SOMACLONAL VARIATION IN TRITICALE (X TRITICOSECALE WITTMACK) CV. "CARMAN".

A Thesis

Submitted to the Faculty

of

Graduate Studies

The University of Manitoba

by

Mark Carlyle Jordan

In Partial Fulfillment of the

Requirements for the Degree

of

Master of Science

Department of Plant Science

February 1984

SOMACLONAL VARIATION IN TRITICALE (X TRITICOSECALE WITTMACK) CV. "CARMAN"

by

Mark Carlyle Jordan

A thesis submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

MASTER OF SCIENCE

ACKNOWLEDGMENTS

I wish to express my gratitude to the following people for their help throughout this project.

Dr. E.N. Larter for his support and guidance given during the whole of my stay here.

Mr. B.R. Oree and Mrs. H. Ikonen for invaluable practical assistance given in the laboratory.

The evening and weekend crew: Jennifer, Monika, Len, Jeff, Meran and Jerry for making working and playing at all hours worthwhile and enjoyable.

Finally, I would like to thank the University of Manitoba for much needed financial assistance.

TABLE OF CONTENTS

PAGE

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Jordan, Mark Carlyle. M. Sc., The University of Manitoba, February, 1984. Somaclonal variation in triticale (X Triticosecale Wittmack) cv. "Carman". Major Professor; E.N. Larter.

Variation in tissue culture systems and in plants regenerated from such systems is a common phenomenon. Such variation may be useful for crop improvement through the production of novel desirable traits, provided that the variation has a genetic basis. An investigation into the range, type and possible usefulness of the variability present in regenerated plants of "Carman" triticale was the basis of the present study.

Callus was initiated from 15 day-old embryos of "Carman" triticale on Murashige and Skoog (MS) media supplemented with $3mgL^{-1}$ 2,4-D. Every four weeks the calli were subcultured to MS media with no added hormones and plants regenerated. Of fourteen plants characterized, eight were chromosomally mixoploid (contained both 2n=42 and 2n>42 cells) and six were euploid (2n=42). The original euploid regenerated plants and their progeny were examined for plant height, number of heads, spike length, fertility, percent kernel protein and electrophoretic banding pattern of prolamins.

A large amount of variation for all traits was observed among the original regenerated plants. The second generation exhibited only slightly less variability than the first, indicating that a large amount of the variation was genetic in nature. Two second generation plants were found to have a significant increase in percent kernel protein relative to "Carman" controls. Electrophoretic studies showed that all of the regenerated plants of both generations had the same prolamin banding pattern as "Carman" triticale but considerable variation existed in the intensity of the bands. This was especially true for the bands coded for by the rye genome, thereby implying changes in prolamin regulatory genes.

In the second and third generations all morphological traits studied were affected by the environment. The genetic component of variation was greatest for spike length, fertility and plant height, while variability for the number of fertile heads per plant did not appear to have an underlying genetic basis.

Only genotype R13 was significantly different from "Carman" in terms of chromosomal instability. The extremely large amount of instability in R13 resulted in decreased fertility and was due to an increased production of wheat univalents relative to "Carman" controls.

The underlying genetic basis of the traits studied as well as the appearance of desirable traits, such as increased kernel protein content, indicate that somaclonal variation may prove useful for triticale improvement.

SECTION I

INTRODUCTION

> "We have good reason to believe that changes in the conditions of life give a tendency to increased variability; and this would manifestly be favorable to natural selection, by affording a better chance of the occurrence of profitable variations."
>
> - Charles Darwin
> The Origin of Species.

The process of speciation is based upon natural selection acting on genetic variation. This basic tenet of biology also applies to mans' attempts to improve his domesticated plants. Man selects from a pool of genetic variation the characteristics which are the most profitable to him. Thus any changes in the conditions of life which lead to increased variability would afford man a better opportunity of finding beneficial characteristics for crop improvement.

There can be no greater change in the conditions of life than the change from the integrated growth of an intact plant to the disorganized growth of a mass of plant cells in culture. Thus it is not surprising that plant cell cultures exhibit a tremendous amount of variability.

This genetic instability *in vitro* has many disadvantages such as the inability to regenerate large numbers of identical plants (cloning genotypes), reduced regeneration capacity, regeneration of sterile plants and the loss of genetic markers.

In spite of these disadvantages, genetic instability in culture could possibly be of use for plant improvement through the generation of mutant cells and the subsequent regeneration of mutant plants (Carlson and Polacco, 1975; Green, 1977). In order to use *in vitro* mutant selection for crop improvement, the traits to be modified must be expressed at the *in vitro* level. This is a problem as many agriculturally important traits such as yield and drought tolerance are not expressed at the cellular level. These traits are very complex and not enough is known about their cellular and biochemical basis thus limiting the development of effective selection techniques (Davies, 1981).

Another limitation to mutant selection is that of mutations being expressed *in vitro* but not in regenerated plants. Davies (1981) quotes an example of mutant tobacco cell culture which accumulated tryptophan due to an altered anthranilate synthetase. Plants regenerated from this culture contained the normal enzyme and showed no increase in tryptophan levels over the control plants.

With the present lack of knowledge of the cellular basis of many important traits, it is not yet possible to control

and direct the variability that occurs in culture. However it is possible that beneficial mutations could occur in culture and be incorporated into regenerated plants. Thus novel beneficial traits may be found in regenerated plants even in the absence of intentional selection.

Triticale (X Triticosecale Wittmack) is a man-made cereal crop species produced from the hybridization of wheat (Triticum sp. L.) and rye (Secale cereale L.). Due to its nutritionally superior protein relative to that of wheat (Hulse and Laing, 1974), triticale has the potential to be of significant importance as an agricultural crop species in many regions of the world. The present study was undertaken with triticale to determine the extent to which genetic variability might be found in plants of this species regenerated from callus tissue. The following specific objectives were pursued:

1) To determine the amount of variability present in triticale plants regenerated from callus cultures.

2) To determine the stability of the variation from one generation to the next.

3) To assess the usefulness of this variation for triticale improvement.

SECTION II

LITERATURE REVIEW

2.1 Variation in Tissue Cultures and in Regenerated Plants - Dicotyledonous Species.

Since the early days of plant tissue culture, it has been observed that cells with different chromosome numbers and karyotypes not found in the intact plant can be found in culture (Partanen, 1963; Sunderland, 1973; D'Amato, 1975). Most of our knowledge on chromosome behaviour is tissue culture comes from dicotyledonous species as these were the first to be successfully cultured.

Sunderland (1977) states that there are two types of nuclear irregularities: (1) chromosomal mutations which can be observed cytologically and (2), gene mutations which can be recognized only by reference to the phenotype. The four basic categories of chromosomal changes are polyploidy, aneuploidy, structural changes and mitotic aberrations. All of these changes have been observed in plant cultures (Bayliss, 1980).

Polyploidy can arise from either endopolyploid or endoreduplicated cells in the original explant or from endomitosis or endoreduplication events which occur during culture initiation and subsequent culture growth (Bayliss, 1980). The degree of polyploidization found in a culture is

often directly related to the hormone composition of the media. Torrey (1961) observed that Pisum sativum L. root explants cultured in the presence of auxin resulted in diploid cultures while root explants cultured in the presence of auxin and cytokinin resulted in primarily polyploid cultures. It was suggested that cytokinins stimulate division of endoreduplicated cells in the explant.

Aneuploidy is most often a result of abnormalities of mitosis which have been shown to occur in plant cultures. Multipolar anaphases, lagging chromosomes, bridges, and fragments can result in the production of aneuploid daughter nuclei (see Sunderland (1977) and Bayliss (1980) for reviews).

It has been suggested that these abnormalities are induced by hormones which must be present in most tissue culture media for the initiation and growth of the culture (Ghosh and Gadgill, 1979). The synthetic auxin 2,4-dichlorophenoxyacetic acid (2,4-D), used widely in culture media, has been shown to induce spindle failure and mitotic abnormalities in intact plants thus resulting in polyploidy and aneuploidy (Mohandas and Grant, 1972).

Bayliss (1975) examined a diploid culture of Daucas carota L. which required 2,4-D for growth and a hypotetraploid habituated line which could grow in the absence of 2,4-D. He found no correlated effect on the frequency of

multipolar mitosis over several 2,4-D concentrations and concluded that mitotic regularity is dependent upon the degree of tissue organization and not upon the presence of 2,4-D itself. Thus 2,4-D only affects mitosis in that it stimulates rapid and unorganized growth.

Changes in chromosome structure are primarily the result of translocations and deletions. These sources of chromosome variation are important in culture as they can lead to the loss of genetic material in the form of acentric fragments, telocentrics and isochromosomes (Sunderland, 1977). Sacristan (1971), working with cultures of Crepis capillaris (L.) Wallr., observed that abnormal karyotypes in cells of the same ploidy level as the original explant emerged within a year of culture and that several of these were due to random translocations and deletions.

The occurrence of chromosomally variant cells has been shown to increase with time in culture (Gupta, 1972; Matthews and Vasil, 1975). This phenomenon is likely the cause of the decreased regeneration potential observed over time (Smith and Street, 1974; Torrey, 1967).

The chromosomal variation found in culture is often present in plants regenerated from tissue cultures. Sacristan and Melchers (1977) for example, regenerated completely aneuploid plants of Nicotiana tabacum L. Similarly, Ogura (1976) regenerated tobacco chimeras (both hypoploid and hyperploid cells were observed), while Browers and Orton (1982)

regenerated celery (Apium graveolens L.) plants which were chromosomally mixoploid.

Much less work has been done on gene mutation as another type of genetic change found in tissue cultures. That such changes do occur is implicated in the regeneration of phenotypically variable plants. Williams and Collin (1976) found that celery plants regenerated from callus had shorter petioles but a larger number of petioles per plant than did plants derived from seed. The regenerated plants were not different from seed-derived plants in terms of chromosome number and chromosome structure.

Lester and Berbee (1977) found within-clone variation for height, number of branches and leaf traits in black poplar trees regenerated from callus culture. Reisch and Bingham (1981) observed considerable variation in growth and morphological characteristics for chromosomally normal dihaploid alfalfa.

2.2 Tissue Culture Studies with Cereal Species

Work on the tissue culture of monocotyledons and especially of the cereal crops has lagged far behind work on the dicotyledons. This is due mainly to the fact that it was not until 1967 that good callus induction and growth of cereal tissue cultures was obtained by using a higher concentration of auxin, usually 2,4-D, than had been used previously

(Carter et al., 1967). Feung et al. (1975) found that monocot cultures metabolize 2,4-D much faster than dicots and therefore need more 2,4-D in order to maintain unorganized growth.

Yajima et al. (1975) found that 2,4-D solubilized artichoke chromatin by specific association with the histones causing DNA-histone dissociation. This dissociation results in the derepression of genes and leads to rapid unorganized growth.

In contrast to the large number of different tissues which can be used to establish tissue cultures in dicotyledons, only a few tissue sources have been successfully used in the cereals, viz. root segments (Gamborg and Eveleigh, 1968), inflorescences (Dudits et al., 1975; Nakamura and Keller, 1982), leaves (Saalbach and Koblitz, 1978; Zamora and Scott, 1983) and embryos (King et al., 1978; O'Hara and Street, 1978; Carew and Schwarting, 1958). Embryos represent the easiest explants with which to establish cultures and most studies on cereal tissue culture utilize them.

The origin of callus from embryos has given variable results. Granatek and Cockerline (1978) stated that callus proliferation proceeds from the arginine-rich histone-complemented mesocotyl. They also suggested that callus formation is a result of a metabolic block in starch utilization as calli accumulated starch. When gibberellic acid was added, the starch disappeared and embryogenesis occurred. Bayliss

and Dunn (1979) also found that callus formation from mature barley (Hordeum vulgare L.) embryos was initiated in the mesocotyl region.

Cummings et al. (1976) reported the initiation of callus from the radicle portion of the immature oat (Avena sativa L.) embryo while the scutellar tissues became necrotic. O'Hara and Street (1978) found callus formation from both the cotyledonary node and in the region of the radicle in mature wheat (Triticum aestivum L.) embryos. Ozias-Akins and Vasil (1983) state that callus from mature wheat embryos arises only from the tissues within or near the procambium of the embryo axis. Previously, these authors reported that callus formation from immature wheat embryos was formed predominantly from parenchyma cells of the scutellum (Ozias-Akins and Vasil, 1982).

The scutellum of cereals is responsive to callus formation only at a particular embryonic stage and produces calli that tend to be compact, nodular and white to yellow in colour (Ozias-Akins and Vasil, 1982; Vasil and Vasil, 1981; Dale and Deambrogio, 1979). Achieving callus formation from the scutellum is important since to-date, only scutellar callus is known to have the potential to consistently regenerate whole plants (Ozias-Akins and Vasil, 1982; Gosch-Wackerle et al., 1979).

Callus not arising from the scutellum is not a true callus but a series of meristematic zones formed in association

with vascular tissues (Ozias-Akins and Vasil, 1983). It has been suggested that these zones represent proliferating presumptive root primordia due to the fact that these cultures will readily form roots on transfer to low auxin media but will rarely form shoots (O'Hara and Street, 1978).

Plant regeneration from cereal callus is of two types: (1) organogenesis and (2) somatic embryogenesis. Organogenesis is the formation of roots and shoots directly from the callus, while somatic embryogenesis involves the production of embryo-like structures which in turn germinate to form roots and shoots. Organogenesis has been said to be the result of the derepression of presumptive shoot primordia which proliferate adventitiously (Brettell et al., 1980). This type of plant regeneration is unfortunately restricted to a few genotypes of each species (Green and Phillips, 1975; Sears and Dechard, 1982) and cultures tend to rapidly lose their potential for regeneration after a few subcultures. Also, since shoot meristems are multicellular, each plant arises from many cell initials which increases the possibility of regenerating chimeric plants, especially in cultures exhibiting chromosomal variability (Bennici and D'Amato, 1978; McCoy and Phillips, 1982).

Somatic embryogenesis on the other hand, gives rise to plants originating from only one cell and chromosome mosaicism in regenerated plants does not occur. The occurrence of somatic embryogenesis in grasses is a new discovery but it

has now been observed in Pennisetum purpureum Schum. (Wang and Vasil, 1982), Zea mays L. (Lu et al., 1982) and wheat (Ozias-Akins and Vasil, 1982).

