THE UNIVERSITY OF MANITOBA

A STUDY OF THE APPLICATION OF NUCLEAR-CYTOPLASMIC RELATIONSHIPS TO THE IMPROVEMENT OF HEXAPLOID TRITICALE

by

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To

My Parents

U Hsam and Nang Bo Keing

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PREFACE

This thesis is divided into five Sections. The first Section deals with the synthesis of the required genetic stock, and the evaluation of their morphological and other agronomic attributes. The second Section is concerned with the comparative studies of genetically identical triticales synthesized with either hexaploid or tetraploid wheat cytoplasm, with reference to cytology, fertility and seed quality. The third Section deals with the interrelationships among agronomic characters. In the fourth Section, biochemical and nutritional properties of triticales as influenced by source of wheat cytoplasm are evaluated, while the fifth Section concerns the quantitative relationships of RNA, protein and histone at the cellular level.

The Sections are written in the editorial style of the Canadian Journal of Genetics and Cytology, and slightly condensed forms are intended for publication in that journal. The main advantage of the format is that it will eliminate the time lapse between the preparation of thesis and the preparation of scientific papers resulting from the investigation.

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ABSTRACT

HSAM, SAI LONG KYIO, Ph.D. The University of Manitoba, February 1974. A study of the application of nuclear-cytoplasmic relationships to the improvement of hexaploid triticale. Major Professor: Dr. E. N. Larter, Department of Plant Science.

A study was conducted on genotypically identical triticale amphiploids differing only in their cytoplasmic source. The F_1 lines employed in this study were obtained from reciprocal plant-to-plant crosses between C_1 (i.e. seeds produced from colchicine-treated hybrids) amphiploids which possessed either hexaploid wheat (Triticum aestivum L. em. Thell., cvs. Manitou and Pitic) or tetraploid wheat (T. turgidum L. vars. turgidum, durum and orientale) cytoplasm. C_2 populations produced from selfed C_1 amphiploid parents were also evaluated.

Triticales possessing hexaploid-wheat cytoplasm were cytologically more stable and exhibited fewer irregularities at meiosis as compared with their counterparts synthesized with tetraploid-wheat cytoplasm. Likewise, results on pollen viability, fertility, yield, and also kernel quality as measured by seed density and alpha-amylase enzyme activity, indicated the consistent superior performance of triticale synthesized with hexaploid-wheat cytoplasm.

A quantitative analysis of amino acid composition of mature kernels, indicated that triticales with hexaploid-wheat cytoplasm had higher amounts of essential amino acids (lysine, histidine, threonine and valine) than genotypically identical triticales with tetraploid-wheat cytoplasm.

In contrast, triticales with tetraploid-wheat cytoplasm had higher amounts of non-essential amino acids (glutamic acid and proline) than those with hexaploid-wheat cytoplasm. A study using sodium-dodecyl-sulfate polyacrylamide gel electrophoresis indicated qualitative as well as quantitative differences in the production of albumins and globulins or enzyme-protein fraction. Higher molecular weight protein subunits ($>$ 34,000 daltons) appeared to be preferably synthesized in triticale with hexaploid-wheat cytoplasm than in those with tetraploid-wheat cytoplasm. Differences in the production of gliadin and reduced glutenin between the reciprocal triticale populations were quantitative.

Microphotometric methods revealed higher levels of total cellular RNA and total cellular protein in triticales with hexaploid-wheat cytoplasm than those with tetraploid-wheat cytoplasm. Triticales with tetraploid-wheat cytoplasm had higher level of nuclear histone than those with hexaploid-wheat cytoplasm.

Factor analysis studies of the interrelationships among agronomic characters, revealed some differences in the inter-character associations between the two triticale populations synthesized with either hexaploid or tetraploid wheat cytoplasm.

Evidence is cited from previous studies which demonstrate the likelihood that beneficial evolutionary changes of cytoplasm have accompanied the evolution of hexaploid wheat, resulting in harmonious co-existence between cytoplasm and what was at one time, an alien D-genome in the hexaploid nucleus. The relevance of these changes to the current problems in triticale development is discussed.

LITERATURE REVIEW

The primary topic of this thesis is concerned with a study of the nucleo-cytoplasmic relationships in triticale (X Triticosecale Wittmack) and their application to the improvement of this potentially new crop species. Therefore, the following section is devoted to a review of the current status of triticale with reference to certain aspects thought most likely to be influenced by nucleo-cytoplasmic compatibility, and also to the role of cytoplasm in inheritance.

1. Triticale — its current status

a) Ploidy level of triticale

Triticale is a man-made species derived from crossing species of wheat (Triticum sp.) and diploid rye (Secale sp.). The name triticale has been coined from a prefix of Triticum and a suffix of Secale (Riley and Chapman, 1957; Briggle, 1969). The F_1 hybrids between wheat and rye are sterile unless the chromosome number is doubled to produce an amphidiploid (Bell, 1950).

Two polyploid forms can commonly be produced in triticale. The octoploid triticales ($2n = 8x = 56$, genomically AABBDDRR) are derived from crosses between hexaploid wheat (T. aestivum L. em. Thell.) and diploid rye. Likewise a hexaploid triticale ($2n = 6x = 42$, genomically AABBRR) results from hybridizing any of the wheat cultivars belonging to the tetraploid species T. turgidum L. with any of the diploid species

of rye. The amphiploids so produced are termed "primary" triticales, in contrast to the so-called "secondary" triticales which are obtained by recombination either between two or more primary triticales (Kiss, 1966); or between octoploid and hexaploid triticales (Kiss, 1966; Pissarev, 1966). To-date, production of primary tetraploid triticale (AARR) has not been successfull (Larter, et al., 1968; Zillinsky and Borlaug, 1971). However more recently, Krolow (1973) provided results on tetraploid triticales which were obtained by hybridizing a hexaploid triticale with rye, followed by selection among the progeny of the F_1 hybrid (ABRR). At this point in time, the true identity of selection among the possible genomes involved (AARR, BBRR, and mixed AB genomes and RR) has not been clearly defined.

The improvement of octoploid triticales had been carried out mainly in Sweden (Müntzing, 1939, 1957, 1966; Müntzing, et al., 1963). However, available evidence indicates that the optimum chromosome number of triticale is at the hexaploid level and accordingly, increased emphasis had been placed on this particular type (Sánchez-Monge, 1959; Kiss, 1966; Krolow, 1966; Larter, 1968; Larter and McGinnis, 1970). At present, the most intensive breeding programs geared to develop hexaploid triticale as a new field crop are those being conducted at the University of Manitoba in Canada, the Kecskemet Vegetable Growing Institute in Hungary, and the Centro de Mejoramiento de Maiz y Trigo (CIMMYT) in Mexico (Larter, et al., 1968; Zillinsky and Borlaug, 1971; and Larter, personal communication).

b) Improving cytological stability and fertility in triticale

It is known that varying degrees of cytological instability exists in hexaploid triticale (Sánchez-Monge, 1959; Nakajima and Zennyozi, 1966; Krolow, 1966). The level of such instability varies with genetic background and the number of generations removed from the original hybrid state (Larter, et al., 1968). Aneuploidy occurred in varying frequencies in the bulk population and even in progenies of 42-chromosome (euploid) plants in hexaploid triticale (Nakajima, 1953, 1965; Nakajima and Zennoyozi, 1966; Krolow, 1966; Tsuchiya, 1968; Tsuchiya and Larter, 1969, 1971; Merker, 1971).

The frequency of aneuploidy in turn, appears to depend on the level of meiotic instability, which includes among others, incomplete pairing and premature desynapsis of bivalents (Müntzing, 1957; Sánchez-Monge, 1959; Riley and Bell, 1959; Larter, et al., 1968; Thomas and Kaltsikes, 1972). Shigenaga, et al. (1971), Larter and Shigenaga (1971), and more recently Merker (1973) have demonstrated that both wheat and rye chromosomes contributed to the anomalous behaviour in hexaploid triticale, not only chromosomes of rye as previously believed (Müntzing, 1939, 1957; Riley and Chapman, 1957; Riley and Bell, 1959; Sánchez-Monge, 1959; Larter, et al., 1968; Tsuchiya, 1970; Shkutina and Khvostova, 1971). However, in octoploid triticale the main contribution to the high frequencies of aneuploids is made by rye chromosomes. This is known from the fact that some lines tend to eliminate the rye chromosomes and revert

to hexaploid wheat (Müntzing, 1957; Shkutina, et al., 1967). This has been confirmed by Pieritz (1970) who made a cytological analysis of aneuploids in two lines of octoploid triticale. He found that in a large majority of the aneuploids, rye chromosomes were involved. Merker (1973) suggested that on the octoploid level there is antagonism between genomes, and in extreme cases resulting in the total elimination of the rye genome. However, in hexaploid triticale no case of elimination of the rye genome and reversion to tetraploid wheat is known, which suggests a better harmony between the genomes at this ploidy level.

Primary triticales are characterized by their partial fertility which has been observed in both the hexaploid and octoploid types (Müntzing, 1939; O'Mara, 1953; Larter, et al., 1968). The reason for such infertility is not yet fully understood. Müntzing (1939), and Muntzing, et al. (1963), observed the meiotic chromosome behaviour of octoploid triticales to be highly irregular and suggested that this in part was the cause of sterility. Moreover, as aneuploids resulting from meiotic instability are of lower fertility than euploids (Riley, 1955; Larter, 1968) they limit yield. Hence it has generally been proposed that an improvement in the meiotic behaviour of triticale would enhance fertility (Müntzing, 1939, 1957, 1963; Sanchez-Monge, 1959). However there is evidence that cytological instability and low fertility are two unrelated phenomena in both hexaploid and octoploid triticales (Riley and Chapman, 1957; Merker, 1971). Similarly, Boyd, et al. (1970), and

Sisodia, et al. (1970), observed that the frequency of meiotic abnormalities was not necessarily reflected in a reduced fertility level. These results support the earlier conclusions reached by Riley and Bell (1959) that among a number of artificially synthesized amphiploids, there was little relationship between fertility and the frequency of univalent and/or multivalent formation. More recently, Hsam and Larter (1973a), showed that there was no statistical association between seed-set and certain meiotic instability variables including the frequencies of univalents, rod bivalents at metaphase I, lagging and excluded chromosomes at telophase I, and micronuclei at the quartet stage.

In a later study, Hsam and Larter (1973b) found that the correlation between meiotic stability and seed-set depends on the levels of these two variables themselves. It appears that a relationship between these two variables exist when both meiotic stability and seed-set are usually low as in newly synthesized amphiploids, or in early generation hybrids. Conversely, no relationship may exist between these variables as meiosis becomes more stable, and seed-set improves as a result of selection. Furthermore Hsam and Larter (1973a) using factor analysis, a multivariate statistical procedure, showed that meiotic instability and fertility are influenced by different causal factors which implied that improving one variable would not concomitantly improve the other.

Thus, from the available evidence, it appears that selecting simultaneously for both meiotic stability and fertility in the early phase of

a triticale breeding program; and for each character separately in advanced lines, would be a rapid and economical method for improving the cytological stability and fertility of hexaploid triticales.

c) Improving seed type in hexaploid triticale

One of the major factors limiting the potential of hexaploid triticale has been poor seed characteristics. Shrivelling of the seed results in low seed weight relative to size. Grain yields are therefore depressed accordingly (Sánchez-Monge, 1959; Larter, 1968; Zillinsky and Borlaug, 1971). Larter, et al. (1968) has reported that any one of the species T. timopheevi, T. persicum or Secale montanum, when included in the parentage of a triticale hybrid, transmit genes for desirable kernel characteristics to the progeny. Unfortunately, some undesirable plant characteristics may accompany the transfer of beneficial ones, viz. male sterility from T. timopheevi, or extreme lateness and chromosome anomalies from S. montanum. Considerable variation in kernel shrivelling does exist however (Müntzing, 1966; Larter, 1968; Zillinsky and Borlaug, 1971), and selection for plumpness is possible.

However, it is only recently that more effort was devoted to identifying the various factors which might be responsible for poor seed development. Klassen, et al. (1971) studying eight triticale lines observed that those with the highest alpha-amylase activities also exhibited the poorest seed type. From an overall consideration of Klassen's (1970) results, poor seed development appears to be the

results of abnormalities in starch synthesis together with some starch breakdown at the latter stages of growth, due to increased levels of alpha-amylase enzyme. However, it appears shrivelling cannot be wholly attributed to the degradative action of amylase, as visual indications of shrivelling were already evident, days before any enzyme activity could be detected (Klassen, 1970). Shealy and Simmonds (1973) using both light and electron microscopic investigations, observed that poor seed development could arise as a result of invaginations or deletions that occur in the aleurone layer of the seed, resulting in subsequent malformation of adjacent endosperm tissue. A cytological study by Kaltsikes (1973) indicated that the rate of endosperm development and especially the disintegration of the antipodal complement appeared to be positively related with the amount of seed shrivelling observed in mature seeds among the five lines he studied. Darvey (1973) analysing rye chromosome addition lines in hexaploid wheat, as well as monosomic analysis of the wheat species, reported that the degree of seed shrivelling caused by individual wheat or rye chromosomes were different. Furthermore, investigations conducted at the University of Manitoba (Klassen, 1970; Kaltsikes and Larter, 1970) have shown that the majority of shrivelled seeds are aneuploid. However, euploid seeds are also shrivelled, making it unlikely that aneuploidy per se is the reason for shrivelling.

The fact that conventional plant breeding methods have not completely overcome seed shrivelling thus far, suggests that the problem

is highly complex involving both genetic as well as genic-cytoplasmic interactions. Possibly, effects of a more general nature, such as could be ascribed to incompatibility between wheat-rye genome and wheat cytoplasm, could result in imbalances in total cellular function, resulting in asynchronous cell divisions or earlier cessation and/or failure of division. The cavities occurring in young endosperm tissue as observed by Klassen (1970) and Shealy and Simmonds (1973), as well as the early disintegration of antipodal cells in shrivelled seeds as observed by Kaltsikes (1973) might arise in this manner. In addition, the desirable changes in the cytoplasm as induced by mutation have produced improved seed type (Sánchez-Monge, 1968) which further suggests the importance of nuclear-cytoplasmic compatibility.

d) Biochemical and nutritional properties of triticale

The importance of cereals in the diet of man may be estimated from the fact that the world obtains approximately half of its protein from this source (Larter, 1969; Redman, 1971). The world production of wheat grain itself constitutes a major portion of the total production, hence it is not surprising that much of the research on proteins has been carried out with particular emphasis on wheat. In recent years however, triticale has been suggested as a potentially important food for humans in that the lysine content of triticale proteins has generally been found higher than that of wheat protein. The total protein level also compare favorably with that of either wheat or barley (Villegas, et al.,

1968, 1970; Larter, et al., 1968).

i) Endosperm proteins

Osborne in 1907 classified wheat proteins into four groups according to their solubility:

Albumin - soluble in water.

Globulin - soluble in dilute salt solution.

Gliadin - soluble in 70% ethanol solution.

Glutenin - soluble in dilute acid or alkaline solution.

The same classification is also being applied in triticale (Chen, 1969). As with all solubility classification schemes there is overlap between the fractions. Thus if flour is successively extracted with large volumes of water, gliadins themselves may be extensively removed due to the fact that they are slightly soluble in water (Chen and Bushuk, 1969b; Redman, 1971). Globulins (Simmonds, personal communcation) may also be slightly or partially extracted with water as salt normally present in the grain will form a solution with water. However, if fairly well defined procedures are followed the four groups may be obtained relatively free from one another and still with their own distinctive properties.

Chen and Bushuk (1969a) reported that the solubility distribution of proteins in one triticale line (University of Manitoba Accession, 6A 190) was intermediate between those of its durum wheat and rye parents (Triticum turgidum L. var. durum cv. Stewart 63, and Secale cereale L.

cv. Prolific respectively). However, a comparison between triticale and a Canadian hard red spring wheat (T. aestivum cv. Manitou), indicated the former to contain significantly more water-soluble protein and less of the insoluble or residue protein. Accordingly, the proteins of triticale flour forms less gluten than those of Manitou.

In recent years, ion exchange techniques, gel filtration and gel electrophoresis have increased our knowledge of protein fractions. Protein of the albumin and globulin classes include the enzyme proteins, of which the amylases and proteases are particularly important from a technological aspect. These fractions probably contain several hundred enzymes, mostly in minute quantities (Redman, 1971). In wheat, both β-amylase (Tipples and Tkachuk, 1965) and α-amylase (Kruger and Tkachuk, 1969) have been characterized to exist in multiple forms. Triticale α-amylase is currently being investigated in the Department of Plant Science (Noll, personal communication). Chen and Bushuk (1969b) reported that triticale protein molecules were 10,000 - 28,000, and 8,000 - 28,000 daltons for the water-soluble and salt-soluble fractions respectively. In addition, the above authors also observed a high molecular weight (MW) fraction of over 150,000 daltons in the salt-soluble extracts. It was suggested that this could well be globulins since molecular weights for this group of wheat proteins as high as 200,000 have been reported (Danielsson, 1949).

Traditionally, the residual proteinaceous material which is left

after a dilute salt solution extraction is termed gluten. Osborne (1907) reported that wheat gluten comprised about 80% of the flour proteins, and was composed mainly of gliadin and glutenin which were present in approximately equal amounts. Furthermore, each was composed of many different molecular species. The viscoelastic properties of dough are thought to arise from both the structure and interactions of these proteins (Bietz and Wall, 1972). Most gliadin proteins have MW's of 16,000 to 50,000 and contain single polypeptide chains whose conformations are stabilized by intramolecular disulfide bonds (Woychik, et al., 1964; Nielson, et al., 1968; Huebner and Rothfus, 1968; and Bietz and Wall, 1972). Glutenin is very heterogenous in molecular size and consists of subunits of MW's of 20,000 to 100,000 linked through intermolecular disulfide bonds into proteins with MW's of 50,000 to 2,000,000 or more (Nielson, et al., 1962; Woychik, et al., 1964; Beckwith, et al., 1966; Crow and Rothfus, 1968; Bietz and Wall, 1972). Recent studies also suggest that glutenin may be a polymer of gliadin, together possibly with other molecules (Lee, 1968; Bietz and Rothfus, 1970).

