Journal of Experimental Botany, Vol. 36, No. 173, pp. 1976–1984, December 1985

Somaclonal Variation in the Gliadin Patterns of Grains of Regenerated Wheat Plants

S. E. MADDOCK, R. RISIOTT, S. PARMAR, M. G. K. JONES AND P. R. SHEWRY

Biochemistry Department, Rothamsted Experimental Station, Harpenden, Herts., AL5 2JQ, U.K.

Received 28 June 1985

ABSTRACT

Maddock, S. E., Risiott, R., Parmar, S., Jones, M. G. K. and Shewry, P. R. 1985. Somaclonal variation in the gliadin patterns of grains of regenerated wheat plants.—J. exp. Bot. 36: 1976–1984.

The banding patterns of the gliadin storage proteins of the grains of 590 regenerated plants from six wheat cultivars were examined by polyacrylamide gel electrophoresis using lactate buffer. Variation additional to that present in control material was observed at a low frequency (~ 1%). Two variant lines showed extensive changes in banding patterns which were accompanied by morphological variation of the plants. More limited variation in the form of an extra ω-gliadin band was observed in a third line. Differences in the seed gliadins were not found in four lines which had shown stable phenotypic changes in height in field trials.

Key words—Wheat, somaclonal variation, gliadins, tissue culture, seed proteins.

Correspondence to: Biochemistry Department, Rothamsted Experimental Station, Harpenden, Herts., AL5 2JQ, U.K.

INTRODUCTION

When plants are regenerated from cultured tissues or protoplasts, they are not always identical to the material from which the cultures were initiated. This phenomenon of somaclonal variation (Larkin and Scowcroft, 1981) is characteristic of *in vitro* growth involving a callus phase, and it has been reported in many species, including crop plants such as potato (Shepard, Bidney, and Shahin, 1980; Wheeler, Evans, Foulger, Webb, Karp, Franklin, and Bright, 1985), tomato (Evans and Sharp, 1983) and the graminaceous species sugar-cane (Heinz, Krishnamurthi, Nickell, and Maretski, 1977; Larkin and Scowcroft, 1983), wheat (Larkin, Ryan, Brettell, and Scowcroft, 1984; Jones, Maddock, Karp, Nelson, Creissen, Foulger, and Bright, 1984), oats (Cummings, Green, and Stuthman, 1976) and rice (Sun, Zhao, Zheng, Qi, and Fu, 1983).

The origins of genetic variability during culture are not yet understood (Karp and Bright, 1985). Changes in both chromosome number and structure have been recorded in regenerated plants, often at high frequencies, and these may be responsible for some of the observed phenotypic differences. However, variation also occurs in regenerants that show no gross cytogenetic abnormalities, indicating more limited genetic changes. Various mechanisms have been proposed that might generate somaclonal variation, including the

mobilization of transposable elements, selective DNA amplification and mitotic recombination, but as yet there is no experimental evidence to support any of them.

In many cases the phenotypic variation observed involves characters for which the genetic basis is not understood, or which are under a complex or polygenic pattern of control. This makes any attempt to determine the underlying molecular mechanisms very difficult, if not impossible. Alterations in characters which are under simple genetic control can be more easily interpreted. The grain storage proteins of the cereals, being the primary products of genes which have been mapped (Payne, Holt, Jackson, and Law, 1984; Shewry, Miflin, and Kasarda, 1984) and in some cases also characterized (Kreis, Shewry, Forde, Forde, and Miflin, 1985), provide an attractive system for studying the origin and molecular basis of somaclonal variation.

Storage proteins account for about half of the total nitrogen of the mature grain, and are usually classified into gliadins and glutenins on the basis of their aggregation properties (Kasarda, Bernardin, and Nimmo, 1976). The gliadins, which are monomeric and are associated by non-covalent hydrogen bonds and hydrophobic interactions, are separated into four groups, called α-, β-, γ- and ω-gliadins, based on their electrophoretic mobility at low pH. These are encoded by six multigenic loci, located on the short arms of chromosomes 1A, 1B and 1D (ω- and γ-gliadins) and 6A, 6B and 6D (α- and β-gliadins; Payne *et al.*, 1984). Thus electrophoresis of gliadins at low pH may give information on events occurring at one or more of these loci.