2.3 Variation in Tissue Culture and Regenerated Plants- Cereals.

Tissue cultures of cereals are not free of the chromosomal alterations which occur with dicotyledons. Scheunert et al. (1978) studied the karyological features of two barley cultures, one slow growing and the other fast growing. The slow growing culture exhibited mainly diploid cells and regular mitoses while the fast growing culture exhibited polyploidy, aneuploidy, C-mitosis, chromosome non-disjunction and lagging chromosomes. They observed no differential action of 2,4-D over a concentration range of 0.5 to 40 p.p.m. The fast growing culture was incapable of plant regeneration and the authors concluded that fast growth is intimately connected with karyological instability and absence of regeneration capacity.

Orton (1980) observed polyploidy, aneuploidy and chromosomal rearrangements in callus and suspension cultures of Hordeum vulgare L.. The extent of the variability was determined by the differentiated state, age and history of the culture.

Dicentric chromosomes, broken chromatids and giant chromosomes have been found in wheat suspension cultures, and

wheat callus cultures consisting of equal numbers of eudiploid and aneuploid cells have been observed (Kao et al., 1970; Shimada et al., 1969). Callus cultures of corn have been found to contain 8 to 15.5% nondiploid cells (Edallo et al., 1981).

With variations in vitro so prevalent in cereal tissue cultures it is to be expected that regenerated plants would also reflect this variability. Cummings et al. (1976) regenerated seven oat plants of which two had the normal 21 bivalents at diakinesis while the other five had aberrant meiotic configurations. McCoy et al. (1982) found micronuclei at the tetrad stage, trisomy, monosomy and partial chromosome loss in regenerated oat plants.

Tetraploidy and trisomy have been found in regenerated corn plants and aneuploidy and chromosome rearrangements have been observed in regenerated barley plants (Edallo et al., 1982; Orton, 1980).

The amount of variation in regenerated plants is usually less than that exhibited in culture. In Orton's (1980) work, cultures exhibited a large amount of polyploidy but no polyploid plants were regenerated. Furthermore, a much lower frequency of aneuploidy and chromosomal rearrangements were found in regenerated plants than in the cultures from which they arose. Thus it appears that regeneration selects for cells with specific chromosome constitutions (Orton, 1980; Sacristan and Melchers, 1969).

Ogura (1978) studied the progeny of normal and chromosomally variable tobacco plants and concluded that there is genetic control of chromosomal chimerism.

In addition to chromosomal variability, regenerated plants often exhibit a large amount of phenotypic variability. Larkin and Scowcroft (1981) proposed that the term somaclonal variation be used for any variation among plants regenerated from a tissue culture cycle. It has been suggested that this somaclonal variation could be of benefit for plant improvement through the generation of novel traits unobtainable through conventional plant breeding (Orton, 1980; Larkin and Scowcroft, 1981).

Somaclonal variation has been useful for sugarcane improvement. Heinz et al. (1977) reports the production of sugarcane plants resistant to eyespot disease through tissue culture. Liu and Chen (1976) found variation in regenerated sugarcane plants for cane yield, sugar yield, stalk number and stalk length.

Variation for plant height, heading date, awn morphology, yellow leaf stripes and fertility was noted by Cummings et al. (1976) for tissue culture-derived oat plants. That these changes were genetic in nature was shown by the fact that many of the changes were heritable and both true-breeding and segregating lines were observed.

Dixon et al. (1982) found corn plants resistant to southern corn leaf blight among regenerated plants from

cultures derived from susceptible plants. Also in corn, Beckert et al. (1983) found significant differences for several morphological traits within tissue culture-derived plants of a single cultivar but the amplitude of the variation was low.

Sears et al. (1982) found significant differences in plant height, vigour, and yield for wheat somaclones. The authors state that most of the observed variation was due to chromosomal instability and that this was likely the cause of most somaclonal variation. However, Larkin and Scowcroft (1981) point out that in several species such as potato and tobacco, variation was observed between plants which were cytologically normal.

Edallo et al. (1981) observed the progeny of 108 diploid corn regenerates and observed simply inherited endosperm and seedling mutants, thus implicating gene mutation as a possible origin of somaclonal variation.

Larkin and Scowcroft (1981) also include deletion, somatic crossing over, sister chromatid exchange, and cryptic virus elimination as other possible origins of somaclonal variation.

2.4 Tissue Culture Studies in Triticale

Tissue culture studies in triticale are limited. Sharma et al. (1980) found distinct genotypic differences in organogenesis between ten triticale lines. The lines differed

markedly in the frequency of plants regenerated and in their response to 2,4-D. Some genotypes favoured no 2,4-D in the regeneration media while others favoured 0.1 mg/l 2,4-D. The explants used were immature embryos cultured at 17±1 days after anthesis.

Sharma et al. (1981) observed that high 2,4-D concentrations in the initiation media resulted in better callus production but, due to a carry-over effect, less plant regeneration occurs when the callus is transferred to regeneration media. The optimum 2,4-D concentration for callus induction and subsequent plant regeneration was 3 mgL^{-1}.

Nakamura and Keller (1982a) found that Kao's media was better than Murashige and Skoog or B5 medium for plant regeneration from immature embryo-derived callus for the triticale cultivars "Welsh" and "Rosner". They found that regenerated plants had higher frequencies of aneuploidy and abnormal chromosome structure than those derived from seed. These abnormalities could be due to induction and proliferation of calli from embryos but might also reflect pre-existing abnormalities in the explanted embryos.

Nakamura and Keller (1982b) also regenerated triticale plants from inflorescence cultures and found that these plants were shorter and less vigorous than the original donor material. Most of the regenerated plants had the euploid chromosome number and the authors suggested that the altered morphology had a physiological rather than a genetic basis.

SECTION II

MATERIALS AND METHODS

3.1 Experiments on Callus Induction and Plant Regeneration

"Carman" triticale plants were grown from seed in a controlled environment at 25°C day and 16°C night temperatures with a 16-hour daylength at a photosynthetic photon flux density (PPFD) of 486 μE m^{-2} sec^{-1}. Approximately 15 g of 16-20-0 fertilizer was added to a 3:1:1 mixture of soil, sand and peat moss, respectively. During growth the plants were verified as euploids by root tip analysis. At various times after anthesis the first spike to appear was removed from the plant. The immature embryos were dissected from the seed under sterile conditions and placed in 7 cm deep screw top vials containing 10 mL of modified Murashige and Skoog (MS) medium (Table 1) plus sucrose and 2,4-dichlorophenoxyacetic acid (2,4-D).

Murashige and Skoog medium was used as the basal medium as this is a standard medium which has been successfully used in wheat and triticale embryo culture (Ahloowalia, 1982; Sears and Deckard, 1982; Sharma et al., 1981). Sucrose was added at a concentration of 20 gL^{-1} as this was found to be the optimum concentration for callus growth from triticale embryos (Sharma et al., 1981). The medium was solidified with 8 gL^{-1} of Difco purified agar.

In order to determine the optimum 2,4-D concentration for callus induction, two replicates of 20, 15-day-old immature embryos were placed on MS medium plus 20 gL^{-1}

TABLE 1. Composition of modified Murashige and Skoog medium used as basal medium.

Ingredient	mgL^{-1} of medium
NH_4NO_3	1650.0
KNO_3	1900.0
$CaCl_2.2H_2O$	440.0
$MgSO_4$	198.0
KH_2PO_4	170.0
$MnSO_4.H_2O$	22.3
$ZnSO_4.7H_2O$	8.6
H_3BO_3	6.2
KI	0.821
$CuSO_4.5H_2O$	0.025
$Na_2MoO_4.2H_2O$	0.25
$CoCl_2.6H_2O$	0.025
Fe-EDTA	25.0
Glycine	1.0
Nicotinic acid	1.0
Pyridoxine-HCl	1.0
Thiamine -HCl	1.0
Agar (purified)	8000.0

sucrose and 3 mgL^{-1} 2,4-D while another two replicates of 20 embryos were placed on MS medium plus 2 gL^{-1} sucrose and 5 mgL^{-1} 2,4-D. The vials were placed in an incubator at 20°C and kept in the dark for four weeks.

The effect of the growth hormones 2,4-D, giberellic acid and kinetin on plant regeneration was also studied. The same experimental procedure as described above was used, utilizing a total of 80, 15-day old embryos. After maintaining the cultures in darkness at 20°C for four weeks, any shoots which had formed were removed along with the original embryo tissue. Each callus (arising from one embryo) was then cut into quarters and one quarter was randomly placed on each of the following nutrient media:

(i) MS medium plus 20 gL^{-1} sucrose and 1 mgL^{-1} gibberellic acid.

(ii) MS medium plus 20 gL^{-1} sucrose, 1 mgL^{-1} gibberellic acid and o.1 mgL^{-1} kinetin.

(iii) MS medium plus 20 gL^{-1} sucrose.

(iv) MS medium plus 20 gL^{-1} sucrose and 2,4-D.

Vials of the first three treatments were placed under fluorescent lights at 30°C with a 16-hour daylength and a PPFD of 152 $\mu E\ m^{-2}\ sec^{-1}$. Vials of the fourth treatment were returned to the dark for further callus growth. Every four weeks the number of regenerated plantlets

for each treatment on regeneration media in the light was recorded.

The optimum embryo age for callus induction was determined by placing 15 embryos of each of four different maturity stages on MS medium supplemented with 20 gL^{-1} sucrose and 3 mgL^{-1} 2,4-D. The age groups included 15, 19 and 28-day old embryos as well as those that were mature. After four weeks the percentage of embryos showing callus plus roots or shoots or callus only was calculated.

3.2 Growth of Regenerated Plantlets

In order to enhance continued development, plantlets which were formed on the regeneration media were transferred to vials containing fresh MS media supplemented with 20 gL^{-1} sucrose. When the shoots reached the top of the vial and roots had formed, the plantlets were transferred to a 10.3 cm (4 inch) diameter pot containing a mixture of soil, sand and peat moss as a 3:1:1 mixture. Approximately 15 g of 16-20-0 fertilizer was added and the plantlets were temporarily covered with a 150 mL glass beaker.

The plantlets were placed under the same light conditions as the regenerating calli and after five days they were moved to the greenhouse. After the plants had become firmly established, root-tips were taken and the plants were transferred to 15 cm (6 inch) diameter pots.

At maturity the plants were measured for plant height, average spike length, number of fertile heads per plant and percent fertility (seeds/florets x100%). Root-tip squashes were prepared using the Feulgen procedure and euploid (2n=42) plants were separated from the aneuploid and mixoploid plants. Four seeds of each euploid plant were grown for the second generation along with "Carman" triticale seeds as a control. The second generation was planted in 15 cm pots in the greenhouse. At maturity the second generation plants were measured for the same characteristics as were the original regenerated plants.

3.3 Protein Content Determination

Total protein content of individual seeds was determined on a single seed basis using the micro-Kjeldahl method (Association of Official Analytical Chemists, 1980). Four well developed seeds of each plant from the first regeneration cycle, and two seeds from each of the second generation plants were used for protein determination. A total of 72 samples were analysed. The seeds were ground using a mortar and pestle and the ground samples were oven-dried for 16 hours at 110°C. Protein calculations were based on a 5.7 N conversion factor and a 0% moisture level.

3.4 Analysis of Variation

3.4.1 First and Second Generation Regenerated Plants

The values for plant height, average spike length, number of fertile heads per plant and percent fertility were used to test for variability in the regenerated plant population. An assessment of inter-plant variability for the above characteristics was carried out by making a comparison between the original regenerated plants and their progeny for each population, as well as between regenerated plants and the "Carman" triticale control.

3.4.2 Second and Third Generation Regenerated Plants

A replicated experiment over two environments was carried out to thoroughly test whether the variation present was due mainly to genetic causes or to environmental conditions during the regeneration process.

Four replicates were used with each replicate composed of four second generation plants of each genotype (ie. seeds from the original regenerated plants), four third generation plants of each genotype (ie. seeds from the second generation plants) and four "Carman" triticale control plants. All seeds were sown in 15 cm pots containing a soil mixture of three parts soil, one part sand, and one part peat moss. Approximately 15 g of 16-20-0 fertilizer were added to each pot. Two of the replicates were randomized and placed in the

greenhouse under a PPFD of 486 μE m^{-2} sec^{-1}, a 16-hour daylength, and a 25°C/16°C day-night temperature regime.

At maturity all plants were measured for plant height, average spike length, number of fertile heads per plant, and percent fertility. An analysis of variance of the derived data from each plant population was carried out using a Statistical Analysis System (SAS) package. Regression and correlation analyses were also computerized using data obtained from second and third generation plants.

3.5 Electrophoresis of Prolamins

Prolamin proteins of single kernels from regenerated plants were examined electrophoretically. Electrophoresis was carried out according to the polyacrylamide gel electrophoresis (PAGE) technique of Bushuk and Zillman (1978) with the following modifications:

(i) Sodium lactate was used instead of aluminum lactate as sodium lactate gives a better resolution of prolamins (Khan et al., 1983). (0.3 g sodium lactate were used in the gel solution and 0.3 g were used in 200 mLs of water for the buffer solution.)

(ii) One mL of a 1.5% hydrogen peroxide solution was used for the catalyst solution instead of a 3.0% hydrogen peroxide solution. This allows more time to pour the gel solution before solidification.

(iii) The gels were run for 5 hours at 100 mA rather than for 6.5 hours at 72-74 mA.

The gels were stained with Coomassie Brilliant Blue and photographed as per Bushuk and Zillman (1978).

An Ortec model 4310 densitometer was used to quantify the band intensities of several gels. In order to eliminate as much of the background contamination as possible, positives were made on 4 x 5 film from 35 mm negatives. The 4 x 5 films bearing the gel image displaying a clean background were then scanned via visible light using the "scan only" mode of the densitometer.

3.6 Meiotic Studies

Pollen mother cells (PMC's) were collected from three plants of each genotype in the second generation, as well as from "Carman" triticale control plants grown under identical conditions in the greenhouse. Spikes were fixed in 6:3:1 (ethanol:chloroform:glacial acetic acid) for three days and then stored in 70% ethanol. Anthers were squashed on slides

in a drop of 1% aceto-carmine. Only anthers containing cells at either metaphase I or the tetrad stage were used. A minimum of 100 cells at metaphase I and tetrad stages of division were scored for the frequency of univalents and micronuclei, respectively.