In a hexaploid triticale line (6A 190) Chen and Bushuk (1969b; 1969c), using the polyacrylamide gel technique showed that all the bands of the triticale proteins were present in the patterns of either the rye or the durum parent. This was in variance with Yong and Unrau (1964) who, using starch gel technique, found several "new" protein bands in triticale extracts.

Contradicting electrophoretic results were also obtained in octoploid triticale. Johnson and Hall (1965) obtained the same starch gel patterns for protein extract from the amphiploid triticale and extract from a mixture of the two parents. On the other hand, Barber, et al. (1968) detected a "new" esterase band in another octoploid triticale that was not present in the patterns of the parents.

ii) Amino acid composition

Amino acid compositions of triticale and its parental species were published by Yong and Unrau (1966), and Chen and Bushuk (1969a). Both groups found that the content of most of the amino acids in triticale was intermediate between those of its parents, durum wheat and rye. Results of Yong and Unrau (1966), showed that triticale contained more leucine and isoleucine than either parent. This observation was not confirmed by Chen and Bushuk (1969a). The two studies referred to above showed that the content of lysine in triticales was intermediate between the values for the rye and durum parents. The values of lysine for the hexaploid wheat used as controls in these studies were significantly lower than the triticale values.

iii) Nutritional quality of triticales

Early studies at the University of Manitoba with chicks and turkeys (Sell, et al., 1962; Sell and Johnson, 1969; Bragg and Sharby, 1970) indicated that triticale was equal to wheat in its nutritional properties.

In contrast, Bixler, et al. (1968) and Guenthner and Carlson (1970) found that the nutritive value of triticale for chicks was below that of wheat and corn.

Studies with swine (Stothers and Shebeski, 1965; Harrold, et al., 1971) showed that triticale seemed to be significantly less palatable than wheat, and this was shown to be related to the high incidence of ergot (Sauer, 1972). Sauer also determined the availabilities of the amino acids in barley, soybean meal, triticale and wheat. He found that the true availabilities vary for the essential amino acids. Lysine was the least available (approximately ranging from 65 to 87%), arginine, histidine and phenylalanine were the most available (approximately ranging from 90 to 95%); and isoleucine, methionine, threonine, valine and leucine were intermediates. However, in all cases triticale grain was comparably favorable to other grains. Of the non-essential amino acids, alanine was the least available and glutamic acid, proline and serine were the most available for all four grains tested.

Nutritive value of triticale for humans was evaluated by extensive studies at the University of Nebraska (Kies and Fox, 1970a, 1970b). Studies showed that triticale protein had a slightly higher nutritional value than wheat protein. Although earlier studies by Kies and Fox (1970a, 1970b) showed that lysine was the limiting factor in triticale as well as in wheat and other cereal grains, recent results from advanced testing of triticale lines at the University of Manitoba demonstrated a mean lysine content (g/100 g protein) of 3.55 (ranging between

2.73 - 4.56) as compared to hexaploid wheat cultivars Neepawa (2.90) and Glenlea (2.81). Selection therefore, has raised the level of lysine in these triticale lines to a point, where this amino acid may no longer be a limiting factor (Larter, personal communication).

Elliott (cited by Zillinsky and Borlaug, 1971) using the meadow vole Microtus pennsylvanicus to determine relative nutritive values of breeding lines of triticale, has shown that considerable diversity exists in the protein efficiency ratio (PER) with values approaching zero to values comparable to that of egg protein. Currently feeding trials with voles, and mice are being conducted at the University of Manitoba, Canada, to evaluate the effects of resorcinol levels contributed by the rye parents. The available results indicate that the high level of resorcinols in diets did not appear to affect weight gain of inbred strains of young white mice. Moreover the high level of resorcinols (0.1%) in triticale did not affect their palatability or appear to cause fatality (Zillman, personal communication). These findings have further enhanced the possibility of hexaploid triticale becoming an established crop species of importance, especially if plant breeders are successful in producing cultivars with high nutritional value.

2. The role of cytoplasm in inheritance

a) Nucleo-cytoplasmic interactions

The cell is the unit of life in which nucleus and cytoplasm form a self-perpetuating reaction system (Michaelis, 1958). Undoubtedly the

nucleus is the seat of genetic information and is of prime importance. On the other hand, expression of this genetic plan occurs principally in the cytoplasm (Bonner, 1959). Hence, they are interrelated making the whole genetic system complete. It is now well established that genetic information is conserved in DNA base sequences. Thus the demonstration of the existence of chloroplast DNA (Chun, Vaughan, and Rich, 1963; and Sager and Ishida, 1963) and subsequently of mitochondrial DNA (Luck and Reich, 1964), elucidates new perspectives on the role of cytoplasm in inheritance.

However, the realization that cytoplasm plays an important role in the control of gene expression is not new. It was decades ago since Hämmerling in 1926 pioneered the investigation of nucleo-cytoplasmic relationships. Since then, evidence has accumulated from studies of enucleate cells (Hämmerling, 1934, 1959, 1963; Harvey, 1940; Goldstein et al. 1960; Tartar, 1961; Brachet, 1961; and Gibor, 1965); nuclear transplantation experiments in animals (Briggs and King, 1957; Gurdon, 1966; Prescott and Goldstein, 1967; and Terra, 1969); cell fusion work (Harris, 1970a, 1970b); reciprocal effects in higher plants (Jinks, 1964; Crane and Nyquist, 1967; Muehlbauer, et al., 1971; Kohel and Benedict, 1972; Singh and Hadley, 1972; Jinks, et al., 1972; Christiansen and Lewis, 1973); as well as cytoplasmic male sterility in plants (see reviews by Chowdhury and Varghese, 1968; Edwardson, 1970; Laser and Lersten, 1972).

Within the Triticinae itself, the well documented studies involving cytoplasmic male sterility and fertility restoration, are prime examples

of the end results of manipulation of nuclear-cytoplasmic relationships (Wilson and Ross, 1962; Schmidt and Johnson, 1966; Kihara, 1968; Maan and Lucken, 1968, 1972; Maan, 1973).

b) Origin of hexaploid and tetraploid wheat cytoplasm

Within the Triticinae, there are five genera, Triticum L., Secale L., Aegilops L., Haynaldia Kanitz, and Agropyron J. Gaert. Nucleo-cytoplasmic interactions between species of the genera were studied mainly by substituting the genome of one species into the cytoplasm of another related species through backcrossing (Kihara, 1951; Fukasawa, 1953).

Several workers have suggested that a knowledge of cytoplasmic differences among the Triticum and Aegilops species may provide information regarding the donor of the B-genome and cytoplasm to the tetraploid and hexaploid wheats (Kihara, 1966; Maan and Lucken, 1967, 1968; Suemoto, 1968). Kihara (1966) suggested that the cytoplasm of hexaploid wheat (AABBDD) may have been derived from an emmer wheat (AABB) since he found that in reciprocal crosses between emmer wheat and Ae. squarrosa (DD), viable seeds were obtained only when emmer was employed as the female parent. Furthermore T. durum and T. aestivum plants with the cytoplasm from the A-genome donor (T. monococcum or T. boeoticum) were male sterile and lacked vigour (Maan and Lucken, 1967, 1968, 1970). Therefore the assumption was made that only the B-genome donor could have contributed cytoplasm to tetraploid and hexaploid wheats (Kihara, 1966; Maan and

Lucken, 1967, 1968).

Suemoto (1968) provided some evidence which indicates that both tetraploid and hexaploid wheat have cytoplasm of Ae. speltoides Tausch or its near relative. In contrast, Maan and Lucken (1971, 1972) reported that durum and T. aestivum in an autotetraploid Ae. squarrosa's cytoplasm were normal in fertility and plant vigour, which suggests squarrosa as the possible cytoplasm donor. However, additional backcrosses (Maan, 1973) indicated that complete substitution of the durum genome into Ae. squarrosa cytoplasm resulted in the development of non-viable seeds. Seedlings could only be obtained by culturing embryos from plants which carried either a whole or telocentric chromosome from the D-genome. Kihara (1973) is of the opinion that the D-genome contains fertility restoring genes for Ae. squarrosa cytoplasm and presents evidence to suggest that the cytoplasm of squarrosa is different from that of emmer wheat.

More recently, Suemoto (1973) reported that the cytoplasm of Ae. speltoides appears to be more closely related to the cytoplasm of T. timopheevi than to the cytoplasm of emmer and common wheats. A review of the literature shows that T. durum or T. aestivum genomes have now been substituted into cytoplasms of the following species: T. monococcum, T. boeoticum, T. timopheevi, T. zhukowskyi, T. araraticum, T. dicoccoides, Ae. speltoides, Ae. squarrosa, Ae. bicornis, Ae. longissima, Ae. sharonensis, Ae. umbellulata, Ae. caudata, Ae. heldreichii, Ae. variabilis, Ae.

ovata, Ae. cylindrica, and S. cereale. According to Maan (1973) sixteen distinct cytoplasms have been demonstrated among these species of which hexaploid and tetraploid wheat apparently possessed the same or similar cytoplasmic source.

c) Importance of hexaploid wheat cytoplasm in hexaploid triticale breeding

Of the two common forms of triticale, the hexaploid and the octoploid, the former is more desirable in terms of field performance (Larter, et al., 1968; Zillinsky and Borlaug, 1971). However, best results in the improvement of 6x triticale are obtained when 6x wheat germ plasm is introduced either by way of 8x triticale x 6x triticale crosses (Pissarev, 1966; Kiss, 1966), or 6x wheat x 6x triticale crosses (Jenkins, 1969) from which 6x triticale derivatives (secondary hexaploid triticales) are ultimately isolated. Pissarev (1966) obtained better seed-set (52%) and seed development in 8x triticale x 6x triticale crosses than in the reciprocals (14.9%). Kiss (1966) reported similar results.

Sisodia and McGinnis (1970) hypothesized that in an established species (such as 4x and 6x wheats) the cytoplasmic nuclear ploidy ratio would be in equilibrium (i.e. 1:1 C:N ploidy ratio). Considering the origin of both the tetraploid and hexaploid wheats, it may be expected that with an increase in nuclear ploidy, the cytoplasm would also adjust accordingly during the course of thousands of years of evolution. Although the cytoplasm of hexaploid wheat is assumed to be of tetraploid

origin (Kihara, 1966, 1968), it remains clear that it has become modified through evolution to co-exist harmoniously with the additional genome contributed by Ae. squarrosa (see Larter and Hsam, 1973 for discussion). However, in a newly synthesized species such as triticale, a harmonious cytoplasmic nuclear relationship may not be obtained, as the source of cytoplasm is entirely dependent upon the female parent being used. Thus a turgidum-triticale and aestivum-triticale would possess a C:N ploidy ratio of 4:6 and 6:8 respectively. It is assumed that the more divergent the C:N ploidy ratio is from 1:1, the more chance there will be of a disturbed nuclear-cytoplasmic relationship. On this basis, secondary hexaploid triticale derived from an 8x x 6x triticale cross would be expected to have a balanced C:N ploidy ratio, and thus perform more efficiently than triticales with an imbalanced C:N ploidy ratio.

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SECTION I

INFLUENCE OF SOURCE OF WHEAT CYTOPLASM ON THE SYNTHESIS AND PLANT CHARACTERISTICS OF HEXAPLOID TRITICALE

INTRODUCTION

Since the earliest published report of an artificially produced wheat-rye hybrid by Wilson in 1875, plant breeders as well as cytologists have investigated various forms of triticale (Triticum sp. x Secale sp.). With the discovery in the mid-1930's, that the drug colchicine, acts as a polyploidizing agent when applied to living plant tissues, triticale research took on new emphasis. Attention was directed toward the synthesis of both the octoploid (AABBDDRR, $2n = 8x = 56$) and the hexaploid (AABBRR, $2n = 6x = 42$) forms (Müntzing, 1966; Larter et al., 1968). Accordingly, triticales so produced possessed hexaploid and tetraploid wheat cytoplasm respectively.

Triticale research in several countries during the last decade has indicated the intensified exploitation of the hexaploid forms. These appeared to be superior to octoploid forms from the standpoint of field performance and their ultimate potential as a commercial crop species (Kiss, 1966; Larter, et al., 1968; Zillinsky and Borlaug, 1971). However, from the standpoint of seed development in particular, the octaploid forms tend to produce a seed type superior to that of the hexaploids. Both Kiss (1966) and Pissarev (1966) suggested intercrossing the 8x and 6x triticales with the eventual isolation of stable hexaploid derivatives (secondary hexaploid triticale) as the best method of improving fertility and other agronomic attributes. Sisodia and McGinnis (1970) also speculated on the importance of hexaploid wheat cytoplasm

in improving the agronomic performance of triticale.

The objective of the present study was to evaluate the effect of hexaploid (6x) versus tetraploid (4x) wheat cytoplasm on various plant characteristics of genetically identical 6x triticale F_1 hybrids, and to assess the extent of nuclear-cytoplasmic interactions in these same hybrids.

MATERIALS AND METHODS

1. Synthesis of C_2 and reciprocal F_1 populations

The basic approach used in this study was first to synthesize triticale F_1 hybrids which were genetically identical, differing only in their cytoplasmic background depending upon whether they possessed either hexaploid or tetraploid wheat cytoplasm. To do this, advantage was taken of chromosomal elimination which takes place in selfed progenies of pentaploid ($2n = 5x = 35$) wheat hybrids produced from hexaploid-tetraploid wheat crosses (Fig. 2a). As shown in Fig. 1, the tetraploid (AABB) wheat parents used for the synthesis of the C_1 amphiploids were initially derived from reciprocal crosses between hexaploid wheat (Triticum aestivum L. em. Thell.) and tetraploid wheat (T. turgidum L.). Two hexaploid wheats (viz. cvs. Pitic and Manitou) and nine tetraploid species were used (Table 1). The pentaploid hybrids produced from these crosses were bagged and self-pollinated. In the F_2 generation, only plants with equal or less than 33 somatic chromosomes as determined from root-tip counts were grown which enhanced the frequency with which the tetraploid AABB components was obtained in the subsequent selfed generation (Fig. 2b). In the following generation only those tetraploid ($2n = 4x = 28$) individuals as verified on the basis of root-tip chromosome counts were saved for use as potential parents (Fig. 2c). At meiosis, these plants were again examined for bivalent formation and those exhibiting 14 bivalents were crossed with rye (Secale cereale L. cv. Centeno) to produce triticale

Figure 1

Diagram showing the synthesis of F_1 and C_2 triticale with either hexaploid or tetraploid wheat cytoplasm.

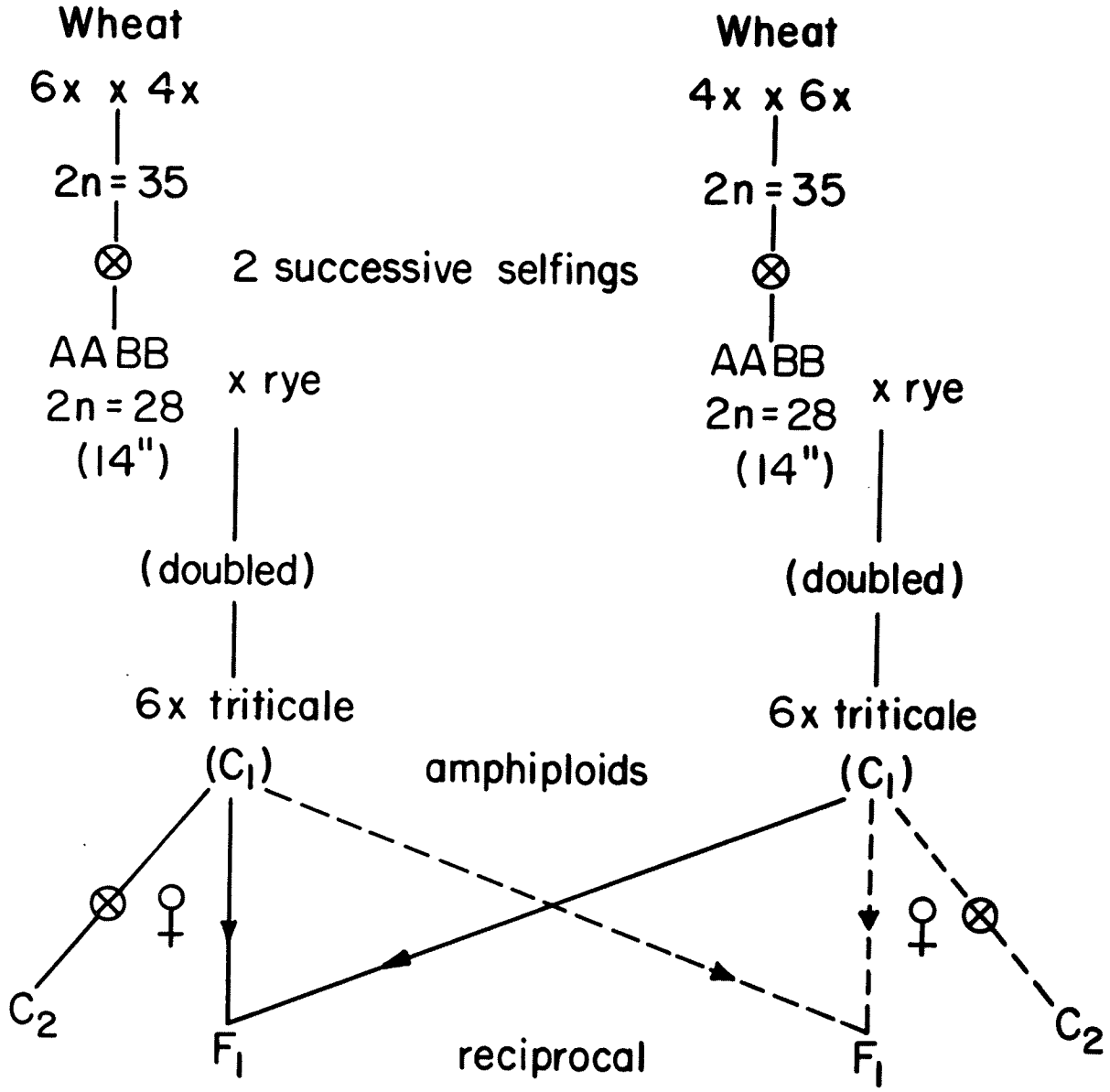

Wheat
6x x 4x
2n = 35
AABB
2n = 28
(14")
x rye
(doubled)
6x triticale
(C1)
2 successive selfings
amphiploids
C2
F1
reciprocal
Wheat
4x x 6x
2n = 35
AABB
2n = 28
(14")
x rye
(doubled)
6x triticale
(C1)
F1
C2