Larkin *et al.* (1984) found extensive variation in the pattern of the gliadin storage proteins amongst regenerated lines of the wheat cultivar Yaqui 50E, in addition to variability in quantitative characters which are probably under multigenic control. We report here a more extensive study of the gliadin patterns of plants regenerated from cultured immature embryos and inflorescences of six cultivars of wheat. This is part of a wider programme to investigate the nature and molecular basis of somaclonal variation.

MATERIALS AND METHODS

Plant material

About 100 lines were screened from each of six wheat cultivars (Angus, Bersee, Chinese Spring, Highbury, Maris Butler and Timmo). Each line was derived from a single wheat plant that had been regenerated from a cultured immature embryo or inflorescence, using the methods described previously (Maddock, Lancaster, Risiott, and Franklin, 1983). In this study, between one and eight plants (lines) were obtained from each explant. The plants regenerated directly from culture have been termed the R_1 generation, and the selfed progeny and subsequent generations R_2, R_3 etc.

A single selfed seed (R_2) from each regenerated plant was selected at random for gliadin analysis. In the case of cv. Highbury, we also analysed a number of seeds from each of four lines which had shown morphological variation in field trials.

Gliadin analysis

Seeds were cut into halves and the embryo halves stored. The endosperm halves were crushed and the gliadins extracted with 4·0 mm^3 mg^{-1} of 70% (v/v) aqueous ethanol (Payne, Holt, Worland, and Law, 1982). After centrifugation for 5 min in a Beckmann microfuge the supernatant was removed and mixed with 0·84 vol of 60% (v/v) glycerol containing basic fushsin as a tracking dye.

20 mm^3 aliquots were separated by polyacrylamide gel electrophoresis using lactate buffer at pH 3·1 (lactate-PAGE), as described by Lafiandra and Kasarda (1985). Gels were fixed and stained in 500 cm^3 of 10% (w/v) trichloracetic acid mixed with 25 cm^3 of ethanol containing 0·4% (w/v) of Coomassie BBR 250.

RESULTS

We have previously reported plant regeneration from cultured immature embryos and inflorescences of 25 cultivars of spring and winter wheats (Maddock *et al.*, 1983). Regenerated

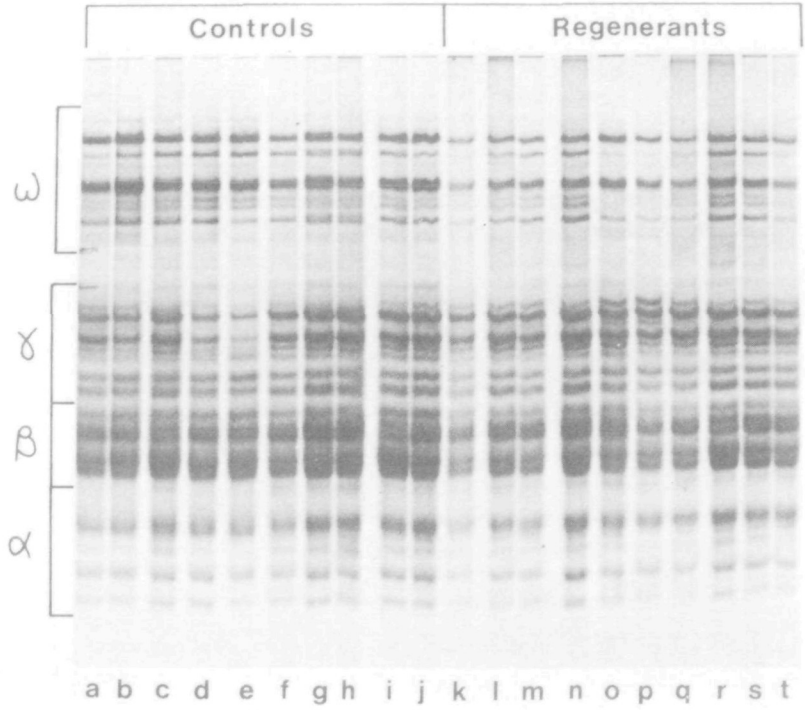

FIG. 1. Lactate-PAGE of gliadin fractions from single control (a-j) and regenerant (k-t) grains of the cv. Angus. The groups of α-, β-, γ- and ω-gliadins are indicated.

lines from six spring cultivars were selected for gliadin analysis: Angus, Bersee, Chinese Spring, Highbury, Maris Butler and Timmo. Five of these have also been assessed for variability in field trials (Maddock and Semple, in preparation). The sixth, Chinese Spring, was selected because it has been used as a standard cultivar for studying the genetics of gliadins (Wrigley and Shepherd, 1973; Kasarda, Bernardin, and Qualset, 1976; Brown, Kemble, Law, and Flavell, 1979), and it would be possible to identify unequivocally any variant loci.

