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Chromosome variation in wheat plants regenerated from cultured immature embryos

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Summary. A cytological study has been made of plants regenerated from cultured immature embryos of four wheat cultivars (*Triticum aestivum*, 2n=6x=42). In total, 29% of the 192 plants examined were aneuploid with a range in chromosome numbers of 38–45. Evidence of chromosome structural changes was also found. This variation occurred in regenerants of all four cultivars, but there were large differences in the proportions of aneuploids arising from individual cultures which meant that no significant differences could be demonstrated between cultivars. Chromosome abnormalities were present in plants regenerated both from embryogenic cultures and from cultures in which the origin of shoots could not be distinctly defined.

Key words: Wheat – Plant regeneration – Chromosome variation

Introduction

Considerable attention has been focused on the potential for crop improvement offered by genetic manipulation techniques involving plant tissue culture. For the exploitation of such techniques it is essential that whole plants can be regenerated routinely from cultured plant tissues or single cells, and in such a way that any introduced changes are transmitted to the regenerants without other undesirable effects. Plants regenerated from tissue cultures, however, often show phenotypic variation (Larkin and Scowcroft 1981). It is important to identify the causes and extent of this 'somaclonal' variation in order to assess the suitability of various regeneration procedures, and as a first step towards either eliminating the variability, or controlling it so that it may be of direct value in plant breeding.

Regeneration from cultured plant tissues and protoplasts often involves a callus phase, and since there is considerable evidence for chromosome instability in callus (Bayliss 1980; Constantin 1981), it is possible that somaclonal variation may have some basis in changes in chromosome number and structure. In addition, some plant tissues are known to undergo changes in ploidy level during differentiation (D'Amato 1977) and it may be that some of the variation is already present in the plant tissues from which regenerating cultures are orginated. It is therefore of considerable relevance to assess the cytological nature of regenerated plants.

In the cereals, there has been only limited success in the culture of isolated protoplasts (Vasil and Vasil 1980; Lu et al. 1981) but plants have been regenerated consistently from cultured immature embryos and inflorescences of a number of species, e.g. maize (Green and Phillips 1975), oats (Cummings et al. 1976), barley (Dale and Deambrogio 1979), wheat (Ozias-Akins and Vasil 1982; Sears and Deckard 1982; Maddock et al. 1983) and pearl millet (Vasil and Vasil 1981; Wang and Vasil 1982). In a recent study of regeneration in wheat we have observed morphological differences between some of the plants obtained (Maddock et al. 1983).

In this report we describe a cytological survey of wheat plants regenerated from cultured immature embryos of four wheat cultivars and present evidence of changes in both chromosome number and structure.

Materials and methods

A cytological examination was made of plants regenerated from cultured immature embryos of one spring (cv. 'Highbury') and three winter cultivars (cvs. 'Fenman', 'Avalon' and 'Copain') of wheat (*Triticum aestivum*) together with the respective control plants which were grown from seed. The method used for regeneration is described elsewhere (Maddock et al. 1983). Shoot-forming cultures were initiated from individual immature embryos. Plants were regenerated from five

different cultures of cvs. 'Highbury' (H7, H22, H27, H30, H50) and 'Copain' (C4, C8, C21, C35, C38), from six cultures of cv. 'Fenman' (F5, F12, F14, F15, F17, F24) and from seven cultures of cv. 'Avalon' (Av19, Av37, Av43, Av45, Av46, Av47, Av54). Metaphase counts were made for up to 12 regenerants from each culture, although in some cases samples were reduced by accidental losses in the glasshouse.

Mitotic chromosome analysis was carried out on root-tip meristems of young plants after pretreatment in a saturated solution of α-bromonaphthalene overnight at 4 °C. Roots were fixed for at least 1 day in 3 : 1 alcohol : glacial acetic acid, hydrolysed in 1N HCl at 60 °C for 5 min and stained in Feulgen. Chromosome counts for each plant were established from at least five well-spread cells in at least two different roots.