C-banding of PMC's at metaphase I was carried out using a modified procedure of Thomas and Kaltsikes (1974). The modification involved diluting the phosphate buffer solution by a factor of one half to speed up the staining time.

SECTION IV

RESULTS AND DISCUSSION

4.1 Experiments on Callus Induction and Plant Regeneration

Table 2 contains the results for the effect of adding either 3 or 5 mL^{-1} of 2,4-D on callus induction from immature embryos of "Carman" triticale. There was no significant difference between the two concentrations in terms of their ability to induce callus production from immature embryos. Sharma et al. (1981) found that 6 mgL^{-1} 2,4-D gave a significantly greater amount of rapid growing triticale callus than did 3 mgL^{-1} 2,4-D. However, due to a carryover effect the higher 2,4-D level prevented plant regeneration on callus subsequently subcultured to regeneration media.

Nakamura and Keller (1982a) reported that callus induction of triticale embryos was promoted as the auxin concentration increased but that high auxin levels resulted in a decrease in subsequent multiple shoot regeneration. Table 3 shows that in the present study the 5 mgL^{-1} 2,4-D application also had an inhibitory effect on subsequent plant regeneration since twice as many plants were regenerated from calli initiated on 3 mgL^{-1} 2,4-D as compared with the 5 mgL^{-1} 2,4-D treatment.

The addition of both giberellic acid and kinetin in the regeneration media severely reduced the amount of plant

TABLE 2. The effect of two concentrations of 2,4-D on primary callus induction from immature embryos of "Carman" triticale.

Replicate*	mgL^{-1} 2,4-D	Number of embryos with callus**	Number of embryos with callus only
1	3	20 (100)***	8 (40)
2	3	20 (100)	10 (50)
		40 (100)	18 (45)
3	5	20 (100)	8 (40)
4	5	20 (100)	11 (55)
		40 (100)	19 (47.5)

*Twenty embryos per replicate.
**May have shoots and/or roots as well as callus.
***Values in parentheses are percentages.
Values taken 4 weeks after culture.

regeneration in the 3 mgL^{-1} 2,4-D group, whereas the results of the gibberellic acid alone were not different from those of the control (Table 3). It was observed that five of the plants regenerated in the absence of hormones had multiple shoots compared with only two multiple shoot plants regenerated on the gibberellic acid treatment.

Sharma *et al*. (1981) found that the addition of 0.1 mgL^{-1} 2,4-D plus 5 mgL^{-1} kinetin gave the highest frequency of plant regeneration followed by the control treatment in which no growth regulator was added. They did not study the effect of gibberellic acid. Nakamura and Keller (1982a), however found that gibberellic acid enhanced shoot bud formation on Kao's medium but not on Murashige and Skoog medium.

Table 4 shows the effect of embryo age on callus induction. A consistent growth pattern emerged from these studies in that embryos less than 13-14 days of age became necrotic and died *in vitro*. Fifteen-day-old embryos produced the highest callus growth among immature embryos followed by that produced from fully mature embryos. Immature embryos older than 15 days exhibited decreased callus formation.

In support of these findings, Nakamura and Keller (1982a) found that 14-day-old triticale embryos gave a higher frequency of callus induction than 18, 21, 28 and 35-day-old embryos. They also noted that in the cultivar "Welsh", embryos 14 days of age gave rise to callus with a higher regeneration capacity than did 18-day-old embryos.

TABLE 3. Effect of various growth hormones on plant regeneration from immature embryo-derived callus of "Carman" triticale.

Concentration of growth hormone in regeneration medium	Concentration of 2,4-D in callus induction medium (mgL^{-1})	Number of plantlets
$1mgL^{-1}$ gibberellic acid	3	6
$1mgL^{-1}$ gibberellic acid + $0.1mgL^{-1}$ Kinetin	3	1
none	3	6
$1mgL^{-1}$ gibberellic acid	5	2
$1mgL^{-1}$ gibberellic acid + $0.1mgL^{-1}$ Kinetin	5	2
none	5	2

Forty embryos used for each 2,4-D concentration. Number of plantlets recorded after 8 weeks on regeneration medium.

TABLE 4. Effect of embryo age on callus induction from embryos of "Carman" triticale.

Age of embryo (Days after anthesis)	Number of embryos cultured	Number showing callus*	Number showing callus only**
11	20	0(0)	0(0)
15	20	20(100)	8(40)
19	15	15(100)	1(6.7)
28	15	15(100)	0(0)
mature	30	27(90)	5(16.7)

*Callus may have shoots and/or roots.
**Callus with no shoots or roots.
Values in parentheses are percentages.
All embryos placed on MS media plus 3 mgL^{-1} 2,4-D and 20 gL^{-1} sucrose.

In light of the results from these experiments further work made use of 15±1 day-old embryos cultured on MS media plus 20 gL^{-1} sucrose and 3 mgL^{-1} 2,4-D (initiation and maintenance (IM) media). For the purpose of plant regeneration, calli were subcultured every four weeks to MS media containing 20 gL^{-1} sucrose in the absence of growth regulators (regeneration (RM) media), as well as to fresh IM media for continued callus growth. Typical callus formation from triticale embryos is shown in Figure 1 while plant regeneration from callus is shown in Figures 2 and 3.

4.2 Morphology of Regenerated Plants and Their Offspring

As shown in Table 5 and Figures 4 and 5, the regenerated plants exhibited considerable variation for the traits studied. For height, average spike length, and fertility most of the regenerated plants were significantly below the values obtained from the "Carman" control plants while the remainder were not significantly different from the control (Table 5). The number of fertile heads per plant showed an opposite trend with all but one of the regenerated plants having significantly more heads than "Carman".

This variation may have an underlying genetic basis or may only be a result of the unique environment to which these plants were subjected, ie. from tissue culture vials directly to pots in the greenhouse. This transfer undoubtedly could have an effect on the subsequent growth and development of the plant.

TABLE 5. Comparison of morphological characteristics between euploid regenerate plants and "Carman" triticale (control).

	Height (cm)	Number of Fertile Heads	Average Spike Length (cm)	Average Fertility (%)+
"Carman"++	92.9±8.9	1.7±0.5	15.1±0.7	58.2±4.1
R1	60.0*	2.0	9.7*	51.0*
R4	47.0*	4.0*	7.7*	50.0*
R5	58.5*	5.0*	5.6*	41.2*
R6	52.0*	3.0*	8.5*	45.5*
R12	98.3	4.0*	14.2*	54.6
R13	91.8	3.0*	12.5*	59.8

*Significantly different from "Carman".
+Fertility measured by $\frac{\text{number of seeds}}{\text{number of florets}}$ x 100%.
++"Carman" values are averages for ten plants. All plants grown in the greenhouse.


A
B
C

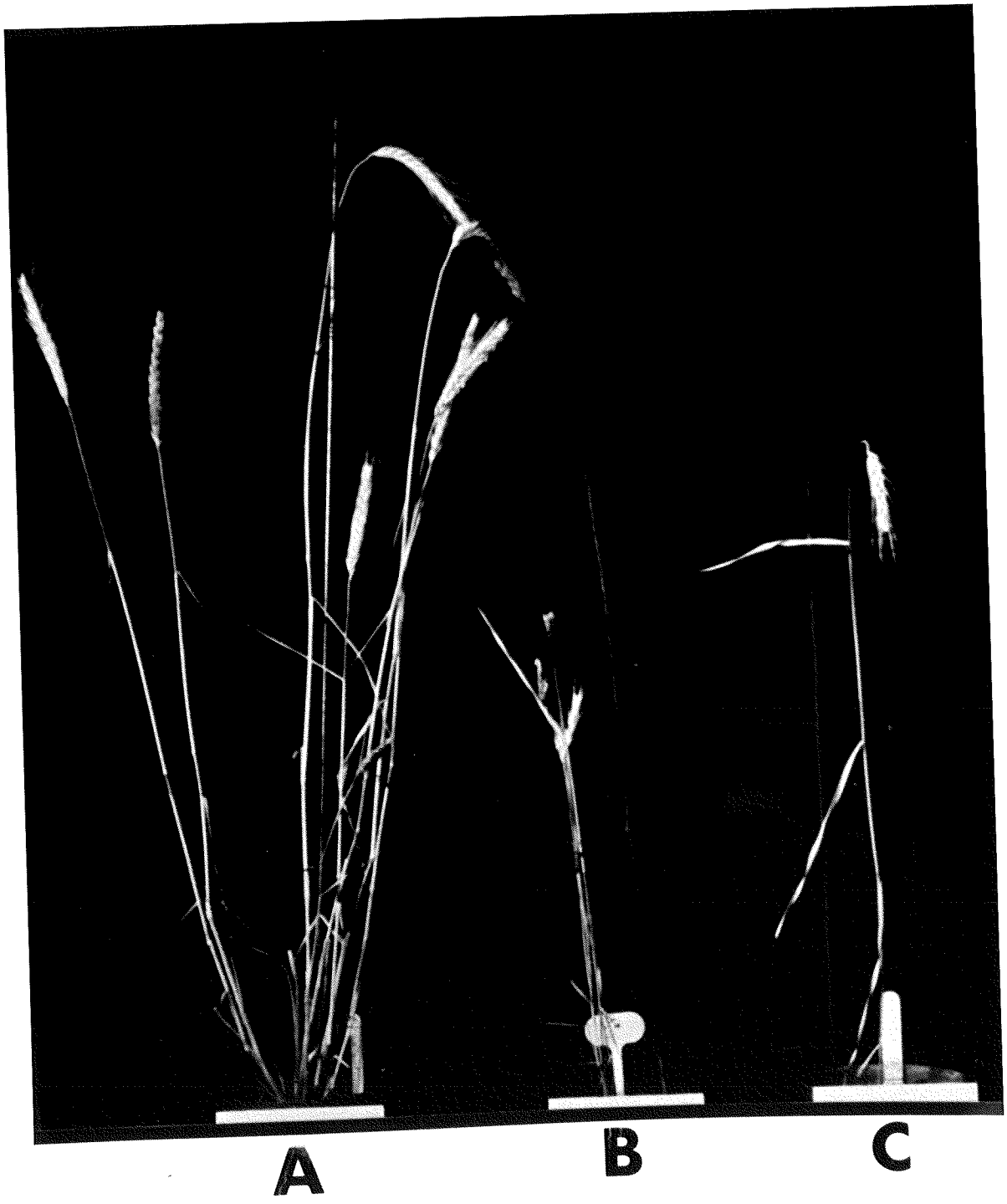

A
B
C

Table 6 shows the data for the second generation regenerated plants and Table 7 compares the means of all the first generation regenerated plants with the means of their progeny for the four traits studied. That the environment to which the original regenerates were subjected played a role in the observed variation can be seen by the fact that for three of the traits the second generation had mean values approaching those of "Carman" and produced smaller standard deviations than plants of the first generation. This indicates that the second generation exhibited less variability and more closely resembled the "Carman" controls than did their parents. Environmental factors do not explain the total original variation expressed since the second generation was still significantly different from "Carman" for all traits studied. This suggests that at least part of the variation may have an underlying genetic basis.

The only trait in which second generation plants did not more closely resemble "Carman" than did the first generation plants was average fertility. The second generation showed a dramatic drop in fertility and this reduction was due to two families: R12 and R13. All second generation families except these two had fertilities which were not significantly different from their first generation parents. Second generation R12 and R13 plants, in contrast, showed a significant reduction in fertility from their parents (Table 8). This reduction was particularly extreme for R13.

TABLE 6. Values of the second generation regenerate plants for the morphological characteristics studied.

	Genotype					
Height (cm)	R1	R4	R5	R6	R12	R13
	90.0	79.0	74.5	73.8	70.5	83.4
	90.5	76.3	77.7	90.0	69.0	82.0
	91.0	59.2	86.2	84.0	93.0	67.1
	81.0	92.0	81.7	80.0	71.5	51.5
	88.1±4.8	76.6±13.5	80.0±5.1	81.9±6.8	76.0±11.4	71.0±14.9
Number of Fertile Heads	1.0	1.0	1.0	1.0	1.0	2.0
	1.0	1.0	1.0	1.0	1.0	1.0
	1.0	1.0	1.0	1.0	1.0	1.0
	1.0	2.0	1.0	1.0	1.0	1.0
	1.0±0	1.3±1.1	1.0±0	1.0±0	1.0±0	1.3±0.5
Spike Length (cm)	12.0	11.6	11.3	11.9	9.3	8.8
	13.6	12.5	12.0	12.7	10.0	6.3
	12.6	12.4	13.1	12.6	12.2	10.5
	10.3	9.9	13.8	12.3	9.5	7.7
	12.1±1.4	12.5±0.74	12.6±1.1	12.4±0.4	10.3±1.3	8.4±.25
Fertility (%)+	35.2	38.1	49.0	35.2	31.4	16.7
	55.6	24.4	29.6	55.0	38.3	0.0
	40.4	45.6	39.7	49.1	50.7	27.5
	45.8	53.1	48.5	41.2	35.2	25.0
	44.3±8.7	40.3±12.2	41.7±9.1	45.1±8.7	38.9±8.4	17.3±12.4

+Fertility measured by $\frac{\text{number of seeds}}{\text{number of florets}}$ x 100%

TABLE 7. Morphological comparison of "Carman" triticale and first and second generation regenerate plants.

	Generation	Mean Height (cm)	Mean Number of Fertile Heads	Average Spike Length (cm)	Average Fertility +(%)
"Carman"	-	92.9±8.9	1.7±0.5	15.1±0.7	58.2±4.1
Regenerate Plants	1	67.9±21.6*	3.5±1.0*	9.7±3.2*	50.4±6.6*
	2	79.0±5.6*	1.1±0.1*	11.4±1.7*	37.9±10.4*

*Significantly different from "Carman" at 95% level by Duncan's Multiple Range Test.

+Fertility measured by $\frac{\text{number of seeds}}{\text{number of florets}}$ x 100%.

TABLE 8. Average percent fertility comparison between Carman and first and second generation regenerate plants.

Plant	Generation	Average Fertility (%) (number of seeds / number of florets x 100%)
"Carman"	-	58.2±4.1
R1	1	51.0*
R1	2	44.3±8.7*
R4	1	50.0*
R4	2	40.3±12.2*
R5	1	41.2*
R5	2	41.7±9.1*
R6	1	45.5*
R6	2	45.1±8.7*
R12	1	54.6
R12	2	38.9±8.4*+
R13	1	59.8
R13	2	17.3±12.4*+

*Significantly different from Carman at 95% level by Duncan's Multiple Range Test.
+Significantly different from generation 1 at 95% level.