Analysis of gliadins from regenerated wheat lines

Angus: 99 regenerated lines and 22 control grains were analysed. Some variation in the intensities of ω-gliadin and γ-gliadin bands was observed in the control and regenerated grains (Fig. 1), but there was no evidence of additional variation in the regenerated lines.

Bersee: Only 91 regenerated lines were available and all were analysed, together with 40 control grains. One line, RB20, differed from the control grains in the patterns of all four groups of gliadins (Fig. 2). Other seeds from this line and their progeny showed the same patterns, indicating that the gliadin loci were homozygous. This line was also analysed by Dr R. J. Cooke at the National Institute of Agricultural Botany (N.I.A.B.), Cambridge, U.K. He showed that the gliadin pattern on starch gel electrophoresis differed from that of all cultivars which are commonly grown in the U.K. This suggests that the line did not originate from contamination. A second plant, RB1, was regenerated from the same immature embryo as RB20. Seed from RB1 showed the typical Bersee gliadin pattern, indicating that RB20 could not have arisen from an off-type in the original seed stock of cv. Bersee.

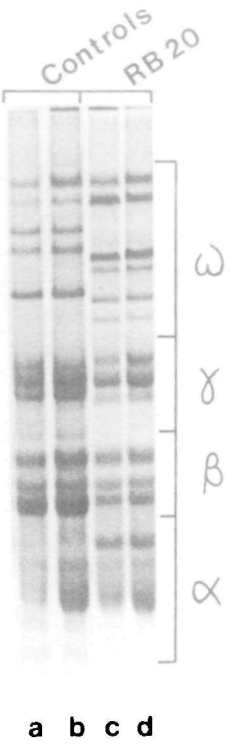

FIG. 2. Lactate-PAGE of gliadin fractions from single grains of the cv. Bersee (a, b) and the regenerant line RB20 (c, d). The groups of α-, β-, γ- and ω-gliadins are indicated.

RB20 also showed morphological variation, in particular reduced height. The R_1 plant and field-grown plants of the R_2 and R_3 generations were all shorter than control plants by 30 cm or more (S. E. Maddock and J. Semple, unpublished observations).

Chinese Spring: 100 regenerated lines and 32 control grains were analysed. No somaclonal variation in gliadin patterns was observed in the regenerated lines, although minor variation in the relative intensities of the slow ω-gliadin bands was observed in the control and regenerated samples (not shown).

Maris Butler: Maris Butler is a multiline, consisting of biotypes which differ in their patterns of α-, β-, γ- and ω-gliadins (see Fig. 3, tracks a–j). Examination of control grains indicated that recombination between the bands had occurred, which is consistent with a low level of outcrossing. It is also possible that some grains were heterozygous for alleles at one or more of the six loci. Because of this variability a larger number of control grains (76) were analysed, together with 100 regenerated lines. No evidence of somaclonal variation was detected (Fig. 3, tracks k–t), although any variation would be difficult to identify in the presence of the high background variability.

Timmo: 100 regenerated lines and 70 control grains were analysed. Some variation in the intensities of bands in the γ- and ω-gliadin regions was observed in both control and regenerated samples (Fig. 4).

Additional variation was found in four regenerant lines. An extra fast mobility ω-gliadin band was observed in line RT26 (Fig. 4, track m), while RT43 showed more extensive differences in the patterns of the β-, γ- and ω-gliadins (Fig. 4, track n). Examination