Meiosis was studied in pollen mother cells (pmcs) from inflorescences fixed in Carnoys fluid (6 : 3 : 1 alcohol : chloroform : glacial acetic acid, mordanted with ferric chloride solution). In order to screen for chromosome structural changes, metaphase I (MI), anaphase I (AI), dyads, AII and tetrads were all studied in detail. In Highbury, where some of the controls were found to carry an interchange, five plants were analysed from each of five cultures, whilst in the other cultivars about ten regenerants from each were screened.

Results

Plant regeneration

Plants were regenerated over a period of up to five months from four wheat cultivars which differed consistently in morphogenetic capacity (Maddock et al. 1983). In cultivars 'Avalon' and 'Fenman' more cultured embryos gave rise to shoot-forming cultures than in cultivars 'Copain' and 'Highbury' (Table 1). The cultures of cultivars 'Avalon' and 'Fenman', chosen at random for the cytological study, were all embryogenic although shoot development was more rapid in the latter cultivar. In cultivar 'Copain' embryoids were clearly evident in four of the five chosen cultures, whilst in cultivar 'Highbury' no embryoids were observed under the dissecting microscope in the five shoot-forming cultures.

Table 1. Variation in chromosome number of regenerated plants from cultivars differing in morphogenetic response

Cultivar	% shoot-forming cultures[a]	Presence of embryoids[b]	% aneuploidy	Range in chromosome nos.
'Highbury'	15	–	33	39–44
'Copain'	40	E+ +	41	37–45
'Avalon'	96	E+ + +	15	40–43
'Fenman'	85	E+ + +	22	41–44

[a] From Maddock et al. (1983)

[b] The % of morphological cultures with embryoids clearly present: E+ + 40–70%, E+ + + 70–100% (from Maddock et al. 1983)

Cytogenetic analysis

Control material. Five control plants of each cultivar were examined and all were found to have the correct chromosome number (2n = 6x = 42). Surprisingly, however, structural chromosome mutations were found in two of the cultivars. One of the Fenman controls had a heteromorphic bivalent at MI, which was considered to be the result of a spontaneous deletion since it was only observed in a single plant. In cultivar 'Highbury' analysis of meiosis revealed a floating interchange in the seed stock, and interchange heterozygotes were found at high frequency amongst the control plants.

Variation in chromosome number amongst regenerated plants. Regenerated plants of all four cultivars were found in which the chromosome number differed from the euploid number of 42 (Table 1). In total, 71% of all regenerants had 42 chromosomes (Fig. 1 a). Amongst the aneuploid plants chromosome numbers ranged between 38 and 45, the most frequent being 41 (Fig. 1 c) and 43 (Fig. 1 b). The majority of regenerants (euploid and aneuploid) had a consistent chromosome count in all the root-tip cells examined. This constancy was confirmed by analysis of meiosis since, in the majority of cases, the same numbers were found in pmcs as in roots, e.g. Fig. 4 c, e (both 2n = 43) and Fig. 5 a (2n = 41). Some exceptions were found, however, and five aneuploid regenerants of cultivar 'Copain' showed variation in chromosome number within the same root-tip. Three of these were regenerants from the culture C21 and two from C8, these being cultures which gave rise to most of the aneuploids found amongst the 'Copain' regenerants (Fig. 2).

The overall frequency and the range of aneuploidy appeared to differ between cultivars (Table 1) but comparisons of aneuploid frequency were complicated by the distribution of the variation between the cultures. Figure 2 shows the distribution of chromosome counts for regenerants from the different cultures. Some gave rise to regenerants which were all euploid, some give rise mostly to euploid plants, and some gave rise to regenerants which were all (or nearly all) aneuploids. Clearly, the number of cultures which gave rise predominantly to aneuploids had a marked influence on the overall estimate of aneuploid frequency. In cultivar 'Copain', where two were of this type, 40% of the regenerants were aneuploids, whilst in cultivar 'Avalon', where no such types were found, the frequency of aneuploidy was lower.