Such variation in progenies regenerated *in vitro* is not unusual in cereal species and the term "somaclonal variation" has been used to describe such variation (Larkin and Scowcroft, 1981). Morphological variability over several generations has been observed in regenerated plants of sugarcane, oats, corn and wheat (Liu and Chen, 1976; Cummings *et al.*, 1976; Beckert *et al.*, 1983; Sears *et al.*, 1982).

In the above analysis the parents and their offspring were grown in the greenhouse at different times and thus environmental differences may have played a role in the variation between the first and second generation regenerated plants. In order to further determine the stability of the variation from one generation to the next, and to further separate genetic from environmental effects, a replicated experiment was conducted under two environmental regimes as described in the Materials and Methods section (Page 21). For all four replications of both the second and third generation plants there were significant differences between genotypes for spike length and fertility (Tables 11 and 12) but not for height or number of fertile heads (Tables 9 and 10).

The block effect was significant for all traits in the four replication analysis for both generations (Tables 9 to 12). This is due to the fact that of the four replications, two were grown under growth-room conditions while two were grown in the greenhouse. Thus all the traits studied were affected significantly by the environment.

TABLE 9. ANOVA values for mean plant height of second and third generation regenerate plants.

Generation	Number of Reps in ANOVA	Environmental Conditions	Mean Square	
			Block	Genotype
2	4	greenhouse growth-room	694.058**	41.778
3	4	greenhouse growth-room	395.807**	94.305
2	2	greenhouse	31.801	64.592
3	2	greenhouse	0.035	106.612*
2	2	growth-room	17.161	17.161
3	2	growth-room	0.483	33.137

*, **Significant at 95% and 99% level respectively by F-test.

TABLE 10. ANOVA values for mean number of fertile heads of second and third generation regenerate plants.

Generation	Number of Reps in ANOVA	Environmental Conditions	Mean Square	
			Block	Genotype
2	4	greenhouse growth-room	2.009*	0.976
3	4	greenhouse growth-room	1.176*	0.176
2	2	greenhouse	0.161	0.744
3	2	greenhouse	0.601	0.030
2	2	growth-room	0.286	0.583
3	2	growth-room	0.161	0.388

*Significant at 95% level by F-test.

TABLE 11. ANOVA values for mean spike length of second and third generation regenerate plants.

Generation	Number of Reps in ANOVA	Environmental Conditions	Mean Square	
			Block	Genotype
2	4	greenhouse growth-room	20.542**	2.155*
3	4	greenhouse growth-room	16.060**	1.901*
2	2	greenhouse	0.206	2.480*
3	2	greenhouse	0.286	2.470**
2	2	growth-room	0.206	1.110
3	2	growth-room	0.315	0.673

*, **Significant at 95% and 99% level respectively by F-test.

TABLE 12. ANOVA values for mean fertility of second and third generation regenerate plants.

Generation	Number of Reps in ANOVA	Environmental Conditions	Mean Square	
			Block	Genotype
2	4	greenhouse growth-room	360.127**	448.665**
3	4	greenhouse growth-room	189.496*	459.739**
2	2	greenhouse	18.515	270.513*
3	2	greenhouse	98.315	448.222**
2	2	growth-room	2.835	230.683**
3	2	growth-room	110.883	90.635

*, **Significant at 95% and 99% level respectively by F-test.

Comparing material from replications grown in the same environment, it is seen that the block effect is nonsignificant for all traits in both generations (Tables 9 to 12). In the second generation there were significant differences for fertility among genotypes in both sets of replications (Table 12). Spike length varied significantly in one set of replications grown in the greenhouse (Table 11). In the third generation significant differences existed in the greenhouse grown set of replications for height, spike length and fertility (Tables 9, 11 and 12).

These results imply that there were genetic differences between the progeny of regenerated plants in both generations and that these differences were modified by the environment. The genetic component of variation was most prominent for spike length and fertility and to a lesser extent for plant height.

Duncan's multiple range tests for the morphological traits over all replications (Tables 13 and 14) show that R13 was the most variable genotype. Other genotypes showed no variation among themselves with the exception of R1 which was significantly different in fertility from both "Carman" and R4 in the second generation (Table 13).

Regression and correlation analysis between the second and third generations over all replications showed highly significant slopes and correlation coefficients for all traits with the exception of fertile head number (Table 15). The highest intergeneration correlation was obtained for

TABLE 13. Mean values of the second generation regenerate plants and "Carman" controls for the various morphological traits studied.

Genotype	Plant Height(cm)	Number of Fertile Heads	Spike Length(cm)	Fertility+ (%)
"Carman"	103.77	2.13	12.58 (a)	56.93 (a)
R1	102.67	2.25	12.65 (a)	43.40 (b)
R4	106.15	1.88	13.03 (a)	55.40 (a)
R5	98.60	3.00	12.30 (a)	49.58 (ab)
R6	98.20	3.00	12.38 (a)	47.80 (ab)
R12	100.42	2.50	12.63 (a)	50.43 (ab)
R13	97.57	3.13	10.75 (b)	25.28 (c)

Values followed by different letters are significantly different by Duncan's multiple range test (alpha=0.05).

+Fertility measured by $\frac{\text{number of seeds}}{\text{number of florets}} \times 100\%$

TABLE 14. Mean values of the third generation regenerate plants and "Carman" controls for the various morphological traits studied.

Genotype	Plant Height(cm)	Number of Fertile Heads	Spike Length(cm)	Fertility+ (%)
"Carman"	103.77	2.13	12.58 (a)	56.93 (a)
R1	102.15	2.58	12.53 (a)	47.15 (a)
R4	101.17	2.60	12.90 (a)	51.85 (a)
R5	102.55	2.75	12.48 (a)	49.38 (a)
R6	103.37	2.35	12.58 (a)	47.00 (a)
R12	107.32	2.33	12.00 (ab)	50.45 (a)
R13	91.62	2.53	10.83 (b)	23.53 (b)

Values followed by different letters are significantly different by Duncan's multiple range test (alpha=0.05).

+Fertility measured by $\frac{\text{number of seeds}}{\text{number of florets}} \times 100\%$

TABLE 15. Slope estimates (b) and correlation coefficients (r) from regression and correlation analysis of second and third generation regenerate plants characterized for four morphological traits.

Morphological Traits							
Height		Number of Fertile Heads		Spike Length		Fertility	
b	r	b	r	b	r	b	r
0.584**	0.611**	0.224(NS)	0.322(NS)	0.837***	0.906***	0.741***	0.737***

**Significant at 99% level.
***Significant at 99.9% level.
Third generation plants were regressed on second generation plants in order to obtain the slope estimates (b).

spike length followed by fertility and plant height. Thus the genetic component of variability was inherited from one generation to the next, particularly for spike length, fertility and plant height. The variation in the number of fertile heads does not appear to have had a genetic basis.

4.2 Cytogenetics

Of fourteen original plants regenerated, six were found to be euploid (2n=42) while eight were chromosomally mixoploid. The mixoploid plants contained hyperploid as well as euploid cells. The hyperploid cells contained very small chromosomes which resembled chromosomal fragments. Only the euploid plants were studied in detail.

Second generation euploid regenerate plants exhibited significant differences between genotypes for the mean number of univalents per cell at metaphase I (Table 16 and Figure 7) and the mean number of micronuclei per tetrad (Table 17 and Figure 6). No significant differences for these characteristics occurred however, within genotypes ie. within the progeny of a single regenerated plant. Thus any changes which occurred in the original regenerated plant relative to chromosome structure, or to genes controlling chromosome pairing, chiasma formation or chromosome movement, were inherited uniformly by the offspring.

Duncan's multiple range test for mean number of univalents per cell at metaphase I (Table 18) shows three different groups at the 95% significance level, with only genotype

TABLE 16. ANOVA for mean number of univalents per cell at metaphase I for second generation regenerate plants of "Carman" triticale.

Source	DF	Mean Square	F
Block	2	0.69	2.80 NS
Genotype	6	2.02	8.26**
Error	12	0.25	

**Significant at 99% level.

TABLE 17. ANOVA for mean number of micronuclei per tetrad for second generation regenerate plants of "Carman" triticale.

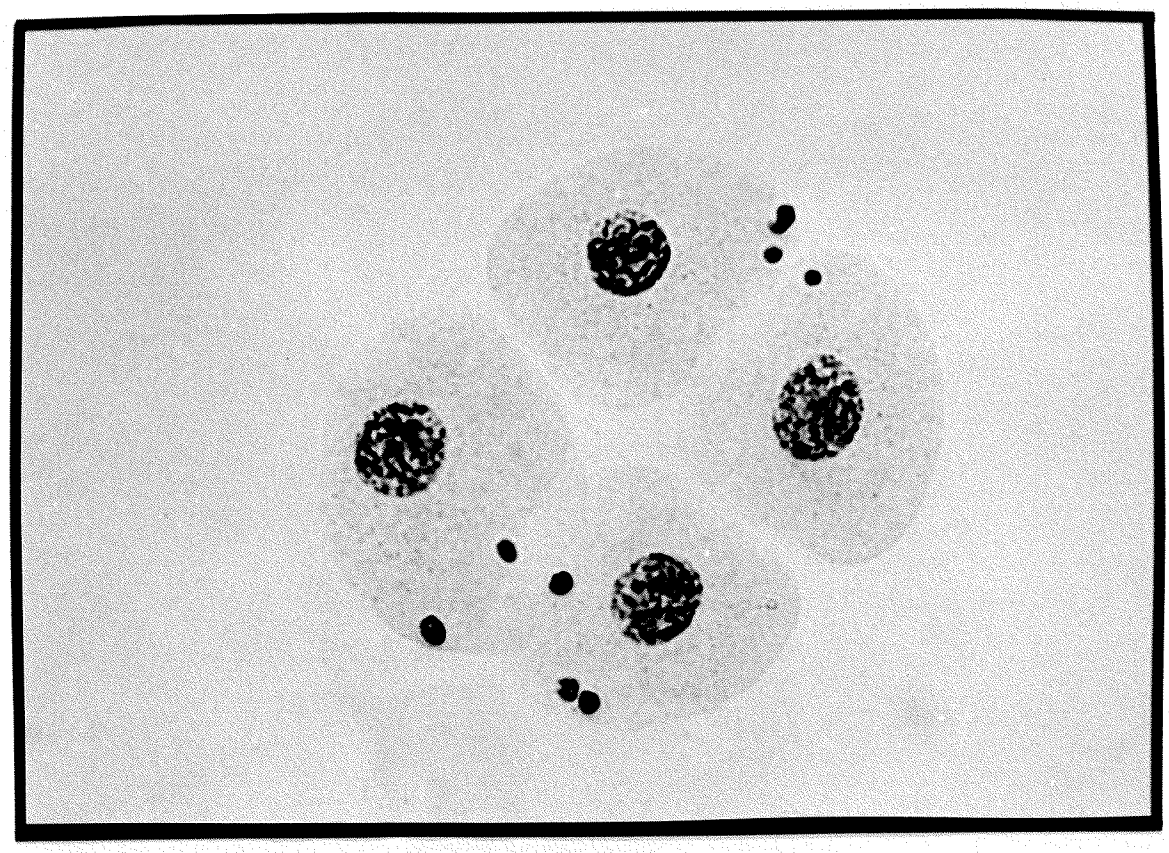
Source	DF	Mean Square	F
Block	2	0.25	0.36 NS
Genotype	6	2.48	3.66*
Error	12	0.68	

*Significant at 95% level.

TABLE 18. Mean number of univalents per cell at metaphase I and mean number of micronuclei per tetrad for "Carman" triticale and second generation regenerate plants of "Carman" triticale.

Genotype	Mean Number of Univalents per Cell	Mean Number of Micronuclei per Tetrad
"Carman"	1.30 (bc)	1.97 (bc)
R1	2.20 (b)	3.23 (ab)
R4	1.13 (c)	1.60 (c)
R5	1.30 (bc)	1.83 (bc)
R6	1.33 (bc)	1.17 (c)
R12	1.50 (bc)	1.53 (c)
R13	3.43 (a)	3.57 (a)

Values followed by different letters are significantly different by Duncan's Multiple Range Test (alpha = 0.05).



R13 being significantly different from "Carman". This situation also holds true for the mean number of micronuclei per tetrad (Table 18).

Sears et al. (1982) stated that the somoclonal variation exhibited for plant height, vigor, and yield among the progenies of regenerated wheat plants was likely due to chromosome instability. In the present study, R13 was the only genotype significantly different from "Carman" in terms of chromosome instability. Similarly, in the second generation R13 was the only genotype significantly different from "Carman" for spike length, and was one of two genotypes significantly different from "Carman" for fertility (Table 13). In the third generation, R13 was the only genotype significantly different from "Carman" for spike length, and fertility (Table 14). Thus the chromosome instability of R13 plants relative to the "Carman" controls, is the most likely cause for the large degree of variation that these plants exhibit when compared to the other genotypes. Even though the production of univalents and micronuclei was quite high in R13, the mean values for the morphological traits were very similar in both the second and third generations (Tables 13 and 14). Thus, even though chromosomally unstable in comparison with "Carman", R13 was stable within itself.

R1 and R4 are the only genotypes (with the exception of R13) to be significantly different from each other in terms of both micronuclei per tetrad and univalent number at metaphase I. All other genotypes had about the same amount of chromosome instability (Table 18) and were similar to one

another in the degree of variability expressed for the various morphological traits studied (Tables 13 and 14).

In general, a population of plants exhibiting chromosome instability can be expected to exhibit some degree of morphological variation. Conversely, plants similar to each other in terms of chromosome stability would be expected to be similar in terms of morphology.

Since the original R13 regenerate plant had a euploid chromosome number, its high degree of meiotic instability could be due to either the loss of a gene or genes controlling synapsis and chiasma formation, to chromosomal structural changes, or to trisomy for one chromosome and monosomy for another.