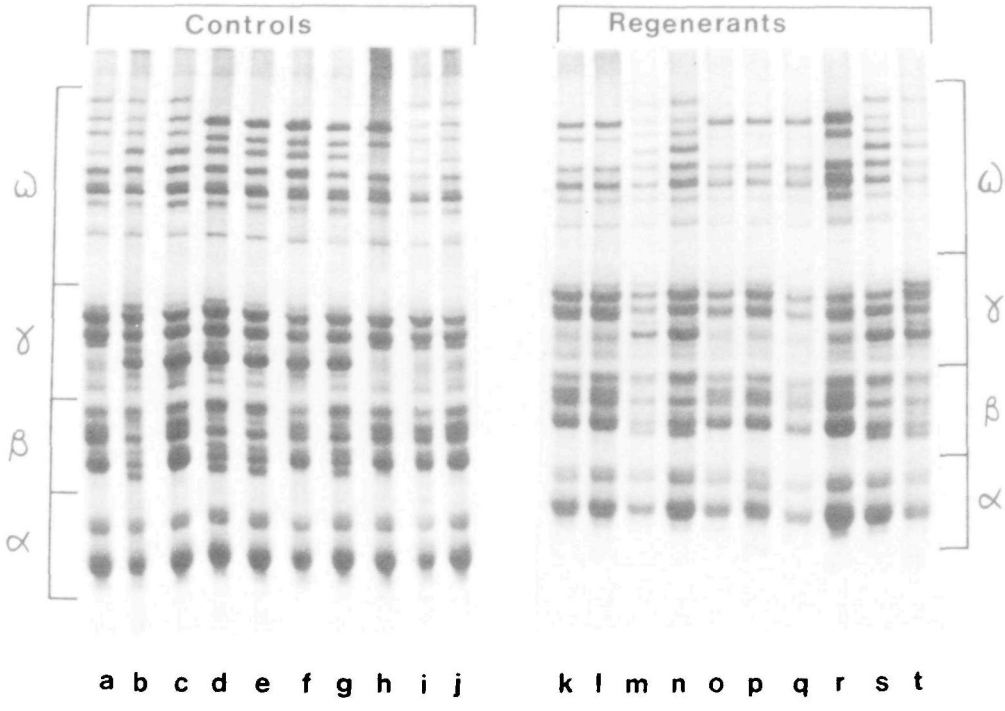

FIG. 3. Lactate-PAGE of gliadin fractions from single control (a–j) and regenerant (k–t) grains of the cv. Maris Butler. Note the presence of biotypes differing in their patterns of α-, β-, γ- and ω-gliadins.

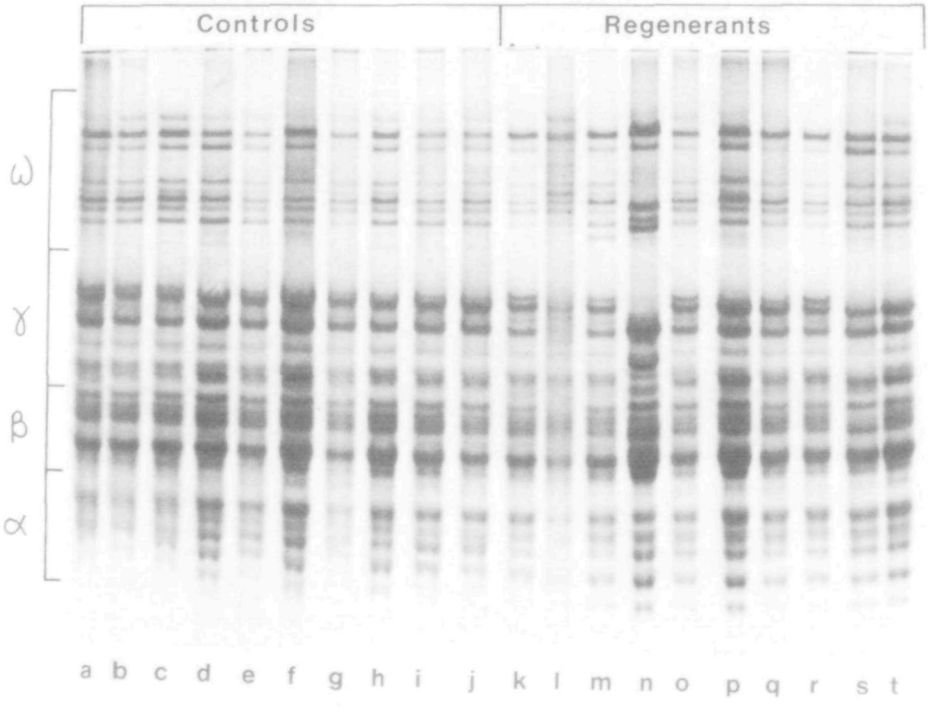

FIG. 4. Lactate-PAGE of gliadin fractions from single control (a–j) and regenerant (k–t) grains of the cv. Timmo. Tracks l, m and n are the lines RT16, RT26 and RT43 respectively, and are discussed in the text. The groups of α-, β-, γ- and ω-gliadins are indicated.