The data were transformed by the binomial error function using the logit link and analysed by the GLIM 3 computer package (Baker and Nelder 1978). An analysis of deviance on the total data (Table 2) showed that no significant differences could be demon-

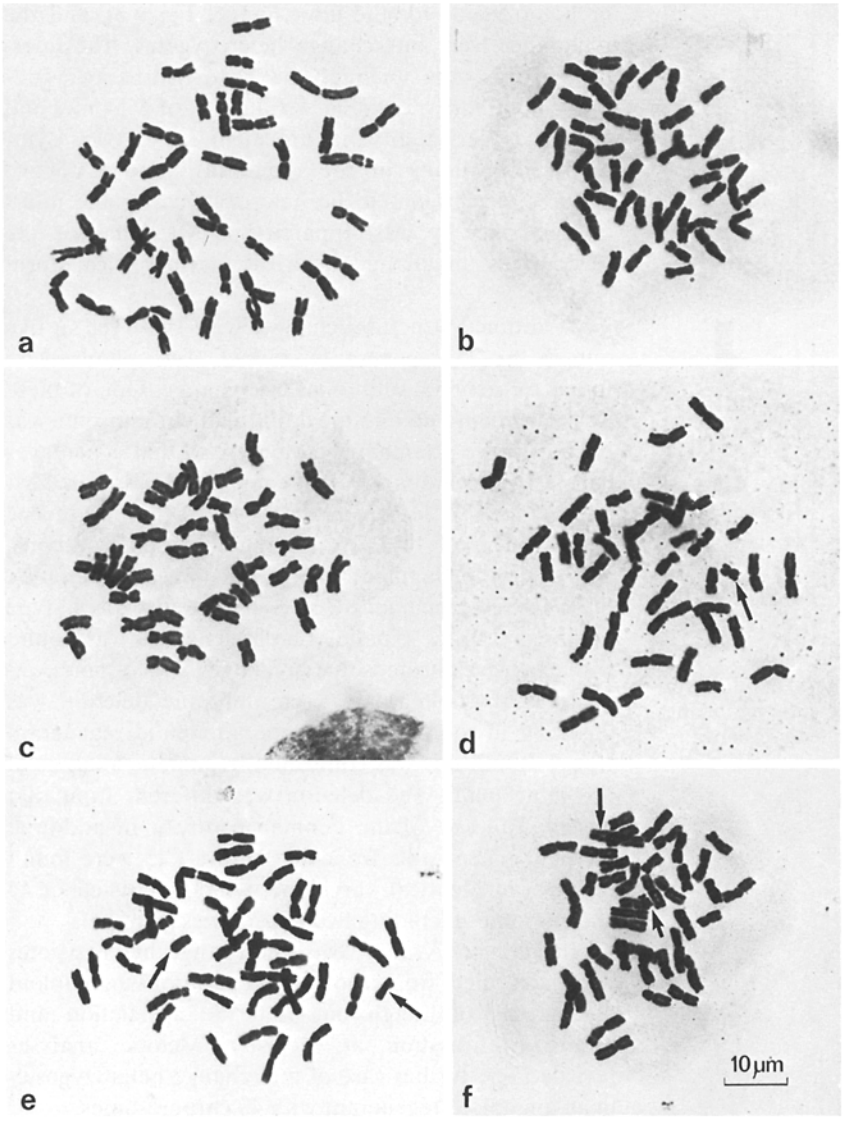

Fig. 1 a–f. C-metaphases in root cells of wheat plants regenerated from cultured immature embryos, showing examples of numerical and structural chromosome variation: **(a)** normal complement 2n = 6x = 42; **(b)** aneuploid, 2n = 43; **(c)** aneuploid, 2n = 41; **(d)** aneuploid with deletion (*arrow*), 2n = 43; **(e)** aneuploid with two telocentrics (*arrows*), 2n = 44; **(f)** interchange heterozygote (*arrows*), 2n = 42

strated because of the large error component, which chiefly resulted from the large amount of variation found within the cultivars. Estimates of success (i.e. the degree to which euploid normals were found amongst the regenerants of different cultures) were compared between the four cultivars. There appeared to be some differences between some cultivars (Fig. 3 a) but the corresponding standard errors (Fig. 3 b) were so large that the differences could not be considered valid.

In cultivar 'Highbury', chromosome counts were obtained for regenerants derived from shoots produced over a time interval of 3–4 months, depending on the culture. Over this period there was no increase in the frequency of aneuploids.