Table 1. Wheats and rye parents used in the synthesis of reciprocal triticales

Univ. Man. Plant Sci. Acc. No.	Species and cultivars		Source	Habit
	Botanical name	Common name		
-	*Triticum aestivum* L. em. Thell cv. Manitou	Common bread wheat	Canada	Spring
-	" cv. Pitic	" feed wheat	Mexico	"
-	*T. turgidum* L. var durum cv. Stewart	Durum wheat	Canada	"
4B 77	" " dicoccum (Schrank)	Emmer "	Percival's Coll. (1955)	"
4B 110	" " turgidum (L.)	Poulard wheat	"	"
4B 233	" " durum (Desf.)	Durum "	Tell-Amara (1957)	"
4B 242	" " orientale (Perc.) (turanicum-Jakubz.)	Khorasan wheat	U.S.D.A. (1955)	"
4B 254	" " polonicum (L.)	Polish "	"	"
4B 280	" " persicum (Vav.)	Persian "	"	"
4B 296	" " abyssinicum	-	Senn (1957)	"
4B 723	" " durum (Desf.)	Durum wheat	India (1961)	"
-	*Secale cereale* L. cv. Centeno	Rye	CIMMYT, Mexico	"

Figure 2

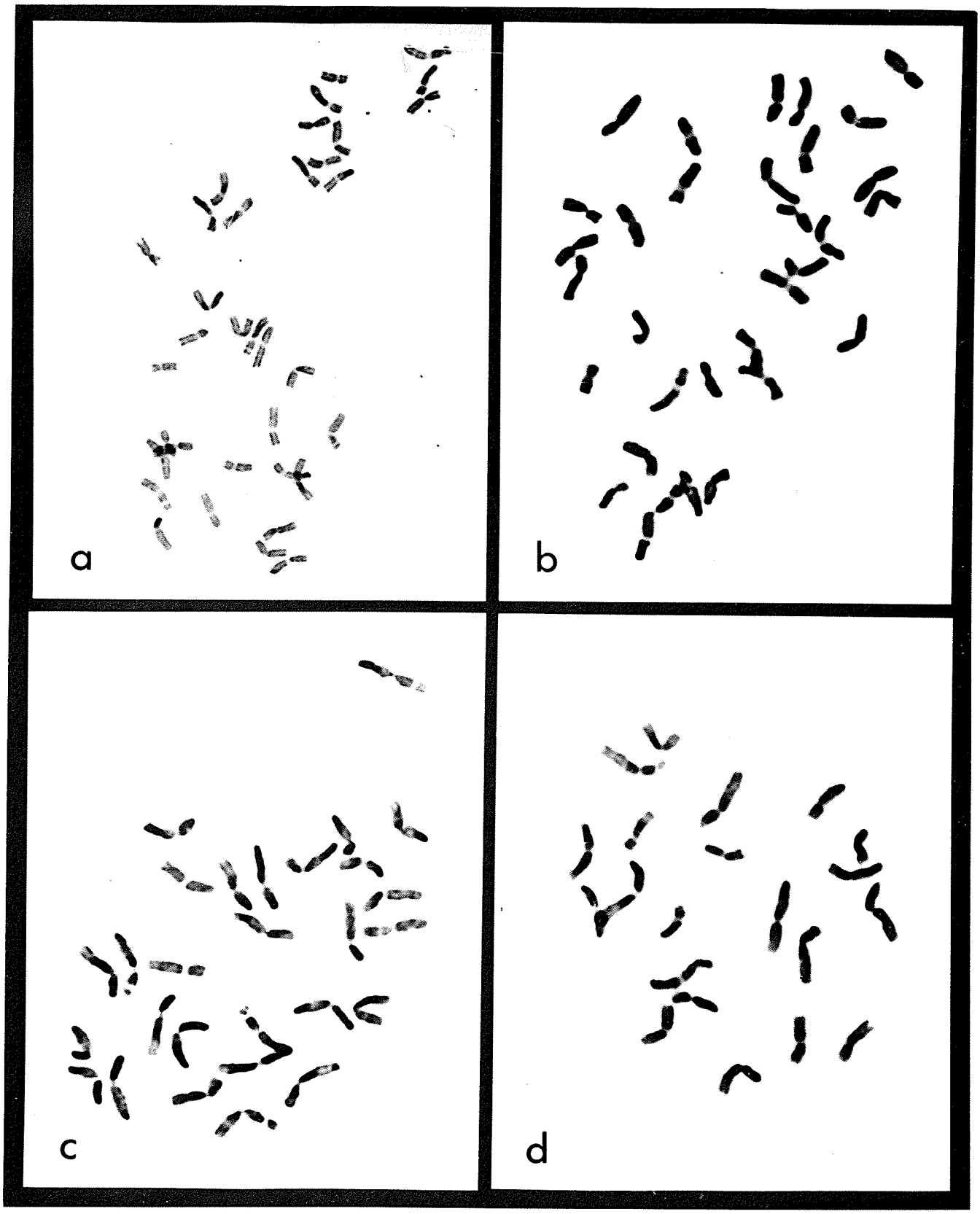
Somatic metaphase I configurations of hexaploid-tetraploid wheat, and wheat-rye hybrids.

(a) Hexaploid-tetraploid wheat F_1 hybrid ($2\underline{n} = 35$; x 970).

(b) Hexaploid-tetraploid wheat F_2 hybrid (a 32-chromosome plant; x 1680).

(c) Hexaploid-tetraploid wheat F_3 hybrid ($2\underline{n} = 28$; x 1680).

(d) Wheat-rye F_1 hybrid ($2\underline{n} = 21$; x 1680).


a
b
c
d

F_1 hybrids. Care was taken to thoroughly mix the pollen from at least 10 rye plants before pollination thereby minimizing the variance introduced by different rye gametes. Concurrently, test crosses between 14-bivalent derived tetraploids and respective tetraploid parents were made and F_1 hybrids were analysed for meiotic behaviour. Only those derived tetraploid parents which produced 14-bivalent hybrids were used as parents in the synthesis of F_1 triticales.

F_1 seeds were set readily but due to endosperm breakdown, embryo culture was necessary (Larter, 1968). Embryos were cultured when the "seeds" started to turn yellow (between fourteen to twenty days after pollination). Slants of 1.0% orchid agar were found to be a satisfactory medium when supplemented with 2 ppm. each of indoleacetic acid and kinetin (Fig. 3a-3c). The chromosome number of each established F_1 plant was verified as $2\underline{n} = 3\underline{x} = 21$ (Fig. 2d) and was subsequently doubled. The doubling treatment consisted of the application of fresh 0.1% aqueous solution of colchicine for twenty four hours using a modified cut-tiller capping method of Bell (1950; Fig. 3d). The C_1 (i.e. seeds produced from colchicine-treated hybrids) amphiploids of common parentage differing in their source of wheat cytoplasm were plant-to-plant reciprocally crossed, thus producing reciprocal F_1 pairs that were genotypically identical but cytoplasmically distinct.

2. Plant characteristics and statistical analysis

The reciprocal F_1 plants obtained as described above, as well as reciprocal C_2 pairs produced from selfed C_1's, were used to study

Figure 3

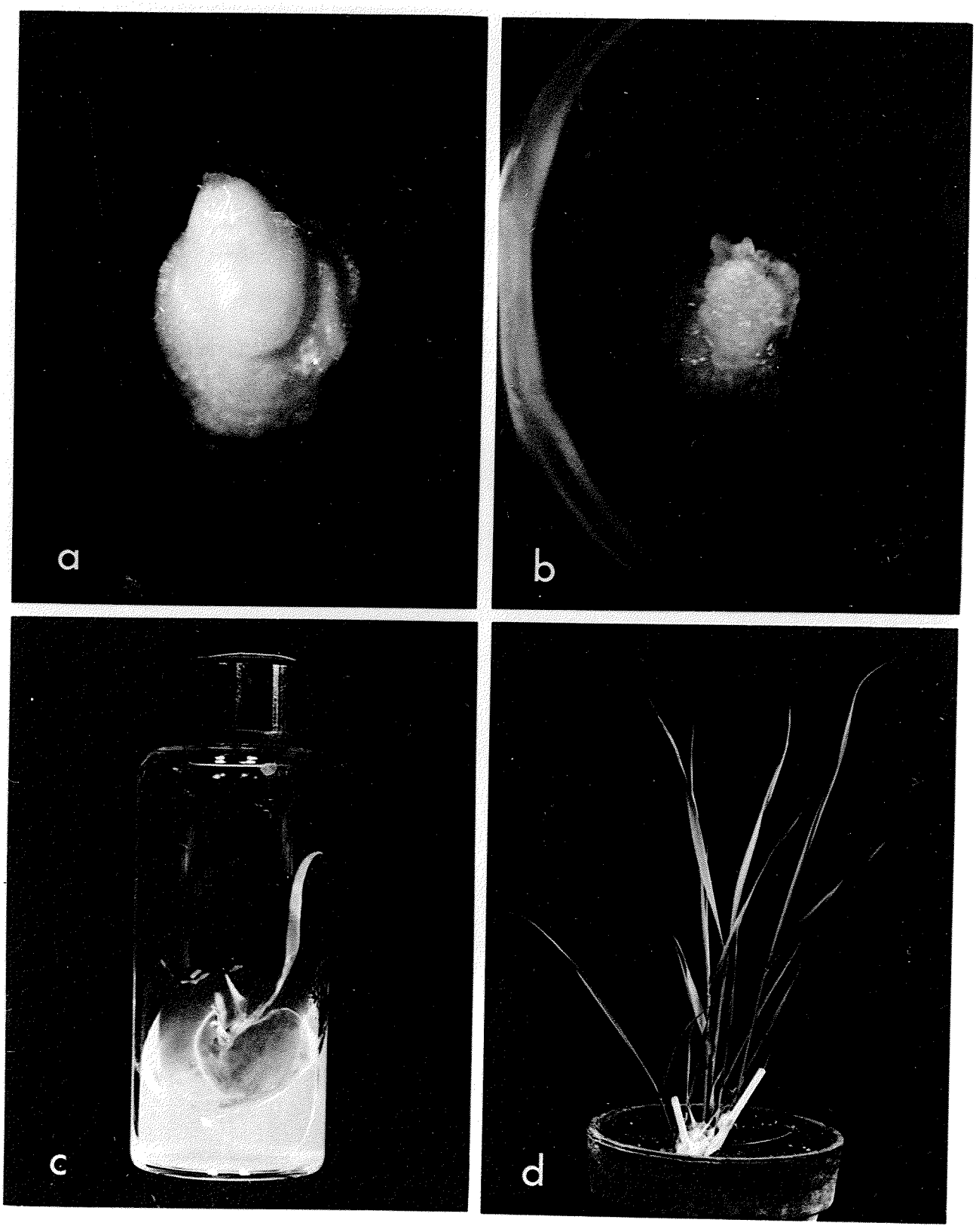
The processes of embryo-culture and doubling of wheat-rye F_1 hybrids.

(a) A normal wheat-rye F_1 embryo in hexaploid wheat cytoplasm.

(b) A deformed wheat-rye F_1 embryo in tetraploid wheat cytoplasm.

(c) A plantlet in orchid agar slant supplemented with IAA and kinetin.

(d) Application of 0.1% colchicine solution using a modified cut-tiller capping method.



morphological and agronomic attributes including growth rate, plant height, the number of fertile tillers and spike characteristics. The plants were screened for euploids and grown in a completely random design in the greenhouse at 16 hours photoperiod and a temperature of about 21°C. The data for the number of fertile tillers were transformed by $\sqrt{\text{datum}}$ to conform to normality as tested by the Kolmogorov-Smirnov test (Sokal and Rohlf, 1969). Data for each of the other characters studied were normally distributed. Unpaired *t*-tests were used to analyse each C_2 and reciprocal F_1 pair, and an analysis of variance was used to analyse C_2 and F_1 populations. Data were also tested for homogeneity according to Bartlett (1937). In the case where variances of each C_2's or reciprocal F_1 pair were not homogeneous, a *t*-test assuming unequal variances as described by Steel and Torrie (1960) was used.

RESULTS

1. Synthesis of C_2 and reciprocal F_1 populations

Although the percentage seed-set obtained from reciprocal crosses between the hexaploid and the tetraploid wheats was generally high, several factors including poor germination, sterility, and necrosis of the resulting pentaploid hybrids resulted in the loss of some lines (Table 2). Necrosis was observed in crosses involving Manitou as one parent and either T. turgidum L. vars. durum cv. Stewart and U. of M. accession 4B 723, or T. turgidum L. var. polonicum as the other. The symptoms appeared late in the hybrids and were absent in the parents. As has been shown previously this lethality is caused by a dominant gene located in either the A or B genome and its complementary gene in the D genome (Nishikawa, 1964; Kihara, 1966; Tsunewaki, 1970). Reciprocal pentaploid hybrids of the same genotype did not differ morphologically including height, spike length, density of spikelets, as well as the density and length of awn. However relative to the respective parents, the pentaploid possessed lax spikes with other characteristics being intermediate between that of the hexaploid and the tetraploid parents (Fig. 4 to 8).

Results showed that meiosis of pentaploids possessed a mean univalent frequency of 6.90 ± 0.11 per sporocyte at metaphase I (Fig. 9a). The mean frequency of trivalents as well as quadrivalents was 0.06 per sporocyte. Assuming a meiotic configuration at MI of 14 bivalents and

Table 2. Behaviour of reciprocal wheat pentaploids used in the synthesis of triticales

Hybrids in 6 x wheat cytoplasm		Hybrids in 4 x wheat cytoplasm	
Pitic x Stewart		Stewart x Pitic	***
" x 77	***	77 x "	***
" x 110		110 x "	
" x 233		233 x "	
" x 242		242 x "	+
" x 254	***	254 x "	
" x 280	++	280 x "	
" x 296	**	296 x "	**
" x 723	+	723 x "	+
Manitou x Stewart	*	Stewart x Manitou	*
" x 77	***	77 x "	***
" x 110		110 x "	
" x 233	+	233 x "	+
" x 242		242 x "	
" x 254	*	254 x "	*
" x 280		280 x "	**
" x 296	***	296 x "	
" x 723	*	723 x "	*

* Necrosis of F_1 plants.

** Poor germination of F_1's.

*** Sterility of F_1's.

\+ Sterility and difficulties met in eliminating D chromosomes.

++ Unsuccessful doubling of the ABR allohaploids.

Figure 4

Spike morphology of wheat and rye parents used in the synthesis of triticales.

(A) Triticum aestivum L. em. Thell. cv. Manitou.

(B) " " " " " cv. Pitic.

(C) T. turgidum L. var. turgidum (UM Acc. 4B110).

(D) " " " " durum (" 4B233).

(E) " " " " orientale (" 4B242).

(F) Secale cereale L. cv. Centeno.


A
B
C
D
E
F

Figure 5

Spike morphology of reciprocal crosses involving Pitic x Triticum turgidum L. var. turgidum.

(A) 6x x 4x wheat (F_1, 2n = 35).

(a) 4x x 6x wheat (").

(B) 6x x 4x wheat (F_3, 2n = 28).

(b) 4x x 6x wheat (").

(C) 6x x 4x wheat F_3/Centeno C_2 (A).

(c) 4x x 6x wheat F_3/ " (A^1).

(D) A x A^1 triticale F_1 hybrid.

(d) A^1 x A " .

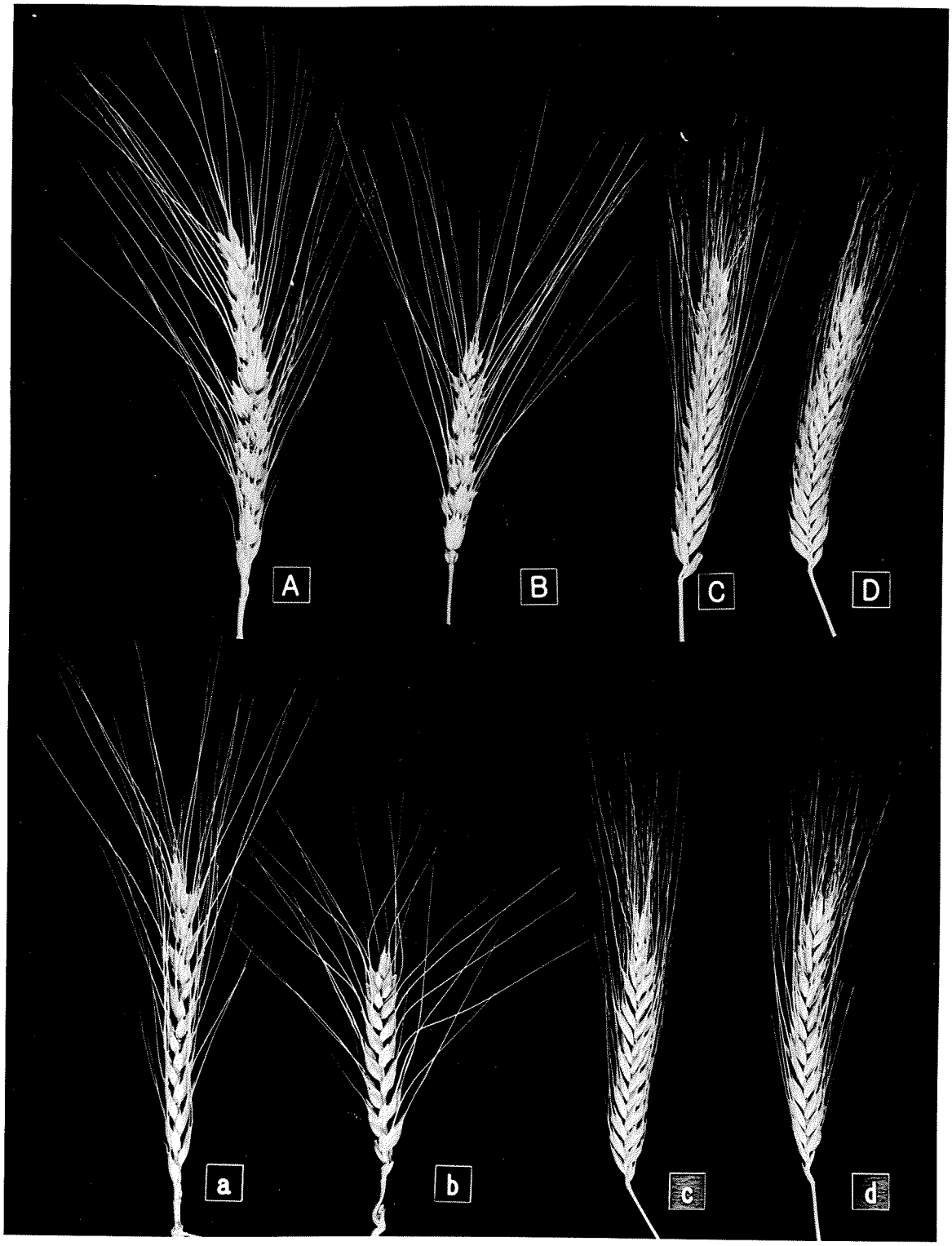

A
B
C
D
a
b
c
d

Figure 6

Spike morphology of reciprocal crosses involving Pitic x *Triticum turgidum* L. var. durum.