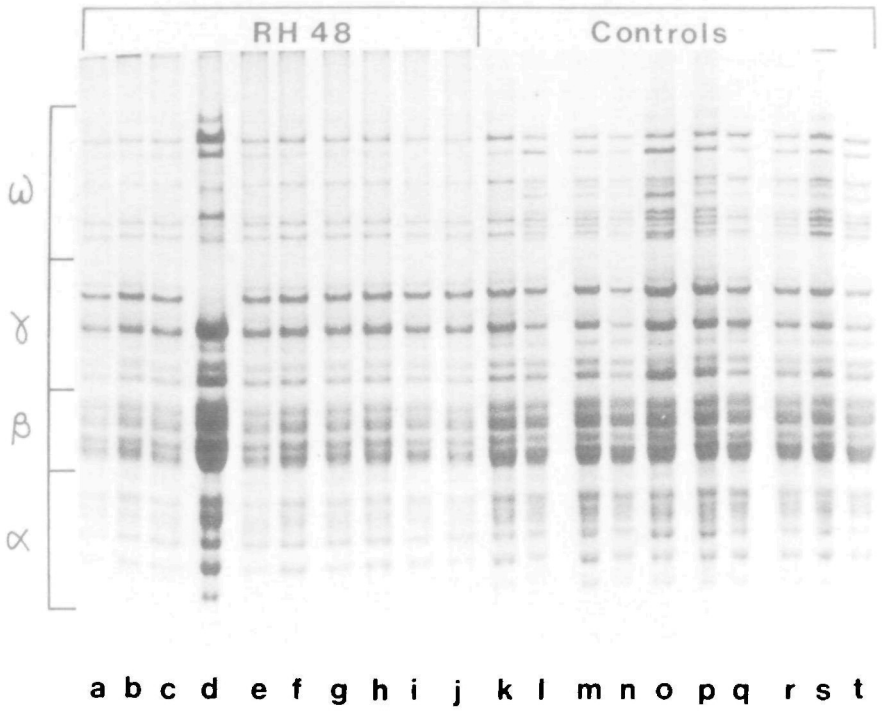

FIG. 5. Lactate-PAGE of gliadin fractions from single grains of the regenerant line RH48 (a–j) and a control sample of the cv. Highbury. Note the presence of two biotypes of Highbury differing in their patterns of ω-gliadins (cf. track k and tracks l–t).

of further R_2 seed of these lines showed that they were homogeneous, and there was no evidence of segregation in the progeny of RT43. Line RT43 was also analysed at N.I.A.B. using starch gel electrophoresis, and the gliadin pattern was found not to correspond to any known U.K. variety. The R_2 and R_3 generations of this line also showed abnormal morphology in the field.

Lines RT13 (not shown) and RT16 (Fig. 4, track 1) gave similar results in which the normal gliadin patterns were indistinct and additional minor bands were present in the ω-gliadin region. Examination of further seeds of these lines showed variability but with most seeds having apparently normal patterns. These lines may have been segregating for a character affecting total gliadin synthesis, rather than the synthesis of specific components.

Highbury: Highbury is again a multiline, but with only two biotypes differing in their patterns of γ- and ω-gliadins (Fig. 5); 36 control grains and 100 regenerants were analysed but no evidence of somaclonal variation was observed.

We also analysed seeds from four lines of cv. Highbury (RH36, RH48, RH84 and RH101) which had shown height variation in the R_2 generation in field trials (S. E. Maddock and J. Semple, unpublished observations). Analysis of 20 R_2 seeds of each line showed only one variant. This was a single seed of RH48, which differed from the sibling and control grains in the patterns of α-, β-, γ- and ω-gliadins (Fig. 5, track d).

Field-grown R_2 material of lines RH48 and RH84 segregated into populations of tall and normal plants, which were harvested and grown on separately. Five R_4 seed from both tall plants and plants of normal height were analysed, but no differences in gliadin patterns were

observed. This shows that the variant gliadin pattern observed in the single seed of RH48 was not associated with the variation in height. Although the pattern of this seed did not correspond to that of any other cultivar being grown in the field trial, the possibility that the seed was a contaminant cannot be ruled out.

DISCUSSION

Our results have shown that some variation can be detected in the gliadin storage proteins of regenerated wheat plants. However, the analyses also revealed considerable heterogeneity in control material, either in the existence of biotypes or more minor variations in relative band intensity. Consequently, variation additional to the background was present in no more than 1% of the regenerated lines. This emphasizes the importance of screening sufficiently large control populations. Sibling and progeny analysis of the variant lines indicated that they had not arisen due to outcrossing of the regenerated plants, and cytogenetic examination of root-tip preparations showed that the variation was not associated with aneuploidy (A. Karp, unpublished observation).