Triticale has always been prone to meiotic irregularities including univalent formation at metaphase I in advanced breeding material. It has been observed via constitutive heterochromatin banding studies that most of the univalents in advanced breeding material are from the rye genome (Thomas and Kaltsikes, 1974). Many hypotheses have been put forth in order to explain the cause of these rye univalents. These include (1) desynapsis due to a lack of crossing over in the inbred rye genome of advanced triticale, (2) interaction between the genes that control chromosome pairing in rye and those that control pairing in wheat, (3) the asynchronous meiotic cycles of the rye and wheat chromosomes in a triticale due to the high DNA content in the rye parent relative to that in wheat, and (4) interference of chromosome pairing by the large blocks of terminal heterochromatin found on the

rye chromosomes (Lelley, 1974; Lelley, 1975; Kaltsikes, 1974; Roupakias and Kaltsikes, 1977; Thomas and Kaltsikes, 1974).

When the meiotic staining technique of Thomas and Kaltsikes (1974) is used, these blocks of constitutive terminal heterochromatin stain as dark bands while the rest of the chromosome remains only lightly stained. As only the rye chromosomes possess these terminal bands, the rye chromosomes in a triticale karyotype can be easily distinguished (Figure 8).

Using the technique on metaphase I cells of "Carman" triticale, it was observed that the proportion of rye:wheat chromosomes was 3.48:1 with 77.69% of the observed univalents being of the rye genome (Table 19). This percentage of rye univalents is in close agreement with the values of 80.56% and 82.14% found by Thomas and Kaltsikes (1974) for inbred lines of hexaploid triticale and for hybrid triticale, respectively. Thus "Carman" behaves similarly to other triticales in that it produces mainly rye univalents.

C-banding of metaphase I cells of the genotype R13 resulted in a rye:wheat proportion of 1.80:1 with only 64.32% of the univalents being from the rye genome (Table 19). Thus the increased meiotic instability in R13 was due to a higher frequency of wheat univalents. This increased frequency could be a result of structural changes in wheat chromosomes or due to alterations in wheat genes regulating synapsis and/or chiasma formation. As chromosome fragments were often found as univalents at metaphase I, chromosome breakage and structural alterations likely played a large role in the increased meiotic instability of genotype R13.

TABLE 19. The distribution of terminal heterochromatin in univalents of "Carman" triticale and in the regenerate genotype R13.

	Total Number of Univalents Observed	Number of Univalents with Terminal Heterochromatin+	Rye:Wheat Ratio
R13	213	137 (64.32%)	1.80:1
"Carman"	242	188 (77.69%)	3.48:1

+Presence of terminal heterochromatin indicates rye chromosomes.

4.4 Protein Content and Electrophoresis

Table 20 contains the mean whole-grain crude protein contents for the regenerated plants as measured by the micro-Kjeldahl technique. In the original first generation regenerates none of the genotypes were significantly different from "Carman" for protein content. However, the standard deviations were generally higher in the first generation plants than in "Carman" suggesting that variation existed among seeds of the original regenerated plants. In the second generation, three plants had significantly different protein contents than "Carman". Two of them, R4-3 and R5-2, had a 6.0% and 5.5% increase, respectively, in percent protein over "Carman". In both cases the increase in protein content was significant. The small standard deviations for these two plants shows that all of the seeds observed from these plants were relatively uniform for the higher protein levels.

An increased seed protein content would be beneficial in triticale. Over the years during which triticale has been researched, the improvement of kernel characteristics has been associated with a lowering of whole grain protein content, ie. selection for plump kernels results in a negative selection for lower protein content (Zillinsky, 1974). Thus, finding a way to increase the protein content of plump kernels would be advantageous. Whether or not the high protein lines derived from the present studies could be used in a

TABLE 20. Mean Percent protein for "Carman" triticale and first and second generation regenerate plants.

Genotype	Generation	% Protein (N x 5.7) (0% Moisture)
"Carman"	--	16.1 ± 0.8
R4	1	14.3 ± 1.1
R4-1	2	13.1 ± 0.6
R4-2	2	14.9 ± 2.1
R4-3	2	22.1 ± 0.2*++
R4-4	2	12.1 ± 0.4
R1	1	14.1 ± 3.7
R1-1	2	17.6 ± 2.7
R1-2	2	13.6 ± 0.1
R1-3	2	14.8 ± 0.8
R1-4	2	17.6 ± 0.3
R6	1	14.6 ± 3.8
R6-1	2	16.2 ± 0.6
R6-2	2	11.6 ± 0.1*
R6-3	2	12.4 ± 1.3
R6-4	2	15.2 ± 4.4
R5	1	16.7 ± 0.1
R5-1	2	12.3 ± 0.5
R5-2	2	21.6 ± 0.9*++
R5-3	2	12.6 ± 1.2
R5-4	2	13.4 ± 1.4
R12	1	13.5 ± 1.5
R12-1	2	15.3 ± 4.7
R12-2	2	12.8 ± 0.5
R12-3	2	13.5 ± 0.6
R12-4	2	12.2 ± 0.4
R13	1	13.6 ± 0.7
R13-1	2	15.9 ± 1.2
R13-2	2	16.2 ± 1.5
R13-4	2	18.0 ± 3.0

*Significantly different from Carman at 95% level by Duncan's Multiple Range Test.
++Significantly greater than Carman at 99% level by Duncan's Multiple Range Test.

breeding program depends on the heritability of the trait. To be useful, the high protein trait must have a genetic basis and must be transferable to the offspring by sexual means.

In order to determine if the high protein trait had a genetic basis, reserve seed of the plants R5-2 and R4-3 was multiplied. Four seeds of each of the two genotypes were grown from this increase along with a "Carman" control. Analysis of protein content was carried out on three seeds of each plant and the results are shown in Table 21. For both genotypes two plants had significantly higher protein content than "Carman" while the two remaining plants were not significantly different from "Carman". This indicates that the high protein trait does have a genetic basis and that the original high protein plants (R5-2 and R4-3) gave rise to offspring which segregated for the trait.

The main components of cereal kernel protein are the endosperm storage proteins. The fraction of these proteins which is soluble in 70% ethanol solutions is known in general terms as the prolamin fraction, or specifically, as the gliadin fraction in wheat and the secalin fraction in rye. This fraction consists of many components which have similar composition and high levels of glutamine and proline. These components can be separated by polyacrylamide gel electrophoresis (PAGE) and the resulting pattern of number, mobility and intensity of bands is distinctive for any given cultivar (Bushuk and Zillman, 1978). For triticale it has been shown

TABLE 21. Percent kernel protein of the offspring of high protein regenerate plants and "Carman" triticale.

Genotype	Offspring Plant Number			
	1	2	3	4
5-2	19.6±0.6*	18.5±1.1	16.8±0.6	19.9±0.9**
4-3	16.4±0.7	19.1±3.1*	18.8±2.3*	15.9±1.0
"Carman"	15.7±1.0	--	--	--

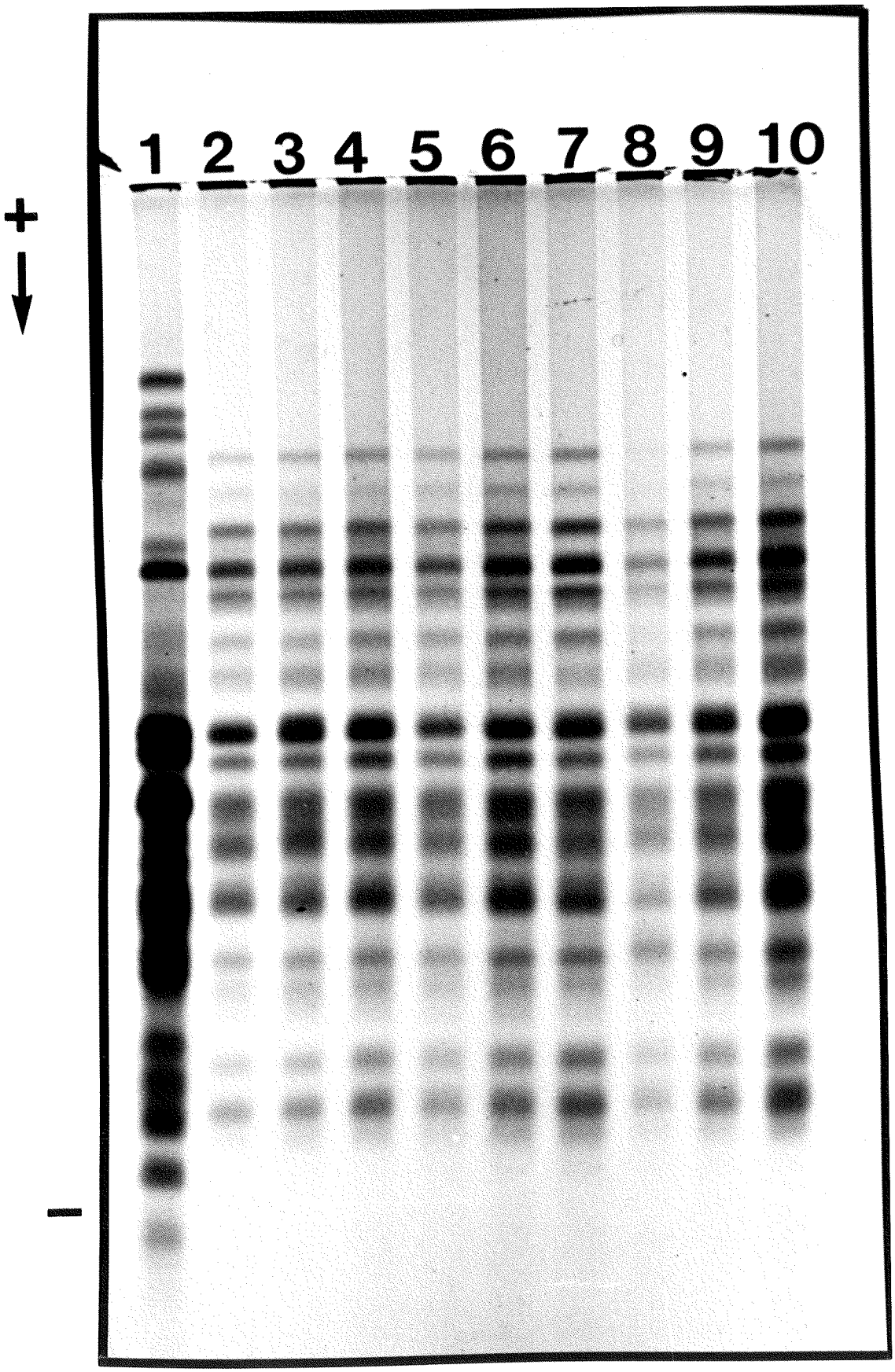
*Significantly different from "Carman" at the 95% level by Duncan's multiple range test.
**Significantly different from "Carman" at the 99% level by Duncan's multiple range test.
Three seeds were analyzed for each plant.

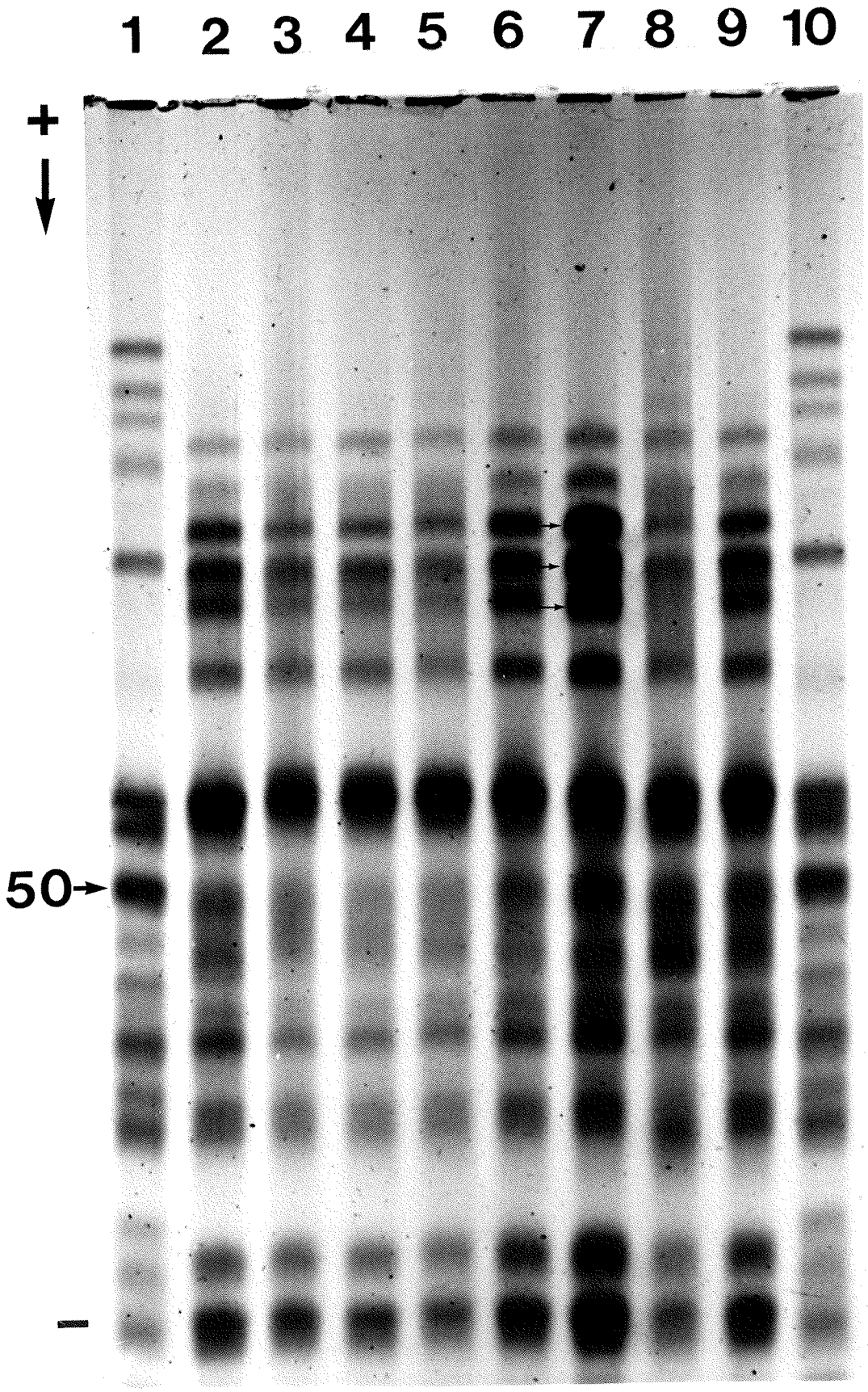
that the prolamin banding pattern is simply the sum of the banding patterns of the wheat and rye parents. No new bands are produced (Chen and Bushuk, 1970).