Changes in chromosome structure in regenerated plants. Evidence of structural chromosome variation was found in all four cultivars. Interchanges were the most commonly observed abnormality and no inversions were found in the sample of regenerants studied.

In cultivar 'Highbury', meiotic screening of regenerants was complicated by the presence of an interchange in the parental plants. Of nine control plants studied at meiosis, two were homozygous (although it was not possible to discern whether they were normal

Table 2. Analysis of deviance on the total data after transformation (D = deviance)

Item	D^2	dF	DMS	DR
Total	100.80	23		
Cultivars	7.49	3	2.50	
Error	93.31	20	4.67	0.54

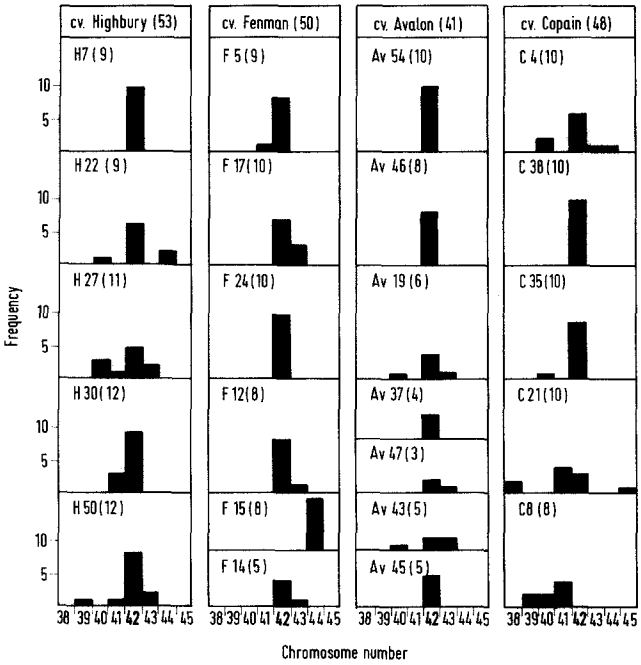

Fig. 2. Frequency distribution of chromosome numbers in plants regenerated from individual cultured embryos of the four wheat cultivars. Numbers of plants screened are shown in *brackets*

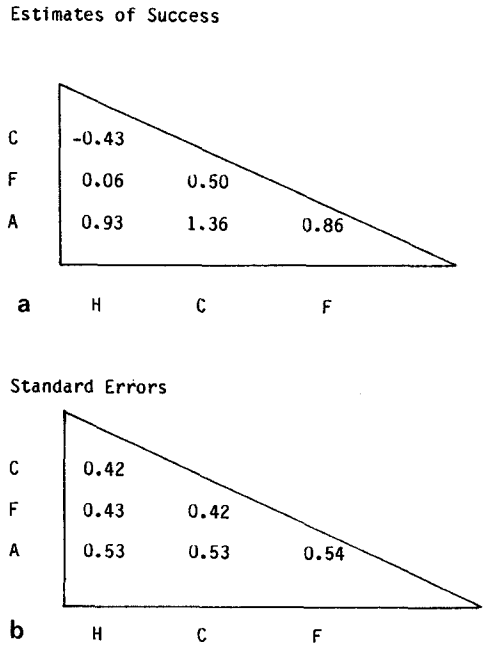

Fig. 3. Comparisons between cultivars of the estimates of success (**a**) and their standard errors (**b**) in the form of *lower triangles*

or homozygous for the interchange; Fig. 4a), and the remainder were interchange heterozygotes. The interchange was very unequal and gave rise to an asymmetrical quadrivalent (Fig. 4b) in 50% of MI pmcs and either to a trivalent and univalent or to two asymmetrical bivalents in the remaining pmcs. All five cultures were found to be heterozygous for the interchange since it was apparent in MI pmcs of the regenerants in its characteristic asymmetrical form (Fig. 4c–e).

Additional structural changes were observed in five out of the 25 regenerants studied. Two plants were found to carry an additional interchange. One of these was also aneuploid and the additional chromosome was related to the interchange complex, so that a pentavalent as well as a quadrivalent could be seen in some MI pmcs (Fig. 4e). Two regenerants had a low frequency (< 5% pmcs) of bridge and fragment configurations, and since the fragment size varied between AI pmcs, these were concluded to be the results of U-type exchange errors. Finally, one regenerant was found with extensive chromosome breakage in 4% of pmcs.