Two of the variant lines (RB20 and RT43) showed extensive variation in the patterns of the different gliadin groups. These changes were homogeneous and stable in the succeeding generation. As a check against accidental contamination, seeds from these lines were tested at N.I.A.B., but could not be identified as corresponding to any of the commonly-grown wheat cultivars. These lines were also different morphologically from controls in field trials, suggesting that in these cases somaclonal variation had affected a number of characters simultaneously. More limited somaclonal variation was found in line RT26, with the appearance of an additional fast mobility ω-gliadin band. Changes such as this will be more easy to investigate experimentally, notably by restriction fragment analysis of the encoding locus.

The low frequency of changes in our material contrasts with the findings of Larkin *et al.* (1984), who reported extensive and stable changes in the gliadin patterns of regenerated plants of the Mexican wheat cv. Yaqui 50E. These changes included the deletion of some bands and the presence of new bands, as well as altered band intensities. Similarly, in field trials (S. E. Maddock and J. Semple, unpublished observations), our lines also showed a much lower incidence of phenotypic variability. These differences in variability may reflect differences between cultivars in their stability during culture. In subsequent work by Larkin and associates, using other cultivars, much less variation was found (P. J. Larkin, personal communication). However, they also observed less extensive variation in later studies with Yaqui 50E, cultured in the same way as the initial material. This might be explained by differences in the stability of individual embryos, since we have observed considerable differences between immature embryos in the amount of cytogenetic abnormality in plants regenerated from them (Karp and Maddock, 1984). The initial study by Larkin *et al.* involved plants obtained from a relatively small number of immature embryos ($\leqslant 5$), whilst our lines were derived from a much larger number of explants (324; 45–63 per cultivar). The effect of a small number of highly variable embryos on the overall level of variation would, therefore, be greater in the material of Larkin *et al.* (1984).

This comparison emphasizes the fact that, at present, somaclonal variation is itself a very variable phenomenon. It highlights the importance of increasing our understanding of the causes of variation in tissue culture and of the factors which may influence it, if we are to attempt to regulate or predict its effects. By using appropriate experimental systems and techniques it is now becoming possible to design studies to investigate these problems, rather than simply to describe the observed effects of somaclonal variation.

Further studies of gliadins and glutelins (the latter by Dr P. I. Payne, Plant Breeding Institute, Cambridge) have indicated that RT43 may derive from a contaminating seed of the cv. Chinese Spring.

ACKNOWLEDGEMENTS

Sheila Maddock thanks Shell Research Ltd. for financial support. We also thank Dr C. N. Law (P.B.I., Cambridge, U.K.) for supplying seed of the cultivars Angus, Bersee and Chinese Spring, Dr D. D. Kasarda (U.S.D.A., Albany, U.S.A.) for providing details of the lactate-PAGE method prior to publication, and Dr R. J. Cooke (N.I.A.B., Cambridge, U.K.) for advice and for running some samples on starch gel electrophoresis.

LITERATURE CITED

BROWN, J. W. S., KEMBLE, R. J., LAW, C. N., and FLAVELL, R. R., 1979. Control of endosperm proteins in *Triticum aestivum* (var. Chinese Spring) and *Aegilops umbellulata* by homeologous group 1 chromosomes. *Genetics*, **93**, 189–200.

CUMMINGS, D. P., GREEN, C. E., and STUTHMAN, D. D., 1976. Callus induction and plant regeneration in oats. *Crop Science*, **16**, 465–70.

EVANS, D. A., and SHARP, W. R., 1983. Single gene mutations in tomato plants regenerated from tissue culture. *Science*, **221**, 949–51.

HEINZ, D. J., KRISHNAMURTHI, M., NICKELL, L. G., and MARETZKI, A., 1977. Cell, tissue and organ culture in sugar cane improvement. In *Applied and fundamental aspects of plant cell, tissue and organ culture*. Eds J. Reinert and Y. P. S. Bajaj. Springer, Berlin. Pp. 3–17.

JONES, M. G. K., MADDOCK, S. E., KARP, A., NELSON, R. S., CREISSEN, G. P., FOULGER, D., and BRIGHT, S. W. J., 1984. Tissue and protoplast culture—a novel way to new crop plant varieties. In *The world biotech report 1984, Volume 1: Europe*. Online Conferences Ltd., Pinner, U.K. Pp. 443–54.