(A) 6x x 4x wheat (F_1, 2n = 35).

(a) 4x x 6x wheat (").

(B) 6x x 4x wheat (F_3, 2n = 28).

(b) 4x x 6x wheat (").

(C) 6x x 4x wheat F_3/Centeno C_2 (B).

(c) 4x x 6x wheat F_3/ " (B^1).

(D) B x B^1 triticale F_1 hybrid.

(d) B^1 x B " .

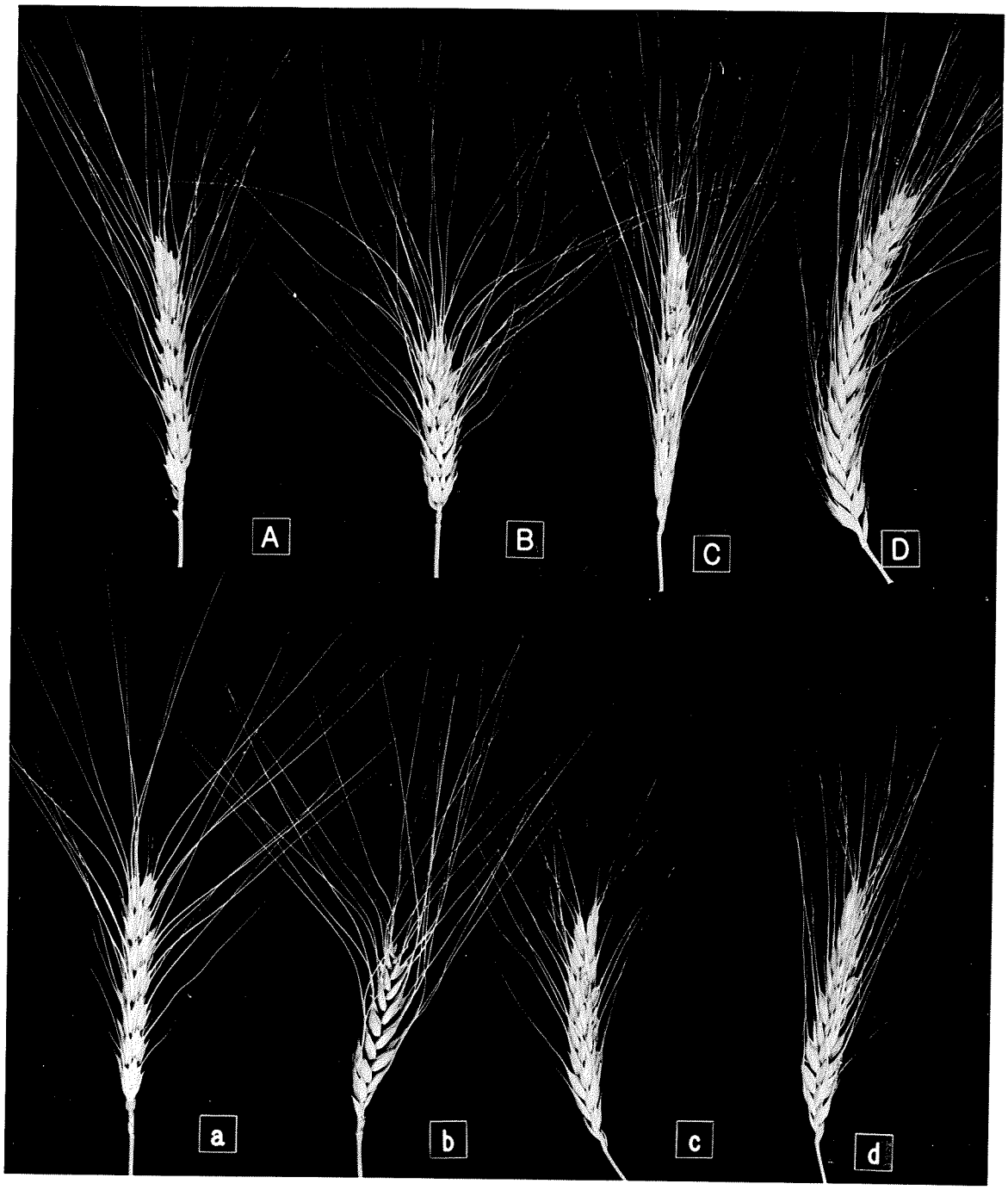

A
B
C
D
a
b
c
d

Figure 7

Spike morphology of reciprocal crosses involving Manitou x Triticum turgidum L. var. turgidum.

(A) 6x x 4x wheat (F_1, 2n = 35).

(a) 4x x 6x wheat (").

(B) 6x x 4x wheat (F_3, 2n = 28).

(b) 4x x 6x wheat (").

(C) 6x x 4x wheat F_3/Centeno C_2 (C).

(c) 4x x 6x wheat F_3/ " (C^1).

(D) C x C^1 triticale F_1 hybrid.

(d) C^1 x C " .


A
B
C
D
a
b
c
d

Figure 8

Spike morphology of reciprocal crosses involving Manitou x Triticum turgidum L. var. orientale.

(A) 6x x 4x wheat (F_1, 2n = 35).

(a) 4x x 6x wheat (").

(B) 6x x 4x wheat (F_3, 2n = 28).

(b) 4x x 6x wheat (").

(C) 6x x 4x wheat F_3/Centeno C_2 (D).

(c) 4x x 6x wheat F_3/ " (D^1).

(D) D x D^1 triticale F_1 hybrid.

(d) D^1 x D " .


A
B
C
D
a
b
c
d

Figure 9

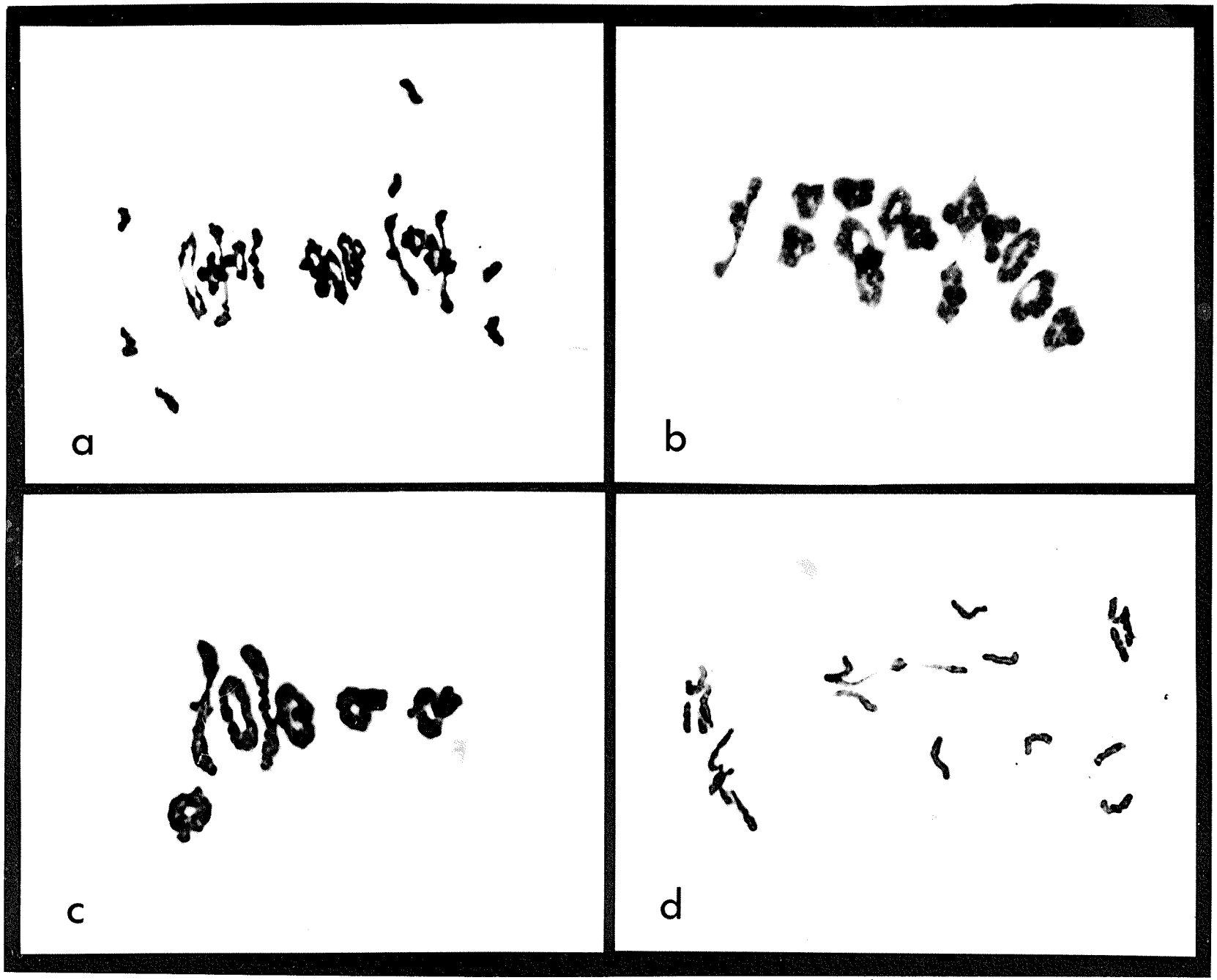
Meiotic metaphase I configurations of wheat and wheat-rye hybrids.

(a) An F_1 pentaploid ($2\underline{n} = 5\underline{x} = 35$, AABBD) wheat hybrid showing 14" + 7' (x 1220).

(b) A test cross between a derived tetraploid and its tetraploid parent showing 14" (x 1530).

(c) Secale cereale L. cv. Centeno rye showing 7" (x 1530).

(d) An ABR wheat-rye F_1 hybrid showing 1" + 19' (x 1060).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7 univalents, the probability of an AI segregation with (14+x) chromosomes toward one pole and (14+7-x) chromosomes to the other is given by the expression of the binomial expression $(0.5 + 0.5)^7$. Thus the probability of each type of gametic and zygotic constitution was calculated by Kaltsikes, et al. (1970) and by Lacadena and Sendino (1970). Table 3 shows the frequencies of somatic chromosome numbers in the F_2 population. Chi-square tests indicated that the observed frequencies of the hybrids with 6x as well as 4x wheat cytoplasms did not fit the expected on the basis of random univalent distribution ($P \leqslant 0.005$). However, it was of interest to note that hybrids with 6x wheat cytoplasm have a higher mean chromosome number than those with 4x cytoplasm ($t = 2.998$, $df = 185$, $P \leqslant 0.01$). A similar situation was again observed in the F_3 generation from the selfed pentaploids (Table 4). It was also noted that 28 chromosomes F_2 plants with both 6x and 4x cytoplasms were cytologically stable, as no aneuploids or chromosome counts other than 28 were obtained.

Genetically, the 28 chromosome plants obtained from the pentaploids (i.e. the derived tetraploids) contained the complete AABB genomes, since test crosses between 14-bivalent derived tetraploids and respective tetraploid parents revealed no univalents in the F_1 (Fig. 9b). Although in a few test crosses a highest mean frequency of 0.30 univalents/sporocyte was observed, this was not considered to be the result of the presence of one or more D genome chromosomes. The presence of

Table 3. Frequencies of somatic chromosome numbers in the F_2 population of pentaploid hybrids as influenced by source of wheat cytoplasm

Chromosome No.	Hybrids in 6x wheat cytoplasm	Hybrids in 4x wheat cytoplasm
28	4	4
29	4	14
30	20	10
31	8	10
32	8	4
33	12	5
34	4	15
35	12	2
36	4	10
37	4	2
38	15	-
39	-	2
40	4	-
41	-	2
42	8	-
TOTAL	107	80
Mean ± S.E.	34.04 ± 0.38	32.44 ± 0.35

Table 4. Frequencies of somatic chromosome numbers in the F_3 population of pentaploid hybrids as influenced by source of wheat cytoplasm

Chromosome No. of F_2 plant	Designation[a]	Chromosome No. in F_3 population									Mean	Total
		27	28	29	30	31	32	33	34	38		
28	I		16								28.00	16
	II		17								28.00	17
29	I	-	-	-							-	-
	II	2	27	20							28.37	49
30	I		16	6	2	1					28.52	25
	II		7	7	4	-					28.83	18
31	I		7	2		1		1	2	1	30.29	14
	II		6	4		-		-	-	-	28.40	10
32	I		5	13	1	3	1				29.22	23
	II		-	-	-	-	-				-	-
33	I		-		-						-	-
	II		2		4						29.33	6

[a] Hybrids in 6x (I) and 4x (II) wheat cytoplasm, respectively.

such would result in a regular occurrence of 2 or more univalents per sporocyte, and/or possibly homoeologous pairing.

A reciprocally derived tetraploid pair ($2\underline{n} = 4\underline{x} = 28$) did not differ among themselves, but exhibited reduced height, smaller spikes, and relatively fewer awns when compared with their hexaploid and tetraploid parents (Fig. 4 to 8). Slight differences in the number, and the length of awns were observed in only those reciprocal crosses involving Manitou and either T. turgidum L. var. turgidum or T. turgidum L. var. orientale.

The rye cultivar used in this study was selected on the basis of its high crossability with wheat (Larter and Hsam, unpublished). Metaphase I of meiosis showed a mean rod-bivalent frequency of 2.14 ± 0.12, and a mean univalent frequency of 0.16 ± 0.08 per sporocyte (Fig. 9c). The sterile ABR F_1 hybrids showed univalents and an occasional bivalent (Fig. 9d). As revealed in Tables 5 and 6, the frequency with which wheat x rye F_1 hybrids were obtained was about 3% higher when the female parent carried hexaploid wheat cytoplasm as opposed to tetraploid wheat cytoplasm. Seed-set, embryo formation, and embryo survival rate were also found to be consistently beneficial in favour of the $6\underline{x}$ cytoplasm. However, notwithstanding difficulties in doubling some of the F_1 wheat-rye hybrids, four reciprocal C_1 triticale lines involving the cytoplasms of two hexaploid wheats (viz. cvs. Pitic and Manitou), and three tetraploid species (T. turgidum L. var. turgidum; T. turgidum L. var. durum;

Table 5. Synthesis of triticale amphiploids as influenced by source of wheat cytoplasm

Hybrid	No. of crossed spikes	Total florets pollinated	No. seeds obtained	No. embryos obtained	No. haploids obtained	No. haploids doubled
Pitic x 110 F_3/Centeno	16	320	63	54	17	6
110 x Pitic F_3/ "	48	960	185	158	11	7
Pitic x 233 F_3/ "	18	360	177	139	52	8
233 x Pitic F_3/ "	25	500	140	95	9	2
Pitic x 280 F_3/ "	23	460	62	51	3	0
280 x Pitic F_3/ "	21	420	136	113	11	1
Manitou x 110 F_3/Centeno	28	560	269	223	35	6
110 x Manitou F_3/ "	28	560	157	131	26	2
Manitou x 242 F_3/ "	18	360	31	17	6	1
242 x Manitou F_3/ "	16	320	117	93	10	7
TOTAL	241	4820	1337	1074	180	40

Table 6. Summary of triticale synthesis as influenced by source of wheat cytoplasm

	Hybrids in	
	6x wheat cytoplasm	4x wheat cytoplasm
% Seeds / Total florets pollinated	29.22	26.63
% Embryos/ "	23.50	21.38
% Embryo survival / Total embryos	23.35	11.36
% Wheat-rye hybrids/Total florets pollinated	5.45	2.40
% Doubled amphiploids/ "	1.00	0.70

T. turgidum L. var. orientale) were eventually obtained. The reciprocal F_1 produced from a plant-to-plant reciprocal C_1 pairs, as well as reciprocal C_2 pairs produced from selfed C_1 lines (Table 7), constituted the required genetic stock.

2. Plant characteristics

a) Growth rate

A minimum number of 9 plants was studied for each reciprocal F_1 or C_2 line. Growth rate was calculated as the ratio of the amount of growth within a given time interval to the height attained at maturity. Rates of growth were found to be significantly different between reciprocal C_2 populations, however the same was not observed in reciprocal F_1 populations (Table 8). In the C_2's, the growth rate of triticales with tetraploid wheat cytoplasm was about 3% higher than the reciprocals for the first 11 days. Later, between thirty to thirty-seven days, the growth rate was again significantly higher. Thus at forty days the total height was about 15% significantly greater ($P \leqslant 0.05$) in favor of triticale carrying tetraploid wheat cytoplasm.

b) Days to heading

With the exception of 1 reciprocal cross (viz. Pitic x T. turgidum L. var. turgidum), there was no significant difference between reciprocal F_1's in number of days to heading (Table 9). In the Pitic - T. turgidum cross, F_1's carrying tetraploid wheat cytoplasm were about 5

Table 7. Parentage of C_2 and reciprocal F_1 populations

Generation	Designation	Parentage
C_2	A	Pitic x T. turgidum (F_3)/Centeno rye
C_2	A^1	T. turgidum x Pitic (F_3)/ " "
F_1	A x A^1	
F_1	A^1 x A	
C_2	B	Pitic x T. durum (F_3)/Centeno rye
C_2	B^1	T. durum x Pitic (F_3)/ " "
F_1	B x B^1	
F_1	B^1 x B	
C_2	C	Manitou x T. turgidum (F_3)/ Centeno rye
C_2	C^1	T. turgidum x Manitou (F_3)/ Centeno rye
F_1	C x C^1	
F_1	C^1 x C	
C_2	D	Manitou x T. orientale (F_3)/Centeno rye
C_2	D^1	T. orientale x Manitou (F_3)/ " "
F_1	D x D^1	
F_1	D^1 x D	

Table 8. Growth rate[a] of C_2 and reciprocal F_1 populations as influenced by source of wheat cytoplasm

Generation	Designation[b]	Days from planting						Total plant growth at 40 days
		5	11	20	30	37	40	
C_2	I	[c]11.9 ± 0.6*	10.4 ± 0.9+	14.4 ± 0.2	15.6 ± 0.8	9.6 ± 0.7*	5.5 ± 1.9	66.5 ± 3.0*
	II	14.5 ± 0.6	13.8 ± 0.9	16.0 ± 1.3	14.4 ± 1.5	14.6 ± 1.1	8.1 ± 1.1	81.4 ± 4.3
F_1	I	11.6 ± 0.6	10.8 ± 0.8	13.8 ± 0.3	14.0 ± 0.3	10.7 ± 1.0	6.3 ± 1.0	64.9 ± 3.2
	II	12.7 ± 0.7	10.1 ± 0.7	15.6 ± 0.8	14.5 ± 0.3	9.8 ± 0.8	7.3 ± 1.1	70.2 ± 2.9

[a] Growth rate (%) = growth in height (cm.) within specific time interval/final height at maturity.