In cultivar 'Fenman' a chromosome deletion was observed in root-tip cells of one aneuploid regenerant (Fig. 1d) and this was confirmed by meiotic analysis of the same plant. The deletion was different from that observed in one of the Fenman controls. In addition, all eight regenerants from the culture F15 were found to have an identical karyotype, which consisted of 44 chromosomes including two telocentrics (Fig. 1e).

In cultivar 'Avalon' two interchange heterozygotes were identified from root-tip preparations of euploid regenerants (although one may be a deletion and awaits confirmation at meiosis). Meiotic analyses revealed one further case of interchange heterozygosity in an aneuploid regenerant with 40 chromosomes.

In cultivar 'Copain' an interchange was seen in a single root of a euploid regenerant (Fig. 1f), but in another root of this plant only 41 chromosomes were present and the small acrocentric visible in Fig. 1f was absent. Further root-tip preparations showed a normal euploid chromosome complement and it was therefore concluded that the interchange was an example of localised instability or mosaicism. Three regenerants which showed a variation in chromosome number within roots did not show the same variation within the inflorescences and meiotic analyses revealed a single count (always aneuploid) for each plant. One of the regenerants, with 44 chromosomes in the root-tip cells, had only 43 chromosomes in pmcs. A detailed examination of meiosis showed that this plant contained an iso-chromosome which formed an iso-ring in the majority of MI pmcs. In some pmcs a trivalent could be seen in addition to the iso-ring (Fig. 5b) and this indicated that the iso-chromosome was not responsible

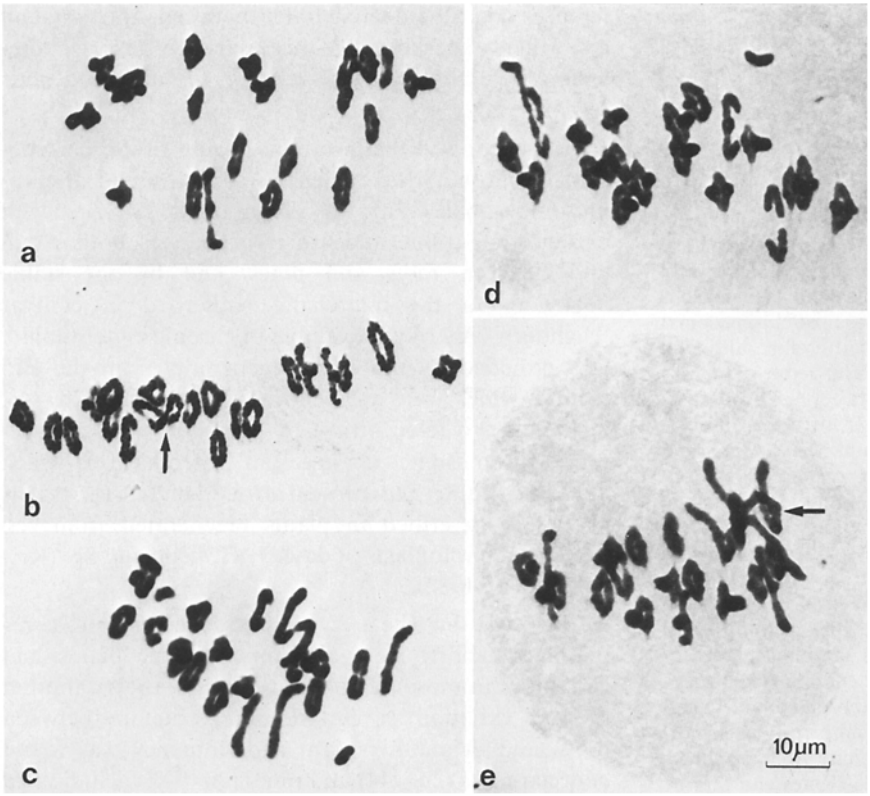

Fig. 4a–e. Chromosome variation at MI of meiosis in normal and regenerated plants of cv. 'Highbury': (**a**) normal control plant 2n = 6x = 42; (**b**) control plant with asymmetrical interchange quadrivalent (*arrow*)-this interchange is also present in regenerants c–e; (**c**) aneuploid regenerant with 'control interchange quadrivalent', 2n = 43; (**d**) euploid regenerant with a trivalent from the 'control interchange'; (**e**) aneuploid regenerant with two interchange multivalents one of the multivalents is from the 'control interchange' and the other from an additional interchange arising in culture. The extra chromosome shows homology with one of the interchange complexes and forms a pentavalent (*arrow*)