KARP, A., and BRIGHT, S. W. J., 1985. On the causes and origins of somaclonal variation. In *Oxford surveys of plant molecular and cell biology, Volume 2*. Ed. B. J. Miflin. Oxford University Press. Pp. 199–234.

——and MADDOCK, S. E., 1984. Chromosome variation in wheat plants regenerated from cultured immature embryos. *Theoretical and Applied Genetics*, **67**, 249–56.

KASARDA, D. D., BERNARDIN, J. E., and NIMMO, C. C., 1976. Wheat proteins. In *Advances in cereal science and technology, Volume 1*. Ed. Y. Pomeranz. American Association of Cereal Chemists, St. Paul, Minn., U.S.A. Pp. 158–236.

———— and QUALSET, C. O., 1976. Relationship of wheat gliadin components to chromosomes in hexaploid wheats (*Triticum aestivum* L.). *Proceedings of the National Academy of Science, U.S.A.* **73**, 3646–50.

KREIS, M., SHEWRY, P. R., FORDE, B. G., FORDE, J., and MIFLIN, B. J., 1985. Structure and evolution of seed storage proteins and their genes with particular reference to those of wheat, barley and rye. In *Oxford surveys of plant cell and molecular biology, Volume 2*. Ed. B. J. Miflin. Oxford University Press. Pp. 253–317.

LAFIANDRA, D., and KASARDA, D. D., 1985. 1- and 2-dimensional (2-pH) system of polyacrylamide gel electrophoresis carried out in a single cell: separation of wheat proteins. *Cereal Chemistry* (In press.)

LARKIN, P. J., RYAN, S. A., BRETTELL, R. I. S., and SCOWCROFT, W. R., 1984. Heritable somaclonal variation in wheat. *Theoretical and Applied Genetics*, **67**, 443–55.

——and SCOWCROFT, W. R., 1981. Somaclonal variation—a novel source of variability from cell cultures for plant improvement. *Theoretical and Applied Genetics*, **60**, 197–214.

———— 1983. Somaclonal variation and eyespot toxin tolerance in sugar cane. *Plant Cell Tissue and Organ Culture*, **2**, 111–22.

MADDOCK, S. E., LANCASTER, V. A., RISIOTT, R., and FRANKLIN, J., 1983. Plant regeneration from cultured immature embryos and inflorescences of 25 cultivars of wheat (*Triticum aestivum*). *Journal of Experimental Botany*, **34**, 915–26.

PAYNE, P. I., HOLT, L. M., JACKSON, E. A., and LAW, C. N., 1984. Wheat storage proteins: their genetics and their potential for manipulation by plant breeding. *Philosophical Transactions of the Royal Society of London, Series B*, **304**, 359–71.

PAYNE, P. I., HOLT, L. M., WORLAND, A. J., and LAW, C. N., 1982. Structural and genetical studies on the high-molecular-weight subunits of wheat glutenin 3. Telocentric mapping of the subunit genes on the long arms of the homeologous group 1 chromosomes. *Theoretical and Applied Genetics*, **63,** 129–38.

SHEPARD, J. F., BIDNEY, D., and SHAHIN, E., 1980. Potato protoplasts in crop improvement. *Science*, **208,** 17–24.

SHEWRY, P. R., MIFLIN, B. J., and KASARDA, D. D., 1984. The structural and evolutionary relationships of the prolamin storage proteins of barley, rye and wheat. *Philosophical Transactions of the Royal Society of London, Series B*, **304,** 297–308.

SUN, Z., ZHAO, C., ZHENG, K., QI, X., and FU, Y., 1983. Somaclonal genetics of rice, *Oryza sativa* L. *Theoretical and Applied Genetics*, **67,** 67–73.

WHEELER, V. A., EVANS, N. E., FOULGER, D., WEBB, K. J., KARP, A., FRANKLIN, J., and BRIGHT, S. W. J., 1985. Shoot formation from explant cultures of fourteen potato cultivars and studies of the cytology and morphology of regenerated plants. *Annals of Botany*, **55,** 309–20.

WRIGLEY, C. W., and SHEPHERD, K. W., 1973. Electro-focussing of grain proteins from wheat genotypes. *Annals of the New York Academy of Science*, **209,** 154–62.