[b] Amphiploids in 6x (I), and 4x (II) wheat cytoplasm, respectively.

[c] Means and standard errors.

+ ($P \leqslant 0.10$), * ($P \leqslant 0.05$) for means within pairs of comparable generations.

n (C_2 = 40 each for I, and II; F_1 = 38 each for I, and II).

Table 9. Summary of days to heading of C_2 and reciprocal F_1 populations as influenced by source of wheat cytoplasm

Generation	Designation	Difference (Hybrids in 6x - hybrids in 4x wheat cytoplasm)	Level of Sig.
C_2	A A^1	- 8.16	**
F_1	A x A^1 A^1 x A	+ 4.59	**
C_2	B B^1	+12.00	**
F_1	B x B^1 B^1 x B	+ 1.0	n.s.
C_2	C C^1	+ 4.7	**
F_1	C x C^1 C^1 x C	- 1.10	n.s.
C_2	D D^1	+ 2.8	**
F_1	D x D^1 D^1 x D	+ 0.8	n.s.

** ($P \leqslant 0.01$).

days earlier maturing than their reciprocal counterparts. This is in direct contrast to the behaviour of the C_2 population of this same cross in which the tetraploid wheat cytoplasm bearing C_2's were 8 days later in maturity than the reciprocal cross. In other C_2 reciprocal pairs, triticales with 6x wheat cytoplasm were significantly later maturing than those with 4x wheat cytoplasm ($P < 0.01$).

c) Plant height

On the overall average for all genotypes, the F_1's and C_2's carrying hexaploid wheat cytoplasm were taller than their reciprocal counterparts by approximately 3 and 14% respectively. As shown in Table 10, in 4 of the 8 comparisons, differences in mean height were statistically significant. In one exception which involved the C_2 population of Manitou x T. turgidum L. var. orientale, a reverse trend was observed but which was not statistically significant. The factorial analysis of variance indicated that differences due to both genotype and cytoplasm were significant for the F_1-plant population (Table 12). However in the C_2's, a highly significant genotype x cytoplasm interaction masked the effects contributed by genotype and cytoplasm alone.

d) Number of fertile tillers

In both the reciprocal F_1 and C_2 populations, the number of fertile tillers was about 20 to 25% higher when the female involved in the cross carried hexaploid wheat cytoplasm. In 3 of the 8 comparisons, differences in the mean number of fertile tillers were statistically significant

Table 10. Plant height (cm.) of C_2 and reciprocal F_1 populations as influenced by source of wheat cytoplasm

Generation	Designation	No. of plants analysed	Mean	SE	Level of Sig.
C_2	A	13	124.10	1.88	$P \leqslant 0.001$
	A^1	13	105.88	2.06	
F_1	A x A^1	10	126.99	1.90	n.s.
	A^1 x A	10	126.45	1.69	
C_2	B	10	126.38	1.67	$P \leqslant 0.001$
	B^1	10	85.67	2.26	
F_1	B x B^1	9	117.82	2.22	$P \leqslant 0.10$
	B^1 x B	9	112.27	1.61	
C_2	C	10	111.84	2.97	$P \leqslant 0.02$
	C^1	10	101.76	2.02	
F_1	C x C^1	9	117.63	2.17	n.s.
	C^1 x C	9	114.06	2.62	
C_2	D	10	98.90	2.11	n.s.
	D^1	10	103.56	1.70	
F_1	D x D^1	10	113.88	1.77	n.s.
	D^1 x D	10	109.53	2.73	

($P \leqslant 0.02$, Table 11). As in plant height, the only exception involved the same C_2 population of Manitou x T. turgidum L. var. orientale in which an opposite trend was observed but which was not significant. A significant correlation was observed between plant height and the number of fertile tillers ($r = 0.30^{**}$ for plants in hexaploid cytoplasm; $r = 0.50^{**}$ for plants in tetraploid cytoplasm; see also Section III of this thesis). As with plant height, a factorial analysis of variance indicated that differences due to genotype as well as cytoplasm were significant at the 0.05% level for the F_1's. In the C_2 populations however, a highly significant genotype x cytoplasm interaction resulted in both the genotypic and cytoplasmic effects being non-significant.

e) Spike characteristics

Spikes from the reciprocal F_1 and C_2 pairs are shown in Fig. 5 to 8. In 7 of the 8 comparisons, of which 4 were statistically significant ($P \leqslant 0.05$), plants in hexaploid wheat cytoplasm had the longer spikes. The exception involved the reciprocal F_1 pairs of Pitic x T. turgidum L. var. turgidum triticales. In general, the member of a reciprocal pair having the longer spikes also possessed numerically more spikelets and florets (Table 13). However, there was one exception involving the reciprocal C_2 and F_1 pairs of Manitou x T. turgidum L. var. orientale in which hybrids carrying hexaploid wheat cytoplasm developed the longer spikes but with fewer floral components than the reciprocal.

Table 11. No. fertile tillers/plant for C_2 and reciprocal F_1 populations as influenced by source of wheat cytoplasm

Genera-tion	Designation	No. of plants analysed	Arithmetic means	Analysed means and SE	Level of Sig.
C_2	A	12	5.9	2.4 ± 0.10	n.s.
	A^1	12	4.6	2.1 ± 0.15	
F_1	A x A^1	10	7.5	2.7 ± 0.10	n.s.
	A^1 x A	10	6.3	2.5 ± 0.09	
C_2	B	10	5.0	2.2 ± 0.09	$P \leqslant 0.001$
	B^1	10	2.3	1.5 ± 0.05	
F_1	B x B^1	10	5.7	2.4 ± 0.06	n.s.
	B^1 x B	10	5.0	2.2 ± 0.14	
C_2	C	10	3.6	1.9 ± 0.10	n.s.
	C^1	10	3.2	1.8 ± 0.08	
F_1	C x C^1	10	4.6	2.1 ± 0.05	$P \leqslant 0.02$
	C^1 x C	10	3.4	1.8 ± 0.10	
C_2	D	10	3.0	1.7 ± 0.10	n.s.
	D^1	10	3.4	1.8 ± 0.09	
F_1	D x D^1	11	5.1	2.2 ± 0.11	$P \leqslant 0.02$
	D^1 x D	11	3.5	1.9 ± 0.08	

Table 12. Mean square values for plant height and fertile tillers/plant of C_2 and reciprocal F_1 populations

Source of variation	Plant height		Fertile tillers/plant		Test against G x C	
	C_2	F_1	C_2	F_1	Height C_2	Tillers C_2
Genotype (G)	692*** (3)	804*** (3)	1.02*** (3)	1.66*** (3)	n.s.	n.s.
Cytoplasm (C)	5565*** (1)	234* (1)	1.32*** (1)	1.53*** (1)	n.s.	n.s.
G x C	1906*** (3)	22 (3)	0.66*** (3)	0.04 (3)		
Error	48 (78)	43 (68)	0.11 (76)	0.09 (74)		

In parenthesis = degrees of freedom.

* ($P \leqslant 0.05$), *** ($P \leqslant 0.005$).

Table 13. Spike characteristics of C_2 and reciprocal F_1 populations as influenced by source of wheat cytoplasm

Generation	Designation	No. of plants analysed	Spike length (cm.)	No. spikelets/spike	No. spikelets/cm.	No. florets/spike
C_2	A	10	[c]10.09 ± 0.32*	29.0 ± 0.52*	2.89 ± 0.08	80.9 ± 3.02
	A^1	10	9.22 ± 0.24	26.6 ± 0.87	2.90 ± 0.12	75.2 ± 2.41
F_1	A x A^1	10	9.81 ± 0.33	25.0 ± 1.28*	2.59 ± 0.05*	71.7 ± 3.77*
	A^1 x A	10	10.30 ± 0.28	28.8 ± 0.42	2.79 ± 0.08	82.2 ± 1.82
C_2	B	8	12.84 ± 0.17***	27.8 ± 0.88**	2.16 ± 0.06***	79.6 ± 2.57***.
	B^1	8	7.48 ± 0.19	20.1 ± 0.58	2.69 ± 0.08	58.0 ± 1.63
F_1	B x B^1	8	9.78 ± 0.15*	23.6 ± 0.53	2.39 ± 0.04+	64.8 ± 1.92
	B^1 x B	8	9.34 ± 0.11	23.3 ± 0.49	2.49 ± 0.04	65.8 ± 1.51
C_2	C	10	10.42 ± 0.36**	26.1 ± 0.75+	2.52 ± 0.07	75.0 ± 2.19+
	C^1	10	9.19 ± 0.19	24.3 ± 0.47	2.64 ± 0.05	68.0 ± 2.89
F_1	C x C^1	10	10.22 ± 0.21	26.1 ± 0.64	2.56 ± 0.06	77.1 ± 3.06*
	C^1 x C	10	9.99 ± 0.23	25.7 ± 0.68	2.57 ± 0.03	70.2 ± 2.73
C_2	D	9	9.08 ± 0.28	20.9 ± 0.75**	2.27 ± 0.06***	60.2 ± 2.18*
	D^1	[a]9	8.88 ± 0.28	24.0 ± 0.68	2.68 ± 0.04	68.4 ± 2.18
F_1	D x D^1	[b]10	10.33 ± 0.06	24.8 ± 0.13	2.41 ± 0.03	69.6 ± 2.60
	D^1 x D	[b]10	10.18 ± 0.18	25.1 ± 0.32	2.43 ± 0.04	70.0 ± 2.92

[a] For No. spikelets/spike N = 10, [b] For No. spikelets/cm. N = 11, [c] Mean ± SE.

+ ($P \leqslant 0.10$), * ($P \leqslant 0.05$), ** ($P \leqslant 0.01$), *** ($P \leqslant 0.001$) for means within pairs of comparable generations.

Differences in spikelet number per spike showed a highly significant genotype x cytoplasm interaction in 5 of the 8 comparisons between reciprocals. Of these, 3 possessed more and 2 possessed fewer spikelets in hexaploid cytoplasm than their counterparts in tetraploid cytoplasm. The density of spikelets expressed as the number of spikelets per centimeter, showed those triticales with tetraploid cytoplasm to have a higher value in all instances. This however was observed to be governed by the relatively shorter length of spikes of triticales with 4x cytoplasm (i.e. compactness) rather than by an increase in the actual number of spikelets per se.

Genotypic x cytoplasmic interactions also played a part in the analysis of floret number per spike, as 3 of the 5 significant differences between reciprocals of both F_1 and C_2 populations; showed hexaploid wheat cytoplasm to be beneficial in 3 cases and tetraploid wheat cytoplasm in the remaining 2 (Table 13). Considering all the spike's characters studied collectively, a significant difference was observed only for the density of spikelets between the reciprocal F_1 populations. For all other characters, a factorial analysis of variance indicated that the genotype x cytoplasm interactions were highly significant. Consequently the main effects (i.e. cytoplasm and genotype) were masked accordingly (Table 14).

Table 14. Mean square values for spike characteristics of C_2 and reciprocal F_1 populations

Source of variation	Spike length		No. spikelets/spike		No. spikelets/cm.		No. florets/spike	
	[a] C_2	F_1	[a] C_2	F_1	[a] C_2	F_1	[a] C_2	[a] F_1
Genotype (G)	4.48*** (3)	1.73* (3)	95.7*** (3)	40.9*** (3)	0.80*** (3)	0.31*** (3)	604*** (3)	478*** (3)
Cytoplasm (C)	67.89*** (1)	0.13 (1)	88.9*** (1)	13.1+ (1)	1.37*** (1)	0.15* (1)	788*** (1)	29 (1)
G x C	25.05*** (3)	0.75 (3)	89.6*** (3)	18.9*** (3)	0.27*** (3)	0.04 (3)	682*** (3)	240** (3)
Error	0.68 (66)	0.45 (68)	4.6 (67)	4.2 (68)	0.05 (66)	0.02 (70)	57 (66)	52 (68)

[a] Both genotype and cytoplasm were non-significant when tested against G x C interaction.

In parenthesis = degrees of freedom.

+ ($P \leqslant 0.10$), * ($P \leqslant 0.05$), ** ($P \leqslant 0.01$), *** ($P \leqslant 0.001$).

DISCUSSION

Plant breeders have generally assumed that nuclear effects are more important than maternal and/or cytoplasmic effects in governing character expression. There is no doubt that this has been a valid assumption. On the other hand, numerous examples are known of maternal inheritance and reciprocal differences in plant hybrids (for a review see Jinks, 1964). Moreover, where one might expect to find significant differences in hybrid reciprocity and valuable application of this phenomenon, is in the synthesis of new species. Triticale is one such species in which the nucleus and cytoplasm from already long-established species of wheat and rye are combined.

In the present study, several important observations were evident during the process of synthesizing the reciprocal triticale populations. Kihara (1968) reported that reciprocal hybrids between 4x and 6x wheat were identical in morphology and fertility. Similar results were observed in most of the eighteen reciprocal 6x x 4x crosses made in the present investigation. In the F_2 generation of pentaploids so produced, it was further observed that parental chromosome combinations occurred more frequently than would be expected on the basis of random distribution of univalents. Similar deviations from the expected patterns of chromosome segregation have been attributed by several investigators to differential functioning of male and female gametes (Thompson and Cameron, 1928; Kihara and Matsumura, 1942). In addition, it appears

that hybrids with 4x wheat cytoplasm reverted more rapidly to the tetraploid condition than did those with hexaploid wheat cytoplasm.

Love (1940) reported that in advanced generations of pentaploids, segregants with 28 chromosomes showed no multivalents and bivalent formation (14") occurred regularly. It was suggested that selective and systematic genome elimination took place in these hybrids (Lacadena and Sendino, 1970). In the present study the chance of obtaining a D-genome chromosome pair substituted for chromosomes either from the A or the B genome is very remote. Kaltsikes (1968) in attempting to produce D genome addition lines from a pentaploid ($2n = 5x = 35$) showed that the probability of obtaining a zygote carrying identical D-genome chromosome gametes is $(0.5)^{14}$ or one in approximately 16,000. These results provide some explanations for the lack of D-genome chromosomes in the 14-bivalent tetraploids derived in the present study. Furthermore, it was observed that the frequency of obtaining wheat-rye F_1 hybrids was higher by about 3% when the female parent carried hexaploid wheat cytoplasm. This is of interest as current breeding procedures in triticale require synthesis of new wheat x rye amphiploids as a means of introducing genetic variability in the breeding population. The utilization of 6x wheat cytoplasm would certainly enhance such a program.

In a study of this nature, involving intergeneric hybrids of different ploidy, it has to be established that (a) the synthesized products which are ultimately used in comparisons are in fact genetically identical,

and (b) that they carry the complete ABR chromosome complement (i.e. free of intergenomic substitutions involving D chromosomes from wheat). It is felt that in this study, both requirements were satisfied — requirement (a) by means of reciprocal plant-to-plant crosses of C_1 amphiploids resulting in identical genotypes of any given reciprocal F_1 pair; requirement (b) by the fact that the tetraploid wheats ($2n = 28$) extracted from reciprocally produced pentaploids, when crossed back to their respective tetraploid parents, revealed no univalents in the F_1. This indicated that the derived tetraploids used in the synthesis of primary triticales were in fact genomically AABB. The difference observed between reciprocal F_1 pairs therefore, was considered to be free of genetic influence. Furthermore the reciprocal C_2 pairs provide additional estimates on the performance of F_1's.

In the present study, differential interactions of an AABBRR nucleus in hexaploid or tetraploid wheat cytoplasm was observed. A beneficial relationship existed in favour of triticales possessing hexaploid wheat cytoplasm when assessed in terms of plant height and fertile tillers. Hsam and Larter (1973) studying the association of agronomic characters in hexaploid triticales observed that both plant height and fertile tillers correlated significantly with yield. A clear relationship was not evident favouring either of the two cytoplasm types regarding spike morphology, as this character appears to be under the influence of the respective genotypes interacting differentially with the same cytoplasm.

In terms of kernel development, seed set, and meiotic stability, Larter and Hsam (1973) have shown that 6x triticales perform more favorably in hexaploid wheat cytoplasm.

The role of cytoplasm in heredity is not as yet completely understood. Recent results as compiled by Sager (1972) suggests a molecular model for cytoplasmic effects per se based on deoxyribonucleic acid (DNA) in mitochondria and chloroplasts. However, with only limited amounts of cytoplasmic DNA present, it seems unlikely that large phenotypic differences in morphological traits would be manifested.

It must be emphasized that in the improvement of triticale as a new potential crop species, it will be necessary to introduce more and more genetic variability by way of synthesizing new wheat-rye amphiploids. On this basis, the synthesis and utilization of triticales in hexaploid wheat cytoplasm should form an integral part of a triticale breeding program.

