altered chromosomes (D, AS, CS and DS group chromosomes) also observed at very low frequencies in cultured cells.

It is imperative from the present study that all the cultured metaphase cells carried structurally altered chromosomes in them and these aberrant cells have a positive selective value in *in vitro* culture systems. Even if latter is not proved, but similar assumption has been made from the study on the culture karyotypes of *Crepis capillaries* (Sacristan 1971). Such type of aberrant cell selection may be comparable to genetic drift. Regenerants from such cultures (Somaclonal variants) might be useful for the selection of useful genetic variants.

Summary

Structural aberrations of chromosomes of 150-day-old cultured cells of barley were examined by karyotypic analysis. Minor and major rearrangements were detected in chromosomes; all the metaphase cells analysed from the culture revealed the presence of aberrant chromosomes in them. Analysis of cultured diploid karyotypes showed the inclusion of 44% structurally aberrated chromosomes. It was observed that certain new karyotypes were originated due to chromosomal rearrangements, while the ploidy level remained same as that of original plant. Tetraploid cells carried greater frequency of aberrant chromosomes than diploid cells. Aneuploid cells of the culture also carried 48% structurally altered chromosomes in them. The significance of this study is discussed.

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