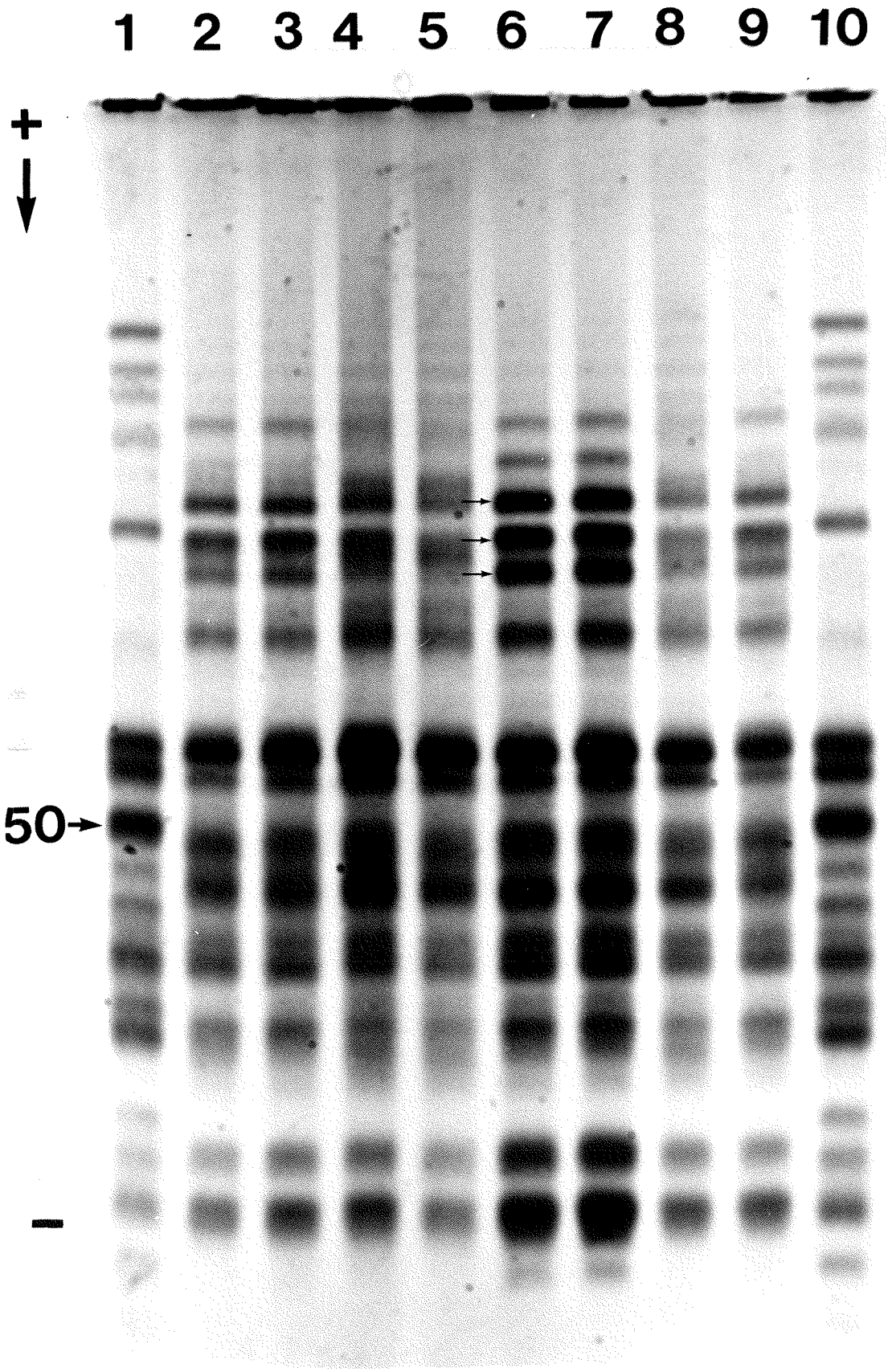
All of the structural genes for the synthesis of the gliadin components of wheat are located on the chromosomes of homoeologous groups 1 and 6 of all three genomes (Kasarda et al., 1976). In rye, the structural genes for secalin synthesis are found on chromosome 1 (Shepherd and Jennings, 1970). Thus any changes in banding pattern in triticale is due to genes on these chromosomes.

The high protein plants derived from tissue culture may have shown increased kernel protein due to changes in the genes controlling prolamin synthesis. If such changes occurred in the structural genes, new banding patterns would appear. If the changes were in regulatory genes, changes in band intensity would result. Figure 9 shows the PAGE banding pattern for eight single seeds of "Carman" triticale. There was variation in band intensity due to seed size variability but the band pattern is the same for all seeds sampled indicating that "Carman" is stable as far as electrophoretic banding pattern of prolamins is concerned.

Figure 10 shows the banding patterns for "Carman" controls, R4 first generation, and R4 second generation plants. The PAGE patterns for Carman controls, R5 first generation, and R5 second generation plants are shown in Figure 11. In both Figures 10 and 11, all samples were of identical weight


1 2 3 4 5 6 7 8 9 10
+
−


1
2
3
4
5
6
7
8
9
10
+
50
–


1 2 3 4 5 6 7 8 9 10
+
50
-

and therefore variations in band intensity were not due to differences in sample size. It can clearly be seen that column 7 in Figure 10 and columns 6 and 7 in Figure 11 show bands of a darker intensity than the other columns but the band pattern is identical. The columns showing the darker bands correspond to the high protein plants R4-3 (column 7, Figure 10) and R5-2 (columns 6 and 7, Figure 11). Thus the increased protein content of these two plants is expressed in the prolamin fraction and is possibly the result of changes in regulatory genes leading to increased prolamin production, and not the result of structural gene changes.

It can also be seen from Figures 10 and 11 that the most striking difference in band intensity between the high protein plants and the other plants occurs in the three bands indicated by the arrows. These bands are coded for by the rye genome. Thus it is possible that regulatory genes of the rye genome have been altered in such a way as to increase the activity of the prolamin structural genes on chromosome 1 of rye.

In order to quantify the differences in band intensity, the gels were analysed using a densitometer. Figures 12 and 13 show the densitometer tracings for plant R4-3 (column 7, Figure 10) and its "Carman" control (column 9, Figure 10) while Figures 14 and 15 show the densitometer tracings for plant R5-2 (column 6, Figure 11) and its "Carman" control (column 9, Figure 11). In both cases, the bands of the high

protein plants show higher peaks than the bands of the "Carman" controls. The differences in band intensity between the high protein plants and the "Carman" controls was quantified in Table 22 on the basis of densitometer chart units. For plant R4-3 the three rye bands marked by arrows in Figure 10 (column 7) were responsible for the greatest differences in band intensity. Similarly, for plant R5-2 (column 6, Figure 11), the three rye bands were responsible for three of the four greatest differences in band intensity. Thus, although all the bands of the high protein plants showed increased levels of protein relative to their "Carman" controls, the largest differences were in the bands coded for by the rye genome.

It may be that an alteration of a rye regulatory gene occurred and that this change resulted in increased prolamin production from the structural genes on chromosome 1R. Darvey and Gustafson (1975) observed that the substitution of wheat chromosome 2D for rye chromosome 2R in hexaploid triticale resulted in a reduction of protein levels and concluded that specific rye chromosomes are important in governing protein content. Homoeologous group 2 chromosomes in wheat have been implicated as possible sites for genes regulating gliadin synthesis (Waines, 1973; Brown and Flavell, 1981). Thus, rye chromosome 2R may also contain prolamin regulatory genes which, when modified, produce the high protein plants derived from tissue culture in the present study.