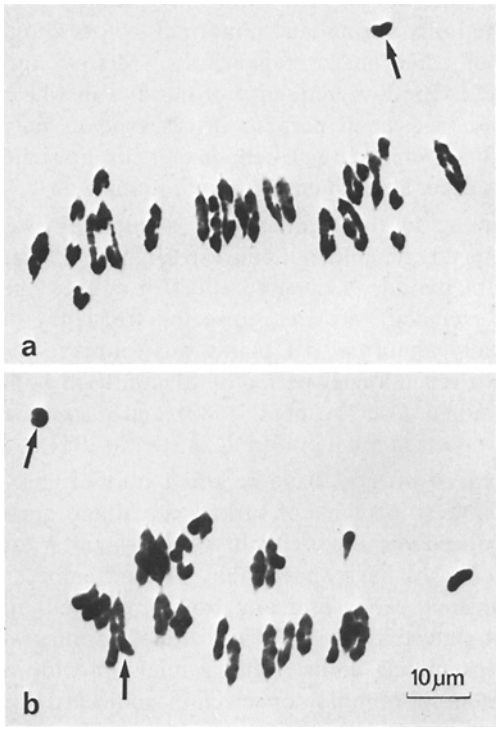

Fig. 5a, b. MI of meiosis in aneuploid regenerants of cv. 'Copain': (**a**) monosomic with a univalent (*arrow*), 2n = 41; (**b**) trisomic, 2n = 43, including an iso-chromosome. The trisomy has given rise to a trivalent (*arrow*) and the iso-chromosome has formed an iso-ring (*arrow*)

for the trisomy present in the meiocytes. It is believed that the iso-chromosome had its origins in the 44th chromosome originally present, although exactly how it arose is uncertain.

Discussion

Variation from the normal (euploid) chromosome number (2n = 6x = 42) was found in plants regenerated from cultured immature embryos of four wheat cultivars. In total, 29% of the 192 plants examined were aneuploid, with a range in chromosome number of 38–45. No changes in ploidy level were found. Although only a small sample of plants was screened at meiosis, this nevertheless provided evidence of structural changes. Interchanges were particularly common and no inversions were observed. A more extensive meiotic analysis is required, however, before the frequency of different structural changes can be assessed, and there may also be minor structural changes which were not detected.

These results are in accordance with a number of reports in the literature of variation in chromosome number and structure amongst plants derived from tissue culture (Constantin 1981), for example, from flower bud callus in *Haworthia setata* (Ogihara 1981) and from ovarian wall callus in a hybrid of *Hordeum vulgare* × *H. jubatum* (Orton 1980).

In the cereals, most success in plant regeneration has been achieved by the culture of immature embryos, but there have been few detailed cytogenetic studies of plants produced in this way. In hexaploid oats (2n = 6x = 42), McCoy et al. (1982) found that cytogenetic alterations were common in regenerated plants at a frequency comparable to our findings with wheat. By contrast, in maize, Edallo et al. (1981) found only two aneuploids amongst 110 regenerants and, similarly, McCoy and Phillips (1982) reported only three cytologically abnormal plants out of 124 regenerants. This lower frequency of abnormality in maize regenerants, compared to oats and wheat, is probably due to the differences in ploidy. As maize is a diploid (2n = 2x = 20) it is less buffered against chromosomal changes and is less tolerant of aneuploidy. Therefore, although aneuploid cells may be present in the culture, viable plants are less likely to be regenerated from these cells.