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SECTION II

CYTOLOGY, FERTILITY AND SEED QUALITY OF HEXAPLOID TRITICALE AS INFLUENCED BY SOURCE OF WHEAT CYTOPLASM

INTRODUCTION

The demonstration in recent years of the existence of cytoplasmic deoxyribonucleic acid has revitalized studies concerning the role of cytoplasm in inheritance. Enucleate and cell fusion experiments involving animal cells, also the increasing volume of evidence from plant species, clearly demonstrate the modifying influence of cytoplasm on the phenotypic expression and physiological behaviour of the organism (Jinks, 1964; Harris, 1970a, 1970b). Within the Triticinae itself, the well documented studies involving cytoplasmic male sterility and fertility restoration, are prime examples of the end results of manipulation of nuclear-cytoplasmic relationships (Schmidt and Johnson, 1966; Kihara, 1968; Maan and Lucken, 1968, 1972; Maan, 1973).

Cytoplasmic-genomic interaction has lately become of practical importance in triticale breeding. Triticale is an intergeneric hybrid in which depending upon the species of wheat used as the female parent, two forms of the amphiploid are produced, viz. the octoploid ($2n = 8x = 56$) and hexaploid ($2n = 6x = 42$). It has been established that to-date the hexaploid types have been agronomically more suitable than the octoploid triticale (Larter, et al., 1968; Zillinsky and Borlaug, 1971). Until recently, primary hexaploid triticales were synthesized by hybridizing tetraploid wheat (Triticum turgidum L.) used as the female, with rye (Secale cereale L.); the hybrid so produced consequently possessing tetraploid wheat cytoplasm. However, available experimental evidence

suggests that the triticale nucleus is more compatible with hexaploid wheat (T. aestivum L.) cytoplasm. Sánchez-Monge (1959), Kiss (1966, 1971), and Pissarev (1966) all report improved seed-set from 8x x 6x triticale crosses compared with reciprocals. Moreover, the hexaploid segregants derived from such crosses (secondary hexaploid triticales) exhibited superior seed development relative to primary types.

The present study was designed to evaluate specifically the extent to which a nuclear-cytoplasmic interaction per se contributed to cytological stability, fertility, and seed quality in hexaploid triticales.

MATERIALS AND METHODS

Basically, the approach used in this study was to determine quantitative differences between genetically identical wheat-rye amphiploids in either hexaploid or tetraploid wheat cytoplasm (C_2 pairs), and between their reciprocal F_1 populations. The parentage and importance of these materials were described by Larter and Hsam (1973) and also in the previous section (Section I) of this thesis. Plants were screened for euploids ($2\underline{n} = 6\underline{x} = 42$) using root-tip Fuelgen squashes, and grown in a completely random design in the greenhouse at 16 hours photoperiod and an average temperature of 21°C.

For meiotic studies, inflorescences were collected in Carnoys II solution, transferred to 70% alcohol after one week and refrigerated. All metaphase plates were studied using acetocarmine-stained squashes of pollen mother cells. Pollen analyses were conducted using the method of Kihara (1958) in which grains with two elliptical sperm nuclei and one round vegetative nucleus were scored as being normal. To ensure maximum viability of pollen, collections were made while the stigmata were still receptive. Several criteria for estimating fertility were used, including kernels per spike, kernels per spikelet, kernels per spike and per spikelet when only the primary and secondary florets were considered, and finally, the percentage of all florets bearing seeds. In addition, seed yield per plant as well as kernel weight were determined. Seed quality and seed development based on seed density and

alpha-amylase enzyme activity were also evaluated. Seed density was measured using seed produced on F_1 plants (i.e. F_2 seed) and on C_2 plants (C_3 seeds). Density (g./cc.) was quantitatively evaluated by measuring liquid displacement upon adding a predetermined weight of seed sample to a 5 ml. volume of paraffin oil. Alpha-amylase activity was measured according to the method of MacGregor, et al. (1971), using a 0.1 g. sample of ground mature grains from approximately 25 seeds. The results were expressed in I.D.C. units. One I.D.C. unit is the amount of enzyme required to lower the absorbance of a standard digest from 0.6 to 0.4 in 100 minutes.

The data for yield/plant were transformed by $\frac{1}{\text{datum}}$ to conform to normality. Data for each of the other characters studies were normally distributed as tested by the Kolmogorov-Smirnov test according to Sokal and Rohlf (1969). Unpaired t-tests were used to analyse each C_2 and reciprocal F_1 pair, and a factorial analysis of variance was employed to analyse the C_2 and F_1 populations. All data were tested for homogeneity as described by Bartlett (1937). In instances where variances of each C_2 or reciprocal F_1 pair were not homogeneous, a t-test assuming unequal variances as described by Steel and Torrie (1960) was used.

RESULTS

1. Cytology

a) Anomalies at meiotic first metaphase

A total of 20 cells from each of 8 plants per F_1 and C_2 population was examined, and the mean number of univalents/cell, rod bivalents/cell, as well as trivalents and quadrivalents/cells were scored.

With one exception, F_1 and C_2 populations derived from crosses in which the female carried hexaploid wheat cytoplasm had fewer univalents than their counterparts derived from tetraploid wheat cytoplasm. The exception involved the C_2 population of a Manitou x turgidum cross (Table 1); however, the difference was not statistically significant. On the other hand, in 6 of the 7 reciprocal F_1 and C_2 pairs in which hexaploid cytoplasm exhibited beneficial effects, differences were significant ($P \leqslant 0.05$, to $P \leqslant 0.005$).

In terms of rod-bivalent frequency, the C_2 population of Pitic x turgidum (Table 1) showed that significant beneficial effect was obtained when the female parent carried hexaploid wheat cytoplasm ($P \leqslant 0.01$). Moreover, in 5 of the remaining 7 comparisons, plant populations with hexaploid wheat cytoplasm exhibited a higher frequency of closed bivalents (i.e. fewer rod bivalents), although the differences were not statistically significant. Only the C_2 population of Pitic x turgidum in hexaploid wheat cytoplasm showed an absence of quadrivalent

Table 1. Chromosomal anomalies as seen at M_I of C_2 and reciprocal F_1 populations

Generation	Designation	No. plants examined[a]	Quadrivalents	Trivalents	Rod bivalents	Univalents
C_2	A	8	0	0	3.67 ± 0.13 **	0.75 ± 0.07***
	A^1	8	0	0.013	4.46 ± 0.12	1.34 ± 0.14
F_1	A x A^1	8	[b]0.06 ± 0.02	0.02 ± 0.01	4.89 ± 0.22	1.82 ± 0.19**
	A^1 x A	8	0.11 ± 0.02	0.05 ± 0.02	5.15 ± 0.17	2.78 ± 0.26
C_2	B	8	0	0.13 ± 0.02	7.38 ± 0.20	1.87 ± 0.21***
	B^1	8	0.013	0.08 ± 0.02	7.18 ± 0.40	6.20 ± 0.52
F_1	B x B^1	8	0	0.13 ± 0.04	5.14 ± 0.30	2.91 ± 0.28***
	B^1 x B	8	0.006	0.11 ± 0.02	5.01 ± 0.28	4.60 ± 0.31
C_2	C	8	0.013	0.09 ± 0.03	7.06 ± 0.33	3.57 ± 0.45
	C^1	8	0.04 ± 0.02	0.04 ± 0.02	7.21 ± 0.30	3.22 ± 0.62
F_1	C x C^1	8	0.07 ± 0.02	0.14 ± 0.05	6.47 ± 0.32	4.16 ± 0.28*
	C^1 x C	8	0.04 ± 0.01	0.15 ± 0.04	6.80 ± 0.32	5.80 ± 0.51
C_2	D	8	0.013	0.05 ± 0.02	6.94 ± 0.42	4.26 ± 0.51
	D^1	8	0.03 ± 0.01	0.08 ± 0.04	7.24 ± 0.42	4.45 ± 0.77
F_1	D x D^1	8	0.013	0.13 ± 0.04	5.82 ± 0.27	5.16 ± 0.31***
	D^1 x D	8	0.006	0.06 ± 0.02	6.49 ± 0.55	7.46 ± 0.58

[a] A total of 20 sporocytes were scored per plant; [b] Mean ± SE/Cell/Plant.

* ($P \leq 0.05$); ** ($P \leq 0.01$); *** ($P \leq 0.005$) for means within pairs of comparable generations.

and trivalent formation. The remainder of the comparisons showed a quadrivalent frequency ranging from 0.0 to 0.15 configurations/cell. However, neither reciprocal F_1's nor C_2's differed significantly in their frequencies of multivalent formation.

b) Chromosome pairing frequency

As was true for the frequency of univalents the chromosome arm-pair frequency was influenced by the source of cytoplasm carried by the female parent (Table 2, Fig. 1a-1d).

With one exception, F_1 and C_2 populations derived from crosses in which the female carried hexaploid wheat cytoplasm had higher numbers of chromosome arm pairs than their reciprocals in tetraploid wheat cytoplasm. The exception involved the C_2 population of a Manitou x turgidum cross; however, the difference was not statistically significant. On the other hand, in 6 of the 7 reciprocal F_1 and C_2 pairs in which hexaploid cytoplasm exhibited beneficial effects, differences were significant ($P \leqslant 0.10$; to $P \leqslant 0.001$). A factorial analysis of variance showed that in both the F_1 and C_2 populations, genotypic as well as cytoplasmic effects were significant ($P \leqslant 0.01$). In the C_2 population however the main effects were not significant when tested against the genotype x cytoplasm interaction (Table 4).

c) Pollen viability

As shown in Table 3, the percentage of viable pollen of both the C_2 and F_1 populations were consistently higher when the female involved

Table 2. Chromosome arm pairs at first metaphase of C_2 and reciprocal F_1 triticale populations as influenced by source of wheat cytoplasm

Generation	Designation	No. plants analysed[a]	Mean	SE	Level of Sig.
C_2	A	8	37.58	0.14	$P \leqslant 0.001$
	A^1	8	36.19	0.10	
F_1	A x A^1	8	35.53	0.42	$P \leqslant 0.05$
	A^1 x A	8	34.03	0.40	
C_2	B	8	32.62	0.36	$P \leqslant 0.01$
	B^1	8	28.54	0.86	
F_1	B x B^1	8	33.83	0.48	$P \leqslant 0.10$
	B^1 x B	8	32.29	0.55	
C_2	C	8	31.28	0.63	n.s.
	C^1	8	31.53	0.86	
F_1	C x C^1	8	31.23	0.50	$P \leqslant 0.02$
	C^1 x C	8	29.25	0.51	
C_2	D	8	30.79	0.86	n.s.
	D^1	8	30.23	1.17	
F_1	D x D^1	8	30.90	0.52	$P \leqslant 0.05$
	D^1 x D	8	27.99	1.02	

[a] A total of 20 sporocytes were analysed per plant.

Figure 1

Meiotic metaphase I configurations and pollen of triticales synthesized with either hexaploid or tetraploid wheat cytoplasm.

(a) M_I of a C_2 triticale involving Pitic x T. turgidum, synthesized with Pitic-cytoplasm (x 1050).

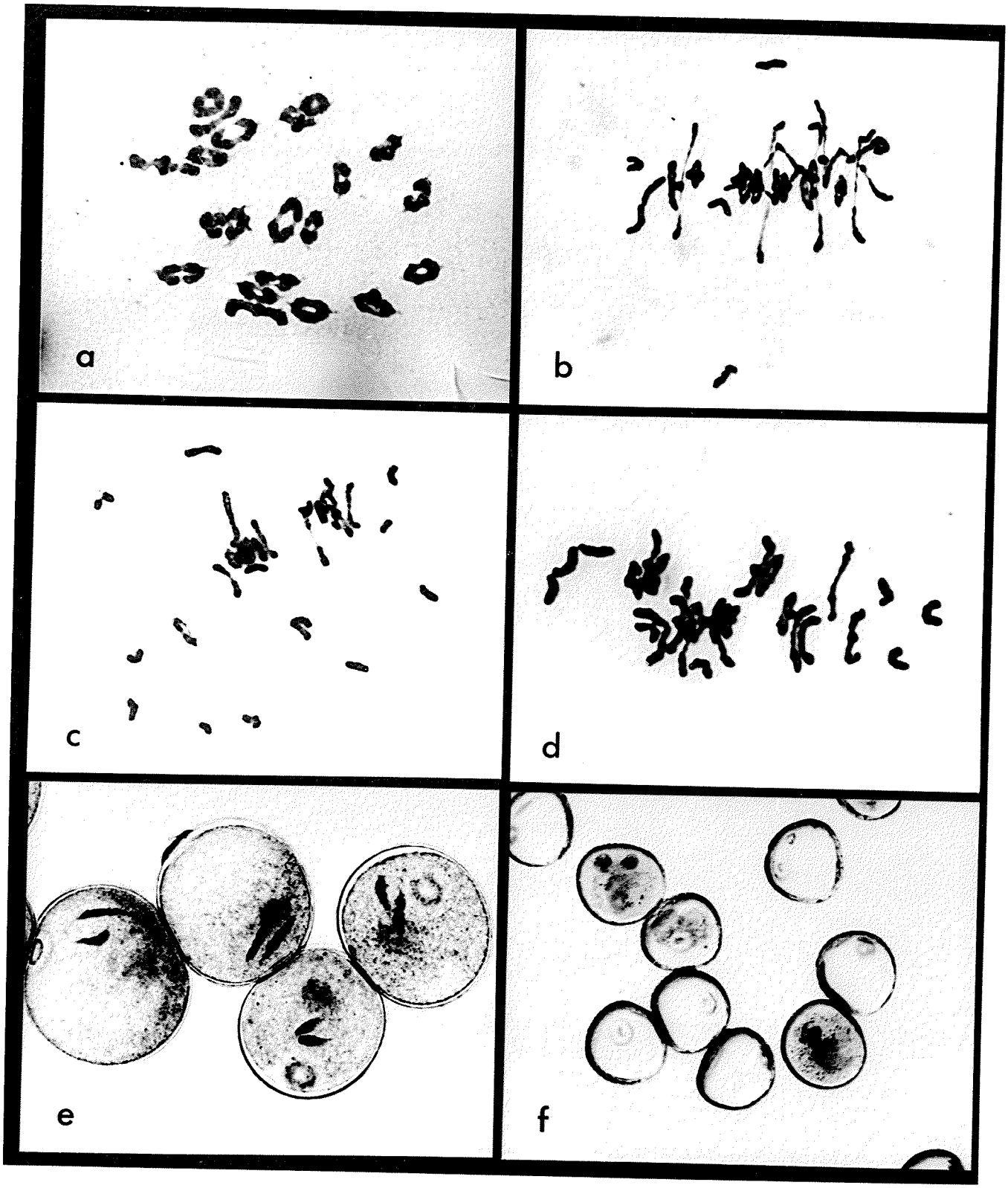
(b) M_I of a reciprocal F_1 triticale involving T. turgidum x Manitou, synthesized with turgidum-cytoplasm (x 870).

(c) M_I of a reciprocal F_1 triticale involving T. orientale x Manitou, synthsized with orientale-cytoplasm (x 860).

(d) M_I of a reciprocal F_1 triticale involving T. turgidum x Pitic, synthesized with turgidum-cytoplasm (x 880).

(e) Normal pollen of a C_2 triticale involving Manitou x T. turgidum, synthesized with Manitou-cytoplasm (x 460).

(f) Abnormal pollen of a reciprocal F_1 triticale involving T. durum x Pitic, synthesized with durum-cytoplasm (x 300).


a
b
c
d
e
f

in the cross carried hexaploid wheat cytoplasm. Statistical significance was attained in all of the eight comparisons ($P \leqslant 0.10$; to $P \leqslant 0.001$). All classes of abnormal pollen as described by Kihara (1958) were observed including those with two round sperm nuclei, as well as binucleate, uninucleate, and empty grains. The highest pollen abnormality was observed in the F_1 population involving durum x Pitic (Fig. 1e, f).

A factorial analysis of variance of both the F_1 and C_2 populations indicated that differences due to genotype and cytoplasm were significant at the 0.05% level, but only at the 5% level when tested against the respective significant genotype x cytoplasm interactions (Table 4).

2. Fertility

Fertility was consistently and significantly lower in those F_1 and C_2 plant populations derived from crosses in which the female carried tetraploid cytoplasm (Table 5). In general, plants with hexaploid cytoplasm show beneficial effects for all the five characters analysed including kernels per spike, kernels per spikelets, kernels per spikelet in only primary and secondary florets, as well as overall percentage of florets bearing seeds.

Of the forty reciprocal comparisons made, only 4 failed to exhibit significant differences. The exceptions involved the C_2 population of Manitou in combination with either T. turgidum or T. orientale.

Table 3. Pollen viability of C_2 and reciprocal F_1 triticale populations as influenced by source of wheat cytoplasm

Generation	Designation	No. plants analysed[a]	Mean (%)	SE (%)	Level of Sig.
C_2	A	12	94.17	0.58	$P \leqslant 0.01$
	A^1	12	87.18	1.58	
F_1	A x A^1	10	87.70	1.85	$P \leqslant 0.10$
	A^1 x A	10	83.24	1.24	
C_2	B	9	85.27	1.86	$P \leqslant 0.001$
	B^1	9	52.75	2.63	
F_1	B x B^1	11	64.27	2.89	$P \leqslant 0.001$
	B^1 x B	11	40.95	3.92	
C_2	C	10	84.66	2.64	$P \leqslant 0.001$
	C^1	10	59.62	4.18	
F_1	C x C^1	9	77.23	2.86	$P \leqslant 0.01$
	C^1 x C	9	64.79	3.06	
C_2	D	10	79.67	4.91	$P \leqslant 0.05$
	D^1	10	64.16	4.46	
F_1	D x D^1	11	70.34	4.03	$P \leqslant 0.05$
	D^1 x D	11	56.06	4.31	

[a] A minimum of 200 pollen were analysed per plant.

Table 4. Mean squares for chromosome arm pairs and percent pollen viability of C_2 and reciprocal F_1 triticale populations

Source of variation	Arm pairs at M_I		Pollen viability		Test against G x C		
					Arm pairs	Pollen	
	C_2	F_1	C_2	F_1	C_2	C_2	F_1
Genotype (G)	149.2*** (3)	98.2*** (3)	1999*** (3)	3896*** (3)	n.s.	n.s.	$P \leqslant 0.05$
Cytoplasm (C)	33.4** (1)	62.7*** (1)	8214*** (1)	3805*** (1)	n.s.	$P \leqslant 0.05$	$P \leqslant 0.05$
G x C	14.1^{+} (3)	$1.73^{n.s.}$ (3)	628*** (3)	305* (3)			
Error	4.13 (56)	2.70 (56)	98 (74)	110 (74)			

In parenthesis = degrees of freedom.