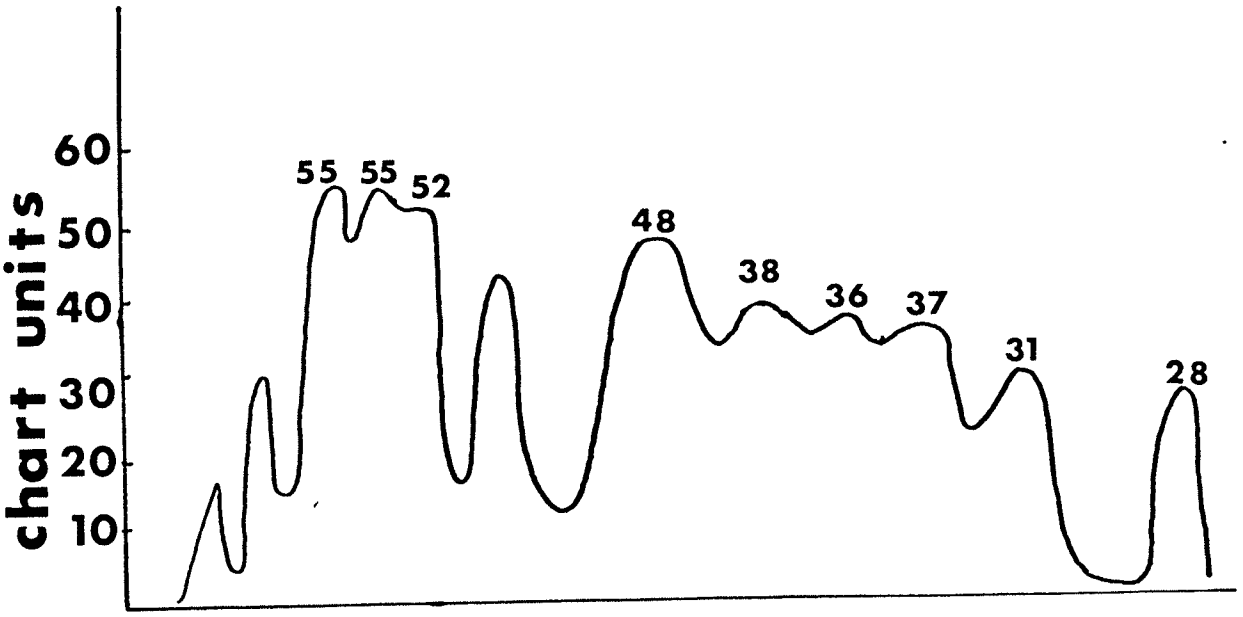


chart units
60
50
40
30
20
10
55
55
52
48
38
36
37
31
28

FIG. 12

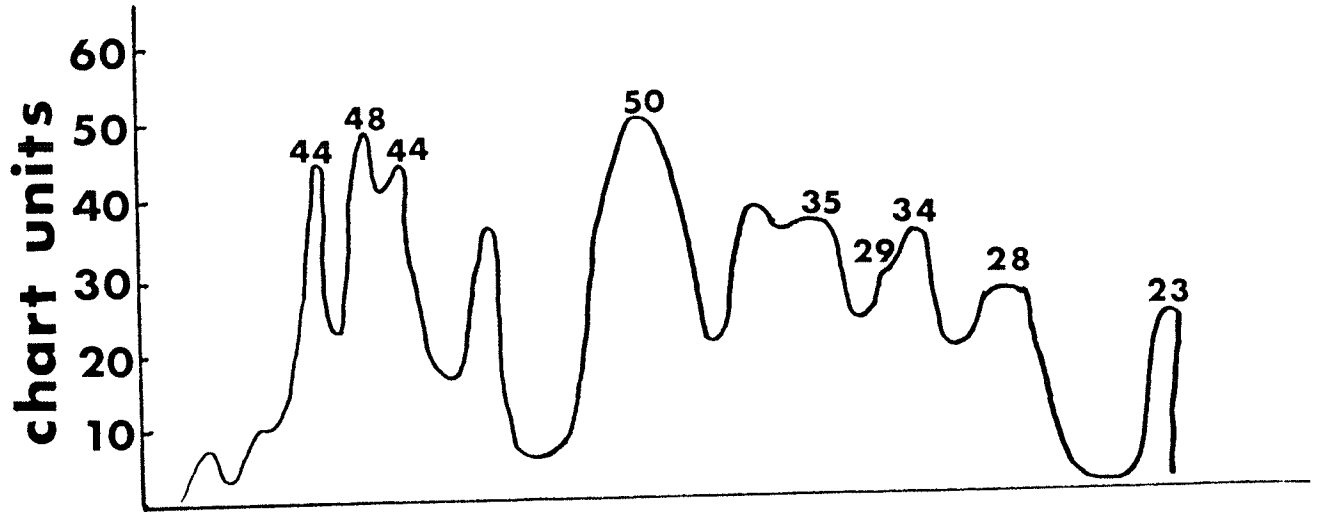


chart units
60
50
40
30
20
10
44
48
44
50
35
29
34
28
23

FIG. 13

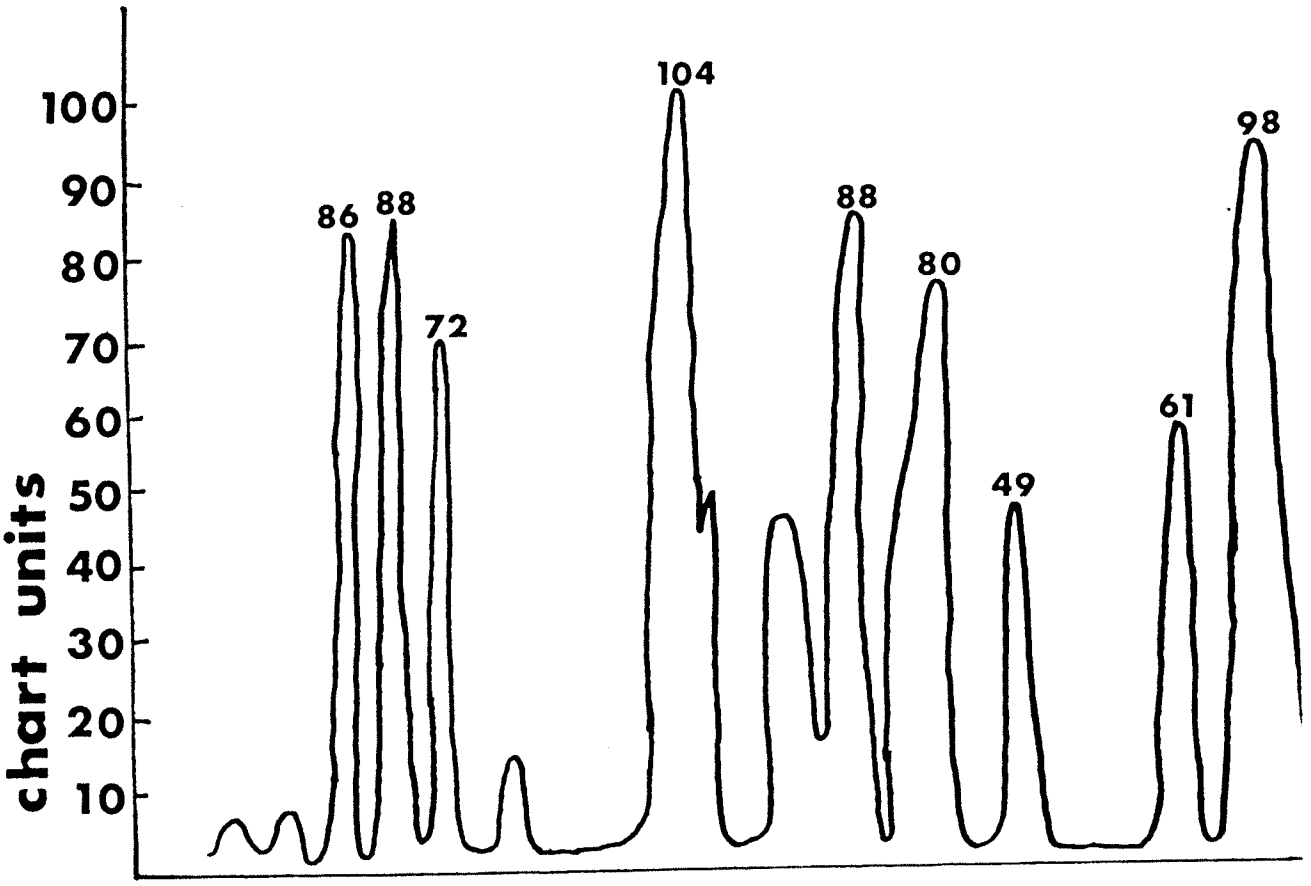


chart units
100
90
80
70
60
50
40
30
20
10
86
88
72
104
88
80
49
61
98

FIG. 14

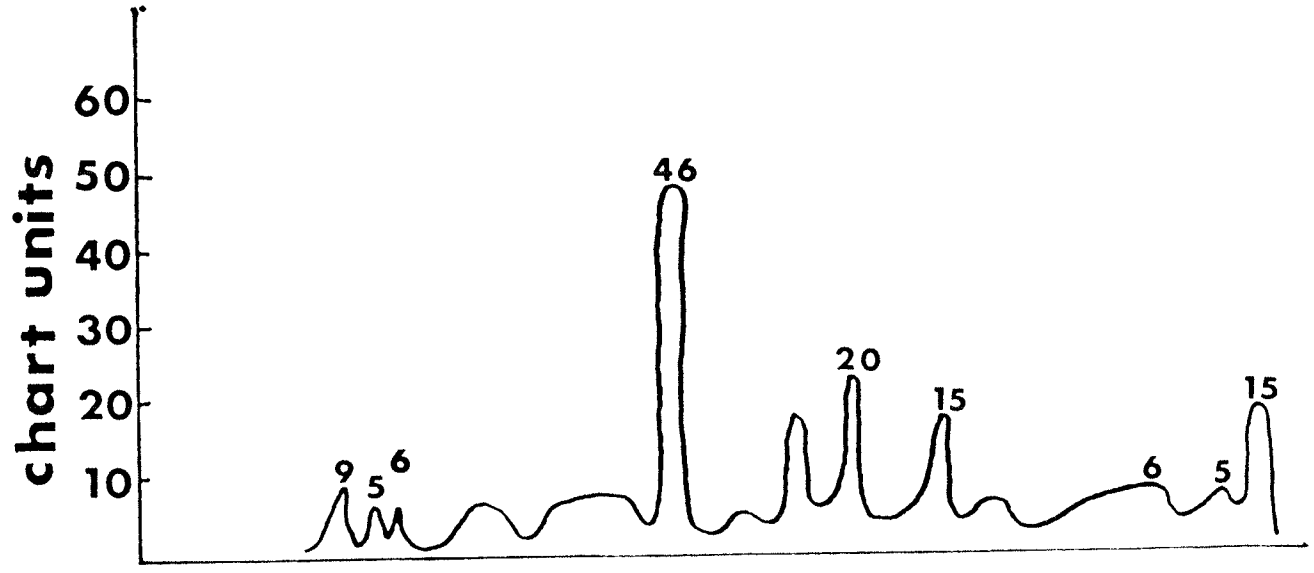


chart units
60
50
40
30
20
10
9
5
6
46
20
15
6
5
15

FIG. 15

TABLE 22. Densitometer chart unit values for electrophoretic bands from densitometer tracings of PAGE patterns of plants R4-3, R5-2 and their "Carman" controls.

Chart Units for Bands					
R 4-3	Carman (Fig 10)	Difference	R 5-2	Carman (Fig 11)	Difference
55	44	11+	86	9	77+
55	48	7+	88	5	83+
52	44	8+	72	6	66+
48	50	-2	104	46	58
38	35	3	88	20	68
36	29	7	80	15	65
37	34	3	49	6	43
31	28	3	61	5	56
28	23	5	98	15	83

+Differences for the arrowed bands of Figures 10 and 11 (rye genome bands).

Although the three rye bands showed the greatest intensity differences, the wheat bands of the high protein plants were also more intense than the wheat bands of their "Carman" controls. Either an altered function of the rye regulatory gene(s) also affected the expression of wheat structural genes, or there could have been simultaneous changes in wheat regulatory genes themselves, leading to increased prolamin production. In either case the prolamin production from the wheat structural genes is less than that of the rye genes.

From an evolutionary standpoint, the wheat and rye genomes are closely related. They have a high level of repeated DNA sequence homology (Flavell et al., 1977). Given this close relationship, it is quite likely that certain rye genes can affect the functioning of wheat genes in the same cell. Lelley (1976) found that rye genes can suppress the action of wheat chromosome 5B thereby promoting homoeologous pairing. In view of the close relationship between the wheat and rye genomes, it is likely that any alteration in rye prolamin regulatory genes would affect mainly the rye prolamin structural genes. Nevertheless, some effect on the wheat prolamin structural genes could also be expected. This is more likely to occur than would simultaneous changes in the function of both wheat and rye prolamin regulatory genes.

SECTION V

GENERAL DISCUSSION

The aim of the present study was to assess the feasibility of using somaclonal variation for triticale improvement. In order for variation to be useful for breeding purposes the novel traits must have a genetic basis and therefore must be able to be sexually transmitted.

The variation which occurs in culture can be due to gene mutation or can be a result of epigenetic change. Epigenetic changes are heritable cellular alterations that do not arise from permanent changes in the cell genome and which are not transmitted meiotically (Meins, 1983). Thus, in order to determine whether phenotypic variation in regenerated plants is genetic or epigenetic in nature requires the production of several sexual generations. If there is high sexual heritability for the variant traits over several generations, then the variation is likely to have a genetic basis. In the present study much of the general morphological variation present in the original plants had disappeared as early as the second generation (Table 7) indicating that part of the variation in the original plants did not have a genetic basis.

Traits with an underlying genetic basis can be of several types. They could be gene mutations inherited in a Mendelian manner, or they could be a result of chromosome alterations and instability. Sears *et al.* (1982) stated that

most somaclonal variation is a result of chromosome instability. Novel traits which are a result of chromosome instability are not likely to be of much value for crop improvement since heritability of the trait would likely be quite low. Furthermore, chromosomal instability is undesirable in commercial cultivars. In addition, plants exhibiting chromosomal instability usually express other undesirable morphological and/or physiological traits. That chromosomal instability did contribute to the observed somaclonal variation in the present study was shown by the fact that genotype R13, which was the only genotype significantly different from "Carman" in terms of chromosomal stability (Table 18), was the most variable genotype morphologically (Tables 13 and 14). All of the variability exhibited by genotype R13 is undesirable, especially the extremely low fertility.

All somaclonal variation is not a result of chromosome instability, however. Novel variants have been found in plants with an otherwise normal chromosome complement, eg. morphological abnormalities in sorghum and sugarcane (Gamborg et al., 1977; Liu and Chen, 1976). This variation is likely a result of gene mutation.

Ideally, for plant improvement the best result would be the regeneration of plants identical to the source plants except for a specific desirable trait. The tissue culture-derived plants resistant to Southern Corn Leaf Blight (Dixon et al., 1982) have come the closest to achieving this goal

among the cereal species. In the present study the plants which had heritably high kernel protein and which were non-significantly different from "Carman" in terms of chromosomal stability, may be of use in a breeding program for improved kernel protein in triticale. The genotypes which were similar to "Carman" for chromosome stability (Table 18) were generally also similar to "Carman" in terms of their morphological traits studied (Tables 13 and 14). This suggests that the high protein plants were unlikely to carry many undesirable traits and would be basically similar to "Carman" in all or most of their agronomic attributes. This would be highly advantageous from a breeding standpoint.

SECTION VI
SUMMARY AND CONCLUSIONS

The best system found for callus induction from "Carman" triticale embryos was the use of 15± day-old embryos cultured in the dark on Murashige and Skoog (MS) media with the addition of 20 gL^{-1} sucrose and 3 mgL^{-1} 2,4-D. Plant regeneration could be obtained by transferring the callus to MS media supplemented with 20 gL^{-1} sucrose in the absence of growth regulators, and by the use of a 16 hour daylength period.

The first generation of regenerated euploid plants were found to vary widely for several morphological traits while second generation plants exhibited somewhat less variability. Two families, R12 and R13, were found to have low fertility in the second generation. The low fertility of R13 was due to extensive chromosomal instability which was a result of an increased production of wheat univalents relative to "Carman" controls. Plants which had a level of chromosome instability significantly greater than that of "Carman" exhibited a high degree of morphological variability, while plants with chromosome instability approximating that of "Carman" were morphologically similar to "Carman".

All morphological traits studied in the second and third generations were affected significantly by the environment. The genetic component of variation was greatest for spike length, fertility and plant height, while variability for

the number of fertile heads per plant did not appear to have a genetic basis.

Two plants, R4-3 and R5-2, had a significantly higher kernel protein percentage than "Carman". This increase in kernel protein was heritable and at least partly due to an increased production of prolamin proteins. The increase in prolamin production was greatest in the electrophoretic bands coded for by the rye genome which indicates a possible alteration in rye prolamin regulatory genes.

The best tissue culture system for the utilization of somaclonal variation in cereal improvement would be one which would generate large numbers of plants morphologically similar to the source plants. Selection for genotypes showing either chromosome stability in culture, or the ability to screen out chromosome abnormalities during the regeneration process, would be beneficial in reducing the number of gross variants. These plants would then be extensively screened for desirable new characteristics. In order for such a system to be achieved, further research is necessary to improve the efficacy of regeneration from callus so that large populations of regenerates can be produced, thereby enabling studies on a field scale.

The present study has shown that somaclonal variation does exist in regenerated plants of "Carman" triticale and that much of the variation is genetic in origin. The production of two plants with high kernel protein, possibly a

result of changes in rye prolamin regulatory genes, illustrates that beneficial desirable traits can be produced from a tissue culture cycle and that such traits may be of significance for triticale improvement.

The chromosomal makeup of the regenerate plants is unknown except for the fact that the original regenerate plants had the euploid chromosome number. An intensive cytological study on the several generations of regenerate plants may shed some light on the reasons for the observed variation. Wheat-rye translocations and/or deletions of terminal heterochromatin may have occurred in these plants. A thorough study of the genetics of the high protein plants may also be useful. The increased protein content may be related to chromosome structural alterations in addition to any changes in regulatory genes which may have occurred.

More progeny of the high protein plants should be grown in order to judge their usefulness for triticale improvement. Quality studies would be useful in assessing the nutritional value of the increased protein. Field trials utilizing the high protein plants would be beneficial since any undesirable traits carried by these plants, such as changes in yield potential or disease resistance, could be detected. Field studies should also be conducted on progenies of the remaining regenerated plants as found in this study so that potentially desirable traits may be detected.

The exact cellular origin of the regenerated plants in the present study is unknown. If somatic embryogenesis has occurred, the regenerated plants can be said to have arisen from a single cell initial. On the other hand, if organogenesis was the basis of plant regeneration, the regenerated plants likely arose from several cell initials. If the different cell initials varied in their genetic makeup, then the resulting plants would be a mixture of several genetic backgrounds which would be undesirable if crop improvement was the ultimate goal of plant regeneration. Thus, it would prove beneficial to study the cellular origin of regenerated triticale plants.

The present study was based upon the progenies of only six euploid regenerate plants. An improved system for plant regeneration would be beneficial in producing a greater number of plants which would afford a better evaluation of the extent of the variability.

The present study serves as a preliminary investigation on heritable somaclonal variation in triticale. That such variation has been found to occur, may have implications for triticale improvement. Determining the ranges of variability which can be produced from triticale tissue culture systems, and establishing the nature of the genetic variability, would be the next logical step in assessing the usefulness of somaclonal variation for triticale improvement.

LIST OF REFERENCES

Ahloowalia, B.S. 1982. Plant regeneration from callus culture in wheat. Crop Sci. 22:405-410.

Association of Official Analytical Chemists 1980. Official methods of analysis (13th ed.). Association of Official Analytical Chemists, Washington, D.C. Sections 47.021 - 47.023, p858.

Bayliss, M.W. 1975. The effects of growth in vitro on the chromosome complement of Daucus carota (L.) suspension cultures. Chromosoma 51:401-411.

Bayliss, M.W. 1980. Chromosomal variation in plant tissues in culture. In: Perspectives in Plant Cell and Tissue Culture. I.K. Vasil (Ed.). Int. Rev. Cytol. Suppl. 11A.

Beckert, M., Pollacsek, M. and Caenen, M. 1983. Study of genetic variability in maize (Zea mays L.) after in vitro generation of plants from callus culture. (in French) Agronomie 3:9-18.