Our results are not consistent, however, with the observations of Shimada and Yamada (1979) and Ozias-Akins and Vasil (1982). In these studies of plant regeneration from immature wheat embryos, chromosome counts were made from root-tip cells of a sample of regenerants and all were found to have 42 chromosomes. Only a small sample (< 20 plants) was studied in the latter report, but Shimada and Yamada examined a total of 46 regenerants from two different cultivars. It is possible that the discrepancy between these and our results is due to different methods of sampling plants for cytogenetic study. Comparisons are also complicated by uncertainty as to whether cultures from which plants are regenerated are necessarily equivalent. For example, in oats morphogenetic callus was considered to originate from proliferation of the mesocotyl region of the embryo (Rines and McCoy 1981; McCoy et al. 1982) whereas in other cereals shoots generally arise from scutellar proliferation. From their studies of a number of cereals and grasses, Vasil and co-workers suggest that plant regeneration may occur via somatic embryogenesis in most species (Vasil and Vasil 1982a), but it is impossible to tell from other reports whether embryogenesis was involved or whether different types of culture gave rise to shoots. We do know, however, that our own wheat cultures are comparable to those of Ozias-Akins and Vasil (1982) (personal communication).

Wheat is an inbreeding cereal and a surprising feature of our study was the large differences found between cultured embryos in the proportions of aneuploids produced. Other workers have not reported such differences but this may be due to different experimental designs. In cultures producing predominantly aneuploid plants chromosome abnormalities may be present in a large proportion of cultured cells, due to changes occurring at an early stage in callus initiation or from preferential growth of abnormal cells in sectors within the callus. Alternatively, the production of different plants with similar chromosome constitutions may result from localisation of shoot formation to particular areas of the callus. In a few cases shoots separated from the callus gave rise to two or more plantlets, either as a result of tillering or possibly because two or more closely adjacent embryoids or primordia had been removed together. In each case the plants were found to have identical chromosome constitutions. The large differences in aneuploidy between different cultures meant that no significant differences could be demonstrated between cultivars, and a further experiment is necessary to test whether aneuploid frequency is related to morphogenetic response.

In addition to the instability giving rise to chromosomal abnormalities, evidence was also found of chromosome stability. This was shown by the consistency of chromosome counts and structural changes, both within and between roots and pmcs, and by the stable behaviour of the interchange inherited in cultivar 'Highbury'. A striking example of chromosome stability was provided by the eight regenerants from the F15 culture, which were all identical in karyotype (2n = 44, including two telocentrics). A karyotype with a structural chromosome change (an interchange) which appeared stable and present at a high frequency has also been described in plants regenerated from cell suspension protoplasts of daylily, *Hemerocallis* sp. (Krikorian et al. 1982).

Amongst the wheat regenerants, exceptions to chromosome stability were also found: three plants had variable chromosome numbers within roots, another showed variation in chromosome structure between roots, and in another plant a discrepancy was found between roots (2n = 44) and pmcs (2n = 43, including an iso-chromosome). In all these cases the variability was not found in the inflorescence and this contrasts with the cytogenetically normal and abnormal sectors found in tassels of three maize regenerants (McCoy and Phillips 1982). The low frequency of mosaics in wheat suggests that in a small number of cases plants may originate from more than one cell, or that chromosome changes can occur subsequent to shoot initiation.

No increase in the frequency of aneuploidy was found amongst regenerants of cultivar 'Highbury', over a five month period. A possible effect of culture age cannot be excluded, however, since the frequency of cytogenetically abnormal oat plants was found to increase over a much longer period of 20 months in both cultivars studied (McCoy et al. 1982) and there are other such reports in the literature (Constantin 1981).

Vasil and co-workers have reported normal chromosome numbers in plants of various cereal and grass species produced via somatic embryogenesis, and Vasil and Vasil (1982b) have shown that somatic embryos and embryogenic callus originate from single cells in cultured immature embryos. They therefore suggest that regeneration via embryogenesis might provide a suitable system for mutation or selection studies. In this investigation with wheat, although embryoids were clearly associated with shoot formation on 17 of the 23 cultures used for plant regeneration, 11 of such cultures still gave rise to plants with chromosome abnormalities. This is therefore an important consideration in

assessing the potential value of this regeneration system for breeding purposes.

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