+ ($P \leqslant 0.10$), * ($P \leqslant 0.05$), ** ($P \leqslant 0.01$), *** ($P \leqslant 0.005$).

Table 5. Fertility of C_2 and reciprocal F_1 populations as influenced by source of wheat cytoplasm

Generation	Designation	No. plants examined	Kernels/Spike	Kernels/Spikelet	Kernels (1,2)/Spike	Kernels (1,2)/Spikelet	% Florets with Seeds
C_2	A	10	[b]43.90 ± 0.9***	1.63 ± 0.06***	41.00 ± 1.6***	1.46 ± 0.03***	55.41 ± 2.5***
	A^1	10	30.20 ± 2.1	1.13 ± 0.06	29.20 ± 2.1	1.08 ± 0.06	40.08 ± 2.3
F_1	A x A^1	10	34.00 ± 1.3*	1.38 ± 0.05***	31.60 ± 1.4+	1.28 ± 0.05***	48.00 ± 1.6***
	A^1 x A	10	29.70 ± 1.0	1.07 ± 0.03	28.20 ± 1.0	0.96 ± 0.04	35.80 ± 1.5
C_2	B	8	38.00 ± 1.5***	1.37 ± 0.04**	36.88 ± 1.6***	1.33 ± 0.04***	47.73 ± 1.3**
	B^1	8	22.38 ± 1.6	1.11 ± 0.07	20.63 ± 1.1	1.02 ± 0.05	38.54 ± 2.6
F_1	B x B^1	8	29.38 ± 1.3**	1.23 ± 0.04**	28.38 ± 1.4**	1.22 ± 0.06**	45.87 ± 1.8***
	B^1 x B	8	23.38 ± 0.8	1.00 ± 0.04	22.75 ± 0.8	0.98 ± 0.04	35.69 ± 1.4
C_2	C	10	38.80 ± 1.9**	1.49 ± 0.06*	36.20 ± 1.7*	1.39 ± 0.06+	51.74 ± 2.1
	C^1	10	30.00 ± 1.9	1.23 ± 0.07	28.80 ± 2.0	1.19 ± 0.08	44.71 ± 3.0
F_1	C x C^1	10	36.80 ± 0.9***	1.41 ± 0.04***	34.80 ± 1.2***	1.34 + 0.05***	48.07 ± 1.3**
	C^1 x C	10	28.50 ± 1.0	1.11 ± 0.04	27.00 ± 0.9	1.05 ± 0.03	41.02 ± 1.9
C_2	D	9	30.67 ± 1.3	1.47 ± 0.06***	28.33 ± 1.3	1.27 ± 0.08	51.06 ± 1.9***
	D^1	9	28.44 ± 1.4	1.19 ± 0.04	27.44 ± 1.6	1.15 ± 0.04	41.51 ± 1.3
F_1	D x D^1	[a]9	32.33 ± 0.9***	1.32 ± 0.03***	30.11 ± 1.3**	1.22 ± 0.04**	47.82 ± 1.7***
	D^1 x D	[a]9	25.89 ± 1.1	1.05 ± 0.05	24.44 ± 0.8	0.94 ± 0.06	35.32 ± 2.3

[a] For kernels (1,2)/Spikelets, and % Florets with seeds N = 10; [b] Mean ± SE.

+ ($P \leqslant 0.10$), * ($P \leqslant 0.05$), ** ($P \leqslant 0.01$), *** ($P \leqslant 0.001$) for means within pairs of comparable generations.

A factorial analysis of variance indicated that differences in all components of fertility due to cytoplasm were significant in both the F_1 and C_2 populations. Differences due to genotype were not observed for either kernels (primary and secondary) per spikelet or for seed-set expressed on a percentage basis for both the C_2 and reciprocal F_1 populations (Table 6).

3. Seed yield

Two yield factors were measured, viz. yield per plant (g.) and 25 kernel weight (g.). As shown in Tables 7 and 8, higher plant yields as well as increased kernel weight were consistently observed in those triticales in which the female parent carried hexaploid wheat cytoplasm.

A factorial analysis of variance indicated that differences in seed yield per plant due to either genotype or cytoplasms were highly significant in both the F_1 and C_2 populations. Insofar as 25 kernel weight was concerned, cytoplasmic effect was significant only in the C_2 population, while genotypic effects were observed to be significant in both the F_1 and C_2 populations (Table 9).

4. Seed quality

In determining seed density, the number of seed samples from each reciprocal cross varied from 10 to 18, with approximately 15 seeds constituting a sample. As shown in Table 10, the density of seed produced on F_1 and C_2 plants carrying hexaploid wheat cytoplasm was consistently higher relative to the reciprocal member of a pair possessing

Table 6. Mean square values for fertility of C_2 and reciprocal F_1 populations

Source of variation	Kernels/Spike		Kernels/Spikelets		Kernels (1,2)/Spike		Kernels (1,2)/Spikelets		% Florets with Seeds		Test against G x C	
	C_2	F_1	C_2	F_1	C_2	F_1	C_2	F_1	C_2	F_1	Kernels/Spike C_2	Kernels (1,2)/ Spike C_2
Genotype (G)	232*** (3)	149*** (3)	0.07+ (3)	0.07*** (3)	207*** (3)	109*** (3)	0.05 (3)	0.05 (3)	97 (3)	50 (3)	n.s.	n.s.
Cytoplasm (C)	1882*** (1)	725*** (1)	1.95*** (1)	1.40*** (1)	1527*** (1)	584*** (1)	1.17*** (1)	1.50*** (1)	1953*** (1)	2091*** (1)	$P \leqslant 0.05$	$P \leqslant 0.10$
G x C	164*** (3)	12 (3)	0.06 (3)	0.006 (3)	196*** (3)	15 (3)	0.06 (3)	0.004 (3)	58 (3)	30 (3)		
Error	25 (66)	11 (66)	0.03 (66)	0.014 (66)	26 (66)	12 (66)	0.03 (66)	0.02 (68)	47 (66)	28 (68)		

In parenthesis = degrees of freedom.

+ ($P \leqslant 0.10$), * ($P \leqslant 0.05$), *** ($P \leqslant 0.005$).

Table 7. Seed yield/plant (g) of C_2 and reciprocal F_1 populations as influenced by source of wheat cytoplasm

Generation	Designation	No. of plants analysed	Aritmetic means	Analysed means and SE	Level of Sig.
C_2	A	10	9.39	0.12 ± 0.01	$P \leqslant 0.01$
	A^1	10	4.29	0.27 ± 0.03	
F_1	A x A^1	10	9.55	0.11 ± 0.01	n.s.
	A^1 x A	10	8.74	0.12 ± 0.01	
C_2	B	9	2.87	0.38 ± 0.04	$P \leqslant 0.01$
	B^1	9	1.82	0.58 ± 0.05	
F_1	B x B^1	9	2.84	0.36 ± 0.02	n.s.
	B^1 x B	9	2.61	0.42 ± 0.05	
C_2	C	9	3.14	0.35 ± 0.04	n.s.
	C^1	9	2.45	0.49 ± 0.08	
F_1	C x C^1	9	3.29	0.33 ± 0.03	n.s.
	C^1 x C	9	2.71	0.41 ± 0.05	
C_2	D	10	2.21	0.49 ± 0.04	$P \leqslant 0.001$
	D^1	10	2.11	0.52 ± 0.06	
F_1	D x D^1	10	3.34	0.32 ± 0.02	$P \leqslant 0.01$
	D^1 x D	10	2.38	0.45 ± 0.03	

Table 8. 25 Kernel weight (g) of C_2 and reciprocal F_1 populations as influenced by source of wheat cytoplasm

Generation	Designation	No. of plants analysed	Mean	SE	Level of Sig.
C_2	A	13	0.93	0.05	n.s.
	A^1	13	0.89	0.06	
F_1	A x A^1	10	1.09	0.06	n.s.
	A^1 x A	10	1.15	0.02	
C_2	B	8	1.25	0.03	$P \leqslant 0.001$
	B^1	8	0.94	0.04	
F_1	B x B^1	9	1.28	0.04	n.s.
	B^1 x B	9	1.24	0.03	
C_2	C	10	1.06	0.03	$P \leqslant 0.05$
	C^1	10	0.94	0.04	
F_1	C x C^1	9	1.00	0.04	n.s.
	C^1 x C	9	0.97	0.04	
C_2	D	8	1.13	0.05	$P \leqslant 0.05$
	D^1	8	0.87	0.09	
F_1	D x D^1	10	1.13	0.03	n.s.
	D^1 x D	10	1.05	0.05	

Table 9. Mean squares for yield/plant and 25 kernel weight of C_2 and reciprocal F_1 populations

Source of variation	Yield/Plant		25 Kernel Weight		Test against G x C
	C_2	F_1	C_2	F_1	25 Kernel wt. C_2
Genotype (G)	0.37*** (3)	0.34*** (3)	0.11* (3)	0.24*** (3)	n.s.
Cytoplasm (C)	0.33*** (1)	0.09*** (1)	0.64*** (1)	0.01 (1)	$P \leqslant 0.10$
G x C	0.02 (3)	0.01 (3)	0.07+ (3)	0.02 (3)	
Error	0.02 (68)	0.01 (68)	0.03 (70)	0.02 (68)	

In parenthesis = degrees of freedom.

+ ($P \leqslant 0.10$), * ($P \leqslant 0.05$), *** ($P \leqslant 0.005$).

cytoplasm from tetraploid wheat (Fig. 2). Differences in mean seed density were statistically significant in 4 of the 8 comparisons, involving the reciprocal F_1's of Manitou x orientale and Manitou x turgidum, and also the C_2's of Pitic x durum and Manitou x turgidum. The factorial analysis of variance of densities of seed from both F_1 and C_2 populations indicated that differences due to genotype and cytoplasm were significant ($P \leqslant 0.05$). The genotype x cytoplasm interaction was also significant for the C_2 population and when the main effects were tested against this significant interaction, cytoplasmic effects remained significantly different (Table 11).

As with seed density a beneficial effect was also observed for plants in hexaploid cytoplasm when analysed for alpha-amylase activity. The mean activity of two plants per F_1 or C_2 population showed that seed samples in tetraploid cytoplasm possessed consistently higher activities than their counterparts in hexaploid cytoplasm (Table 10).

A factorial analysis of variance indicated that variations in alpha-amylase due to cytoplasm were significant for both the C_2 and F_1 populations ($P \leqslant 0.05$). The genotypic effect was significant only in the C_2 population (Table 11). As would be expected, a highly significant negative correlation was observed between seed density and alpha-amylase activity ($P \leqslant 0.01$, Table 10).

Table 10. Seed quality of C_2 and reciprocal F_1 triticale populations as influenced by source of wheat cytoplasm

Generation	Designation	Seed density[a] No. of plants	Seed density[a] Mean ± SE	α-Amylase activity[b] No. of plants	α-Amylase activity[b] Mean ± SE
C_2	A	13	1.225 ± 0.03	2	6.57 ± 0.3
	A^1	13	1.223 ± 0.01	2	20.67 ± 4.7
F_1	A x A^1	10	1.239 ± 0.03	2	11.91 ± 0.7*
	A^1 x A	10	1.182 ± 0.02	2	46.17 ± 0.2
C_2	B	8	1.207 ± 0.03**	2	8.50 ± 1.2
	B^1	8	0.927 ± 0.07	2	36.30 ± 27
F_1	B x B^1	9	1.217 ± 0.03	2	10.86 ± 4.5
	B^1 x B	9	1.153 ± 0.03	2	23.30 ± 4.6
C_2	C	10	1.143 ± 0.04+	2	44.48 ± 8.5
	C^1	10	1.004 ± 0.07	2	55.54 ± 8.5
F_1	C x C^1	9	1.224 ± 0.03***	2	24.17 ± 5.2+
	C^1 x C	9	1.031 ± 0.03	2	56.92 ± 0.1
C_2	D	8	1.225 ± 0.03	2	3.17 ± 0.3
	D^1	8	1.095 ± 0.08	2	20.00 ± 12
F_1	D x D^1	9	1.209 ± 0.03**	2	18.36 ± 7.3+
	D^1 x D	9	1.025 ± 0.05	2	51.33 ± 1.3

Regression coefficient of seed density on α-amylase b = -136.9***
" " " α-amylase on seed density b = -0.004***
r = -0.73 **

[a] g/cc; [b] IDC units/mg.

+ ($P \leqslant 0.10$), * ($P \leqslant 0.05$), ** ($P \leqslant 0.01$), *** ($P \leqslant 0.005$) for means within pairs of comparable generations.

Table 11. Mean square values for seed quality of C_2 and reciprocal F_1 populations

Source of variation	Seed density		α-Amylase activity		Test against G x C	
					Seed Density	α - Amylase
	C_2	F_1	C_2	F_1	C_2	F_1
Genotype (G)	0.105** (3)	0.037* (3)	1253.9* (3)	402.7** (3)	n.s.	n.s.
Cytoplasm (C)	0.369** (1)	0.287** (1)	1217.4* (1)	3159.9** (1)	$P \leqslant 0.10$	$P \leqslant 0.025$
G x C	0.061* (3)	0.025 (3)	53.2 (3)	109.5* (3)		
Error	0.020 (70)	0.009 (66)	269.5 (8)	31.1 (8)		

In parenthesis = degrees of freedom.

* ($P \leqslant 0.05$), ** ($P \leqslant 0.005$).

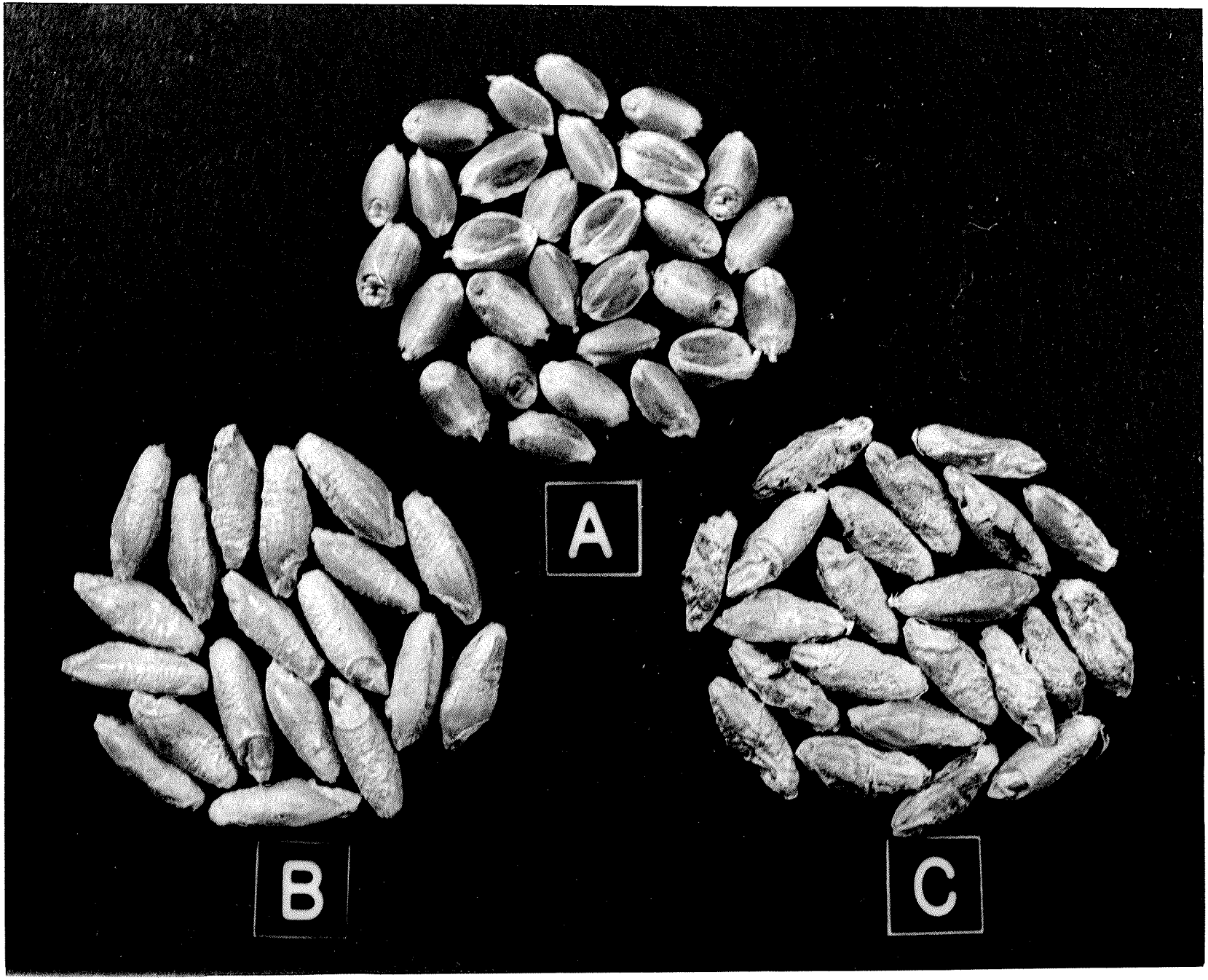
Figure 2

Kernel development of triticales possessing either hexaploid or tetraploid wheat cytoplasm, as compared to hexaploid wheat.

(A) Triticum aestivum L. em. Thell. cv. Manitou.

(B) Triticale possessing hexaploid wheat cytoplasm.

(C) Triticale possessing tetraploid wheat cytoplasm.


A
B
C

DISCUSSION

For the improvement of triticale as a new crop species, more and more genetic variability must be introduced into the breeding populations. Accordingly, triticale workers throughout the world are utilizing various breeding techniques in order to incorporate new germ plasm from wheat and rye. Sisodia and McGinnis (1970b) outlined various methods of accomplishing this, one of which involves the synthesis of "secondary hexaploid triticale" which either directly or indirectly utilizes the germ plasm of hexaploid wheat. From 8x x 6x triticale or 6x wheat x 6x triticale, for example, the cytoplasm of the derived secondary hexaploid triticale is of common-wheat origin. Improved seed-set and seed development on hexaploid derivatives from 8x x 6x triticale crosses have been reported by Kiss (1966), Pissarev (1966), and Sisodia and McGinnis (1970a).