Bennici, A. and D'Amato, F. 1978. In vitro regeneration of durum wheat plants. I. Chromosome numbers of regenerated plantlets. Z. Pflanzenzuchtg. 81:305-311.

Brettell, R.I.S., Wernicke, W. and Thomas, E. 1980. Embryogenesis from cultured immature inflorescences of Sorghum bicolor. Protoplasma 104:141-148.

Browers, M.A. and Orton, T.J. 1982. Transmission of gross chromosomal variability from suspension cultures into regenerated celery plants. J. Hered. 73:159-162.

Brown, J.W.S. and Flavell, R.B. 1981. Fractionation of wheat gliadin and glutenin subunits by two-dimensional electrophoresis and the role of group 6 and group 2 chromosomes in gliadin synthesis. Theor. Appl. Genet. 59:349-359.

Bushuk, W. and Zillman, R.R. 1978. Wheat cultivar identification by gliadin electrophoregrams I. Apparatus, methods and nomenclature. Can. J. Plant Sci. 58:505-515.

Carew, D.P. and Schwarting, A.E. 1958. Production of rye embryo callus. Botan. Gaz. 119:237-239.

Carlson, P.S. and Polacco, J.C. 1975. Plant cell cultures-genetic aspects of plant improvement. Science 188:622-625.

Carter, O., Yamada, Y. and Takahashi, E. 1967. Tissue culture of oats. Nature 214:1029-1030.

Chen, C.H. and Buskuk, W. 1970. Nature of proteins in triticale and its parental species III. A comparison of their electrophoretic patterns. Can. J. Plant Sci. 50:25-30.

Cummings, D.P., Green, C.E. and Stuthman, D.D. 1976. Callus induction and plant regeneration in oats. Crop Sci. 16:465-470.

D'Amato, F. 1977. Cytogenetics of differentiation in tissue and cell cultures. In: Applied and Fundamental Aspects of Plant Cell, Tissue, and Organ Culture. J. Reinert and Y.P.S. Bajaj (Eds.) Sprinter Verlag, New York, pp 343-357.

Dale, P.J. and Deambrogio, E. 1979. A comparison of callus induction and plant regeneration from different explants of Hordeum vulgare. Z. Pflanzenphysiol. 94:65-77.

Darvey, N.L. and Gustafson, J.P. 1975. Identification of rye chromosomes in wheat-rye addition lines and triticale by heterochromatin bands. Crop Sci. 15:239-243.

Davies, D.R. 1981. Cell and tissue culture: potentials for plant breeding. Phil. Trans. R. Soc. Lond. B 292:547-556.

Dixon, L.K., Leaver, C.J., Brettell, R.I.S. and Gengenbach, B.G. 1982. Mitochondrial sensitivity to Drechslera maydis T-toxin and the synthesis of a variant mitochondrial polypeptide in plants derived from maize tissue cultures with Texas male-sterile cytoplasm. Theor. Appl. Genet. 63:75-80.

Dudits, D., Nemet, G. and Haydu, Z. 1975. Study of callus growth and organ formation in wheat (Triticum aestivum) tissue cultures. Can. J. Bot. 53:957-963.

Edallo, S., Zucchinali, C., Perenzin, M. and Salamini, F. 1981. Chromosomal variation and frequency of spontaneous mutation associated with in vitro culture and plant regeneration in maize. Maydica 26:39-56.

Feung, C., Hamilton, R.H. and Mumma, R.O. 1975. Metabolism of 2,4-dichlorophenoxyacetic acid VII. Comparison of metabolites from five species of plant callus tissue cultures. J. Agric. Fg. Chem. 23:373-376.

Flavell, R.B., Rimpau, J. and Smith, D.B. 1977. Repeated sequence DNA relationships in four cereal genomes. Chromosoma 63:205-222.

Gamborg, O.L., Shyluk, J.P., Brar, D.S. and Constabel, F. 1977. Morphogenesis and plant regeneration from callus of immature embryos of sorghum. Pl. Sci. Lett. 10:67-74.

Gamborg, O.L. and Eveleigh, D.E. 1968. Culture methods and detection of glucanases in suspension cultures of wheat and barley. Can. J. Biochem. 46:417-421.

Ghosh-Wackerle, G., Avivi, L. and Galun, E. 1979. Induction, culture and differentiation of callus from immature rachises, seeds and embryos of Triticum. Z. Pflanzenphysiol. 91:267-278.

Granatek, C.H. and Cockerline, A.W. 1978. Callus formation versus differentiation of cultured barley embryos: hormonal and osmotic interactions. In vitro 14:212-217.

Green, C.E. 1977. Prospects for crop improvement in the field of cell culture. Hort. Sci. 12:131-134.

Green, C.E. and Phillips, R.L. 1975. Plant regeneration from tissue cultures of maize. Crop Sci. 15:417-421.

Gupta, S. 1972. Tissue culture of Nigella sativa I. The behaviour of nucleus. Experientia 28:441-443.

Heinz, D.J., Krishnamurthi, M., Nickell, L.G. and Maretzki, A. 1977. Cell, tissue and organ culture in sugarcane improvement. In: Applied and Fundamental Aspects of Plant Cell, Tissue and Organ Culture. J. Reinert and Y.P.S. Bajaj (Eds.) Springer Verlag, New York, pp.3-17.

Hulse, J.H. and Laing, E.M. 1974. Nutritive value of triticale protein. International Development Research Centre, Ottawa.

Kaltsikes, P.J. 1974. Univalency in triticale. In: Triticale. Proc. Int. Symp. El Batan, Mexico. IDRC-024e, Ottawa, pp 159-167.

Kao, K.N., Miller, R.A., Gamborg, O.L. and Harvey, B.L. 1970. Variations in chromosome number and structure in plant cells grown in suspension cultures. Can. J. Genet. Cytol. 12:297-301.

Kasarda, D.D., Bernardin, J.E. and Qualset, C.O. 1976. relationship of gliadin protein components to chromosomes in hexaploid wheats (Triticum aestivum L.). Proc. Natl. Acad. Sci. U.S.A. 73:3646-3650.

Khan, K., McDonald, C.E. and Banasik, O.J. 1983. Polyacrylamide gel electrophoresis of gliadin proteins for wheat variety identification-procedural modifications and observations. Cereal Chem. 60:178-181.

King, P.J., Potrykus, I. and Thomas, E. 1978. In vitro genetics of cereals:problems and perspectives. Physiol. Veg. 16:381-399.

Larkin, P.J. and Scowcroft, W.R. 1981. Somaclonal variation -a novel source of variability from cell cultures for plant improvement. Theor. Appl. Genet. 60:197-214.

Lelley, T. 1974. Desynapsis as a possible source of univalents in metaphase I of triticale. Z. Pflanzenzuchtg. 73:249-258.

Lelley, T. 1975. Genic control of pairing of rye chromosomes in triticale. Z. Pflanzenzuchtg. 75:24-29.

Lelley, T. 1976. Induction of homoeologous pairing in wheat by genes of rye suppressing chromosome 5B effect. Can. J. Genet. Cytol. 18:485-489.

Lester, D.R. and Berbee, J.G., 1977. Within-clone variation among black poplar trees derived from callus culture. For. Sci. 23:122-131.

Liu, M-C. and Chen, W-H. 1976. Tissue and cell culture as aids to sugarcane breeding. 1. Creation of genetic variation through callus culture. Euphytica 25:393-403.

Lu, C., Vasil, I.K. and Ozias-Akins, P. 1982. Somatic embryogenesis in Zea mays L. Theor. Appl. Genet. 62:109-112.

Matthews, P.S. and Vasil, I.K. 1976. The dynamics of cell proliferation in haploid and diploid tissues of Nicotiana tabacum. Z. Pflanzenphysiol. 77:222-236.

McCoy, T.J., Phillips, R.L. and Rines, H.W. 1982. Cytogenetic analysis of plants regenerated from oat (Avena sativa) tissue cultures; high frequency of partial chromosome loss. Can. J. Genet. Cytol. 24:37-50.

McCoy, T.J. and Phillips, R.L. 1982. Chromosome stability in maize (Zea mays) tissue cultures and sectoring in some regenerated plants. Can. J. Genet. Cytol. 24:559-565.

Meins, F. 1983. Heritable variation in plant cell culture. Ann. Rev. Plant. Physiol. 34:327-346.

Mohandas, T. and Grant, W.F. 1972. Cytogenetic effects of 2,4-D and amitrole in relation to nuclear volume and DNA content in some higher plants. Can. J. Genet. Cytol. 14:773-783.

Nakamura, C. and Keller, W.A. 1982a. Callus proliferation and plant regeneration from immature embryos of hexaploid triticale. Z. Pflanzenzuchtg. 88:137-160.

Nakamura, C. and Keller, W.A. 1982b. Plant regeneration from inflorescence cultures of hexaploid triticale. Pl. Sci. Lett. 24:275-280.

O'Hara, J.F. and Street, H.E. 1978. Wheat callus culture:the initiation, growth and organogenesis of callus derived from various explant sources. Ann. Bot. 42:1029-1038.

Ogura, H. 1976. The cytological chimeras in original regenerates from tobacco tissue cultures and in their offsprings. Jap. J. Genet. 51:161-174.

Ogura, H. 1978. Genetic control of chromosome chimerism found in a regenerate from tobacco callus. Jap. J. Genet. 53:77-90.

Orton, T.J. 1980. Chromosomal variability in tissue cultures and regenerated plants of Hordeum. Theor. Appl. Genet. 56:101-112.

Ozias-Akins, P. and Vasil, I.K. 1982. Plant regeneration from cultured immature embryos and inflorescences of Triticum aestivum L. (wheat):evidence for somatic embryogenesis. Protoplasma 110:95-105.

Ozias-Akins, P. and Vasil, I.K. 1983. Callus induction and growth from the mature embryo of Triticum aestivum (wheat). Protoplasma 115:104-113.

Partaen, C.R. 1963. Plant tissue culture in relation to developmental cytology. Int. Rev. Cytol. 15:215-243.

Reisch, B. and Bingham, E.T. 1981. Plants from ethionine-resistant alfalfa tissue cultures: variation in growth and morphological characteristics. Crop Sci. 21:783-788.

Roupakias, D.G. and Kaltsikes, P.J. 1977. The effect of telomeric heterochromatin on chromosome pairing of hexaploid triticale. Can. J. Genet. Cytol. 19:543-548.

Saalbach, G. and Koblitz, H. 1978. Attempts to initiate callus formation from barley leaves. Pl. Sci. Lett. 13:165-169.

Sacristan, M.D. 1971. Karyotypic changes in callus cultures from haploid and diploid plants of Crepis capillaris (L.) Wallr. Chromosoma 33:273-283.

Sacristan, M.D. and Melchers, G. 1977. Regeneration of plants from 'habituated' and 'Agrobacterium-transformed' single-cell clones of tobacco. Mol. Gen. Genet. 152:111-117.

Scheunert, E-U., Shamina, Z.B. and Koblitz, H. 1978. Karyological features of barley callus tissues cultured in vitro. Biologia Pl. 20:305-308.

Sears, R.G., Gao, M.W., Guenzi, A.C., Hatchett, J.H. and Browder, L.E., 1982. Somaclone variation in wheat. Agronomy Abstracts 1982.

Sears, R.G. and Deckard, E.L. 1982. Tissue culture variability in wheat: callus induction and plant regeneration. Crop Sci. 22:546-550.

Sharma, G.C., Bello, L.L. and Sapra, V.T. 1980. Genotypic differences in organogenesis from callus of ten triticale lines. Euphytica 29:751-754.

Sharma, G.C., Bello, L.L., Sapra, V.T. and Peterson, C.M. 1981. Callus initiation and plant regeneration from triticale embryos. Crop Sci. 21:113-118.

Shepherd, K.W. and Jennings, A.C. 1970. Genetic control of rye endosperm proteins. Experientia 27:98-99.

Shimada, T., Sasakuma, T. and Tsunewaki, K. 1969. In vitro culture of wheat tissues. I. Callus formation, organ redifferentiation and single cell culture. Can. J. Genet. Cytol. 11:294-304.

Smith, S.M. and Street, H.E. 1974. The decline of embryogenic potential as callus and suspension cultures of carrot (Daucus carota L.) are serially subcultured. Ann. Bot. (London) 38:223-241.

Sunderland, N. 1973. Nuclear cytology. In: Plant Tissue and Cell Culture. H.E. Street (Ed.) Oxford:Blackwell pp. 161-190.

Sunderland, N. 1977. Nuclear cytology. In: Plant Tissue and Cell Culture. H.E. Street (Ed.) Second Edition. Univ. of California Press, Berkeley. pp.177-205.

Thomas, J.B. and Kaltsikes, P.J. 1974. A possible effect of heterochromatin on chromosome pairing. Proc. Nat. Acad. Sci. (U.S.A.) 71:2787-2790.

Torrey, J.G. 1961. Kinetin as a trigger for mitosis in mature endomitotic cells. Exp. Cell Res. 23:281-299.

Vasil, V. and Vasil, I.K. 1981. Somatic embryogenesis and plant regeneration from tissue cultures of Pennisetum americanum and P. americanum x P. purpureum hybrid. Amer. J. Bot. 68:864-872.

Waines, J.G. 1973. Chromosomal location of genes controlling endosperm protein production in Triticum aestivum cv. "Chinese Spring". Proc. Fourth Int. Wheat Genet. Symp. Columbia. E.R. Sears and L.M.S. Sears (Eds.) University of Missouri Press. pp873-877.

Wang, D-Y. and Vasil, I.K. 1982. Somatic embryogenesis and plant regeneration from inflorescence segments of Pennisetum purpureum Schum. (napier or elephant grass). Pl. Sci. Lett. 25:147-154.

Williams, L. and Collin, H.A. 1976. Growth and cytology of celery plants derived from tissue cultures. Ann. Bot. (London) 40:333-338.

Yajima, Y., Yasuda, T. and Yamada, Y. 1975. Solubilization of Jerusalem artichoke tuber chromatin by 2,4-dichlorophenoxyacetic acid. Phytochemistry 14:1939-1943.

Zamora, A.B. and Scott, K.J. 1983. Callus formation and plant regeneration from wheat leaves. Pl. Sci. Lett. 29:183-189.

Zillinsky, F.J. 1974. Improving seed formation in triticales. In: Triticale: Proc. Int. Symp. El Batan Mexico. IDRC-024e, Ottawa. pp.155-157.