In the present study, differences between reciprocal crosses having the same genotype but with different cytoplasms are used to measure nuclear-cytoplasmic interactions in triticale, as well as the effects of cytoplasm per se. Since the conditions under which the test material was grown was controlled as closely as possible it can be assumed that the confounding of genotype or cytoplasmic effects with environmental influence was minimal. The validity of the assumption that differences observed between the reciprocal F_1 and C_2 population were free of genetic influences has been stated (Larter and Hsam, 1973; and Section I

of thesis). Maternal influence was considered to be absent in measurements involving seeds and seed development, since comparisons were made on seed produced on reciprocal F_1 plants (i.e. F_2 seeds), and C_3 seeds produced on C_2 plants.

In the present study, differential interaction of an AABBRR nucleus in hexaploid or tetraploid wheat cytoplasm was demonstrated. A clearly consistent beneficial relationship existed in favour of triticales possessing hexaploid-wheat cytoplasm compared to their reciprocals when assessed in terms of meiotic stability, pollen viability, seed-set, seed yield, kernel development and lower alpha-amylase enzyme activity. These findings are of importance, since partial sterility and shrivelling of seeds due to poor development of the endosperm are the major factors limiting yields in today's triticale (Zillinsky and Borlaug, 1971). More recently Shealy and Simmonds (1973) using electron microscopy to study the relationship between developmental morphology and grain shrivelling in hexaploid triticale, observed that poor seed development could arise as a result of invaginations or deletions that occur in the aleurone layer of the seed. This, in turn, resulted in subsequent malformation of adjacent endosperm tissue. Klassen, et al. (1971) studying 8 triticale lines observed that those with the highest alpha-amylase activities also exhibited the poorest kernel type and lowest seed density. In addition, Klassen (1970) suggests that precocious release of alpha-amylase may lead to premature digestion of starch

granules, thus loss of kernel weight. We tentatively suggest that these problems involve a genetic-cytoplasmic interaction rather than being entirely of a genic nature.

As for the original source of cytoplasm of hexaploid wheats, the donor species within the Triticinae has not been clearly defined. From experiments involving reciprocal crosses between emmer and Aegilops squarrosa L., Kihara (1966) is of the opinion that emmer wheats were the donor. Suemoto (1968) presents evidence which indicates that both emmer and hexaploid wheat have cytoplasm of Ae. speltoides Tausch. or its near relative. In contrast, Maan and Lucken (1971, 1972) reported that the initial hybrids involving either durum or T. aestivum in Ae. squarrosa's cytoplasm were normal in fertility and plant vigour, which suggests squarrosa as the possible cytoplasm donor. However, they noted that upon carrying out additional backcrosses in order to complete the substitution of the durum genome into Ae. squarrosa cytoplasm, the frequency of occurrence of non-viable seeds increased (Maan, 1973). Seedlings could be obtained only by culturing embryos from plants which carried either a whole or telocentric chromosome from the D genome. Kihara (1973) is of the opinion that the D genome contains restoring genes for squarrosa cytoplasm, and presents evidence to suggest that the cytoplasm of squarrosa is different from that of emmer wheat.

Nevertheless, assuming the cytoplasm of hexaploid wheat to be of tetraploid origin, it remains clear that it has become modified through

evolution to co-exist harmoniously with the additional genome contributed by Ae. squarrosa. Tarkowski (1972) as cited by Kiss and Tréfas (1973), is currently engaged in determining by electron microscopy the morphological and structural differences between cytoplasms of hexaploid triticale (essentially tetraploid wheat cytoplasm) and that of hexaploid wheat. Furthermore, that modification of the genomic-cytoplasmic components did occur in today's hexaploid wheats was demonstrated by the work of Kerber (1964) and Kaltsikes, et al. (1969), who, by repeated backcrossing extracted the AB tetraploid genomes from cultivars of hexaploid wheat. On the basis of chromosomal pairing in hybrids of extracted x normal tetraploids, they concluded that genomically the two tetraploid wheats were identical. Nevertheless the extracted forms phenotypically resembled their hexaploid parent more closely than any forms of tetraploid wheat. More recently, the work of Thomas and Kaltsikes (1972) has shown that triticales synthesized from extracted tetraploids were more regular in their chromosome behaviour than triticales produced from the natural tetraploid wheat species T. turgidum L. var. durum. The authors attributed this difference to the adaptation that had taken place in the bread wheats in response to the presence of the alien D genome. We suggest further that since the extracted tetraploids possessed essentially hexaploid wheat cytoplasm, interaction of an ABR nucleus with this cytoplasm should also be considered.

It has been shown that induced mutational changes in durum-triticale's cytoplasm produced beneficial effects when assessed in

terms of endosperm development (Sánchez-Monge, 1968). Also as suggested by Sisodia and McGinnis (1970a), problems associated with wheat-rye hybrids could be due to the imbalance of nuclear-cytoplasmic (C:N) ploidy ratio. Established species including common wheats would be expected to have a C:N ploidy ratio of 1:1. Thus a turgidum-triticale and aestivum-triticale would possess a C:N ratio of 4:6, and 6:8 respectively. According to this hypothesis one would expect that the more divergent the C:N ratio is from 1:1, the more chance there will be of a disturbed nuclear-cytoplasmic relationship. A secondary hexaploid triticale derived from an 8x x 6x triticale cross, as well as the triticales carrying hexaploid cytoplasm as do those used in the present study, would be expected to have a balanced C:N ratio.

On the basis of these findings, the synthesis and utilization of secondary hexaploid triticale should be used as a technique in the breeding methodology of a triticale program. Equally important is the realization by workers engaged in the area of interspecific and intergeneric hybridization that co-adaptation of nucleus and cytoplasm is accomplished only after many generations of intensive selection and that man-made allopolyploids are still relatively new and unadjusted compared to today's established or native crop species.

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SECTION III

INTERRELATIONSHIPS AMONG AGRONOMIC ATTRIBUTES IN HEXAPLOID TRITICALE AS INFLUENCED BY SOURCE OF WHEAT CYTOPLASM

INTRODUCTION

Basically, triticale is a man-made species derived from crosses between species of the genera Triticum and Secale. Depending on the wheat parents used, two forms of triticale, viz. the hexaploid ($2n = 6x = 42$) and the octoploid ($2n = 8x = 56$) are obtained. Accordingly, triticales possess either tetraploid or hexaploid wheat cytoplasm respectively. Kiss (1966, 1971), and Pissarev (1966) obtained an improvement of the hexaploid derivatives when $6x$ wheat cytoplasm is introduced by way of $8x \times 6x$ triticale crosses. In our own work, as reported by Larter and Hsam (1973; and Sections I and II of this thesis) we observed that identical triticale genotypes performed more efficiently in $6x$ wheat cytoplasm than in $4x$'s, when assessed in terms of meiotic stability, seed-set, seed yield, seed development, and certain agronomic attributes including number of fertile tillers and plant height.

Utilizing the information obtained from our previous investigations, the objective of the present study was twofold: (1) to compare the association between agronomic characters in triticales possessing either $6x$ or $4x$ wheat cytoplasm; and (2) to explain such associations in terms of certain causal influences (factors). Such an approach would be helpful in revealing and evaluating the unidentified sources of common variation for the characters concerned.

MATERIALS AND METHODS

The C_2 and reciprocal F_1 populations of triticales synthesized in either hexaploid or tetraploid wheat cytoplasm (for parentage see Larter and Hsam, 1973; and in Section I of this thesis) were grown in the greenhouse in a completely random design.

The following agronomic characters were measured per plant:

(1) Number of spikelets per spike.

(2) Spike length (cm).

(3) Yield per plant (g).

(4) 25 Kernel weight (g).

(5) Seed density (g/cc) which was quantitatively evaluated by measuring liquid displacement upon adding a predetermined weight of seed sample to a 5 ml. volume of paraffin oil (Larter and Hsam, 1973).

(6) Plant height (cm), measured from the ground to the tip of the tallest spike excluding awns.

(7) Number of fertile tillers per plant.

(8) Number of kernels per spike.

(9) Number of florets per spike.

(10) Percentage of florets bearing seeds.

(11) Number of kernels per spikelet.

(12) Percentage of viable pollen, as determined by the method of Kihara (1958) in which grains with two elliptical sperm nuclei and one spherical vegetative nucleus were scored as viable.

Values for all spike characteristics and seed-set were based on the mean of 2 spikes per plant. The values for plants carrying hexaploid wheat cytoplasm, and those which carried tetraploid wheat cytoplasm were separately subjected to correlation and factor analyses. In the latter, extraction of the original factor matrix was by means of principle factoring using the mathematical techniques described by Cattell (1965). The factor matrix so obtained was rotated to a more easily interpretable structure by the varimax method (Harman, 1967). The criterion used to determine the number of factors to be retained was based on a 4% constant limit. As such, individual factors accounting for less than 4% of the total variation were not included.

RESULTS

1. Comparison of mean values

As shown in Table 1, mean values for all characteristics studied were higher in triticales possessing hexaploid wheat cytoplasm relatives to their counterparts carrying tetraploid cytoplasm. Of the various characters studied, yield per plant showed the highest coefficients of variation (C.V.) of approximately 70% in both triticale populations followed by the number of fertile tillers per plant (C.V. = 35%). In general, plants possessing 6x wheat cytoplasm showed higher variability for morphological characters including height, spike length, number of spikelets and number of florets per spike. On the other hand their counterparts in 4x wheat cytoplasm possessed a higher variability for seed characteristics including seed yield and also for pollen viability. Yield and seed-set were low in both populations since growing conditions were sub-optimal in the greenhouses. Notwithstanding this, triticales synthesized with 6x wheat cytoplasm was approximately 15% higher in percentage of viable pollen, and 10% higher in percentage of fertile florets.

2. Simple phenotypic correlations

A total of sixty-six correlations were possible among the twelve characters studied. Of these nineteen and forty-four significant correlations were obtained for triticales in common wheat (6x) and

Table 1. Means and coefficients of variation for 12 agronomic characters in two genetically identical triticale populations differing only in their cytoplasm

	Triticales with 6x wheat cytoplasm[1]		Triticales with 4x wheat cytoplasm[1]	
	Mean	C.V.(%)	Mean	C.V.(%)
Spikelets/Spike	25.41	13.12	25.09	11.79
Spike Length (cm.)	10.16	13.20	9.40	11.37
Yield/Plant (g.)	4.84	70.35	3.54	70.47
25 Kernel Weight (g.)	1.09	15.20	1.01	17.97
Seed Density (g./cc.)	1.21	7.84	1.08	15.67
Plant Height (cm.)	115.46	15.01	108.48	12.14
Fertile Tillers/Plant	5.14	38.36	4.10	42.86
Kernels/Spike	36.09	16.04	27.73	17.98
Florets/Spike	72.73	13.69	71.21	12.46
% Florets With Seeds	49.76	11.14	39.20	15.91
Kernels/Spikelet	1.42	11.34	1.11	15.42
% Viable Pollen	81.08	16.25	66.50	25.92

[1] n = 70 plants.

emmer wheat (4x) cytoplasm respectively (Table 2). Except for one case, all the significant correlations observed in plants possessing common wheat cytoplasm were also observed in those synthesized with emmer cytoplasm. The exception involved a negative correlation, significant at the 5% level, between 25 kernel weight and percentage of viable pollen. In the correlation patterns, some distinct similarities as well as differences were observed between the two populations (i.e. those synthesized in common and emmer cytoplasms respectively). In both populations, percent viable pollen correlated significantly with yield per plant, and kernels per spike ($P \leqslant 0.01$). Although there was no obvious basis for a cause-effect relationship, percent viable pollen also correlated significantly with the numbers of spikelets per spike and florets per spike. However in both populations no correlation was observed between viable pollen and either kernels per spikelet or percentage of fertile florets. In addition, yield per plant showed no correlation with fertility including percentage florets with seeds and kernels per spikelets, which themselves were highly correlated ($P \leqslant 0.01$). Moreover, in addition to the difference in the total number of significant correlations, one most striking difference between the two plant populations was in the correlation pattern of seed density. In the population possessing emmer cytoplasm, a significant correlation was observed between seed density and many of the characters studied. Exceptions involved components of fertility including kernels per spike, kernels per

Table 2. Phenotypic correlation matrix among agronomic attributes of triticales synthesized in either common or emmer wheat cytoplasm[1]

	Character	1	2	3	4	5	6	7	8	9	10	11	12
1	Spikelets/Spike		0.69**	-	-	-	-	-	0.73**	0.99**	-	-	0.41**
2	Spike Length	0.66**		-	-	-	-	-	0.46**	0.68**	-	-	-
3	Yield/Plant	0.60**	0.38**		0.38**	-	0.38**	0.69**	0.24*	-	-	-	0.48**
4	25 Kernel Weight	-	0.27**	0.36**		-	-	-	-	-	-	-	-0.25*
5	Seed Density	0.30*	-	0.42**	0.41**		-	-	-	-	-	-	-
6	Plant Height	0.63**	0.63**	0.63**	0.46**	0.42**		0.32**	-	-	-	-	-
7	Fertile Tillers/Plant	0.42**	0.32**	0.76**	0.37**	0.50**	0.53**		-	-	-	-	-
8	Kernels/Spike	0.51**	0.31**	0.29*	-0.26*	-	-	0.28*		0.75**	0.50**	0.55**	0.40**
9	Florets/Spike	0.93**	0.62**	0.63**	-	0.33**	0.59**	0.43**	0.48**		-	-	0.45**
10	% Florets With Seeds	-	-	-	-0.40**	-	-	-	0.74**	-		0.99**	-
11	Kernels/Spikelet	-	-	-	-0.39**	-	0.25*	-	0.76**	-	0.95**		-
12	% Viable Pollen	0.47**	-	0.50**	-	0.25*	0.29*	0.30*	0.38**	0.40**	-	-	

[1] Values appear above the diagonal belong to triticales synthesized in common (6$\mathfrak{X}$) wheat cytoplasm, and those below to triticales synthesized in emmer (4$\mathfrak{X}$) wheat cytoplasm.

* ($P \leqslant 0.05$), ** ($P \leqslant 0.01$).

spikelet, and percentage of florets bearing seeds, as well as spike length. However, in the population possessing hexaploid wheat cytoplasm, no association was observed between seed density and any of the characters studied.

3. Factor analysis

In the population possessing hexaploid wheat cytoplasm, 7 factors were extracted which produced the inter-correlations among the various characters (Table 3). The 7 factors accounted for 96% of the total variability, and resulted in high communalities ($\geqslant$ 0.80). The communality was the amount of variance expressed by a character as accountable on the basis of all factors taken collectively. For purposes of interpretation, only those factor loadings greater than 0.5 were considered important. Using this criterion, only kernels per spike loaded on more than one factor. The largest or the most important factor (Factor 1) contained spikelets per spike, florets per spike, kernels per spike and spike length. As stated earlier, kernels per spike also possessed high loadings in factor 2 which contained fertility variables including percentage of florets bearing seeds and kernels per spikelets. Factor 3 contained seed yield and the number of fertile tillers per plant. Factors 4 to 7 arranged in order of importance contained one character each, viz. seed density, percentage of normal pollen, plant height, and 25 kernel weight respectively.

Table 3. Varimax rotated factor matrix for 12 agronomic characters of triticales synthesized in common (hexaploid) wheat cytoplasm (factor loadings x 10^3)

	Factors							Communalities
	1	2	3	4	5	6	7	
Factor 1								
Spikelets/Spike	953	- 97	74	- 3	-178	- 62	- 67	0.96
Spike Length	840	-130	-122	1	105	91	206	0.80
Florets/Spike	948	- 72	77	8	-225	- 55	- 56	0.97
Kernels/Spike	762	593	36	35	-180	- 40	-120	0.98
Factor 2								
% Florets with Seeds	-104	985	- 29	23	17	10	- 82	0.99
Kernels/Spikelets	- 62	989	- 28	34	- 49	13	- 72	0.99
Factor 3								
Fertile Tillers/Plant	41	-156	956	- 6	46	-111	- 37	0.96
Yield/Plant	- 17	176	796	89	-415	-199	-216	0.93
Factor 4								
Seed Density	10	49	39	990	- 76	- 56	77	1.00
Factor 5								
% Viable Pollen	276	34	131	84	917	- 38	-112	0.96
Factor 6								
Plant Height	28	- 18	205	59	- 46	972	- 36	1.00
Factor 7								
25 Kernel Weight	11	-140	-143	84	114	36	961	0.99

A total of 5 factors were extracted for the population possessing tetraploid wheat cytoplasm (Table 4). Together, these 5 factors accounted for approximately 89% of the total variability. The communalities ranged from 0.74 for plant height to 0.98 for kernels per spike, and also for seed density. Using the previous criterion for interpretation of factor loading, no characters except plant height loaded on more than one factor (Table 4). The most important factor (Factor 1) included spikelets per spike, florets per spike, spike length and plant height. This result indicated that these 4 characters were simultaneously influenced by some common underlying cause. Similarly, factor 2 contained attributes of fertility including kernels per spikelet, kernels per spike and percentage of florets bearing seeds. Factor 3 contained yield per plant, 25 kernel weight and the number of fertile tillers per plant. Percent normal pollen and seed density were each a factor by themselves, which indicated that within the characters studied, these aforementioned two characters were individually influenced by causes which were identified as separate from those influencing other characters.

As was true with correlation studies, some distinct similarities and differences existed in the inter-character association between the two plant populations. In both populations, seed density and percent viable pollen were each a factor by themselves. However in terms of importance, seed density accounted for more than 9% of the total variability when plants possessed hexaploid wheat cytoplasm, and less than

Table 4. Varimax rotated factor matrix for 12 agronomic characters of triticales synthesized in emmer (tetraploid) wheat cytoplasm (factor loadings x 10^3)

	Factors					Communalities
	1	2	3	4	5	
Factor 1						
Spikelets/Spike	874	- 11	203	350	76	0.93
Spike Length	870	15	154	-215	59	0.83
Florets/Spike	851	- 43	223	313	108	0.89
Plant Height	633	-132	534	- 24	183	0.74
Factor 2						
Kernels/Spikelet	-148	973	- 41	22	47	0.97
% Florets with Seeds	-143	966	- 58	19	- 72	0.96
Kernels/Spike	443	844	90	243	20	0.98
Factor 3						
Fertile Tillers/Plant	199	102	846	126	215	0.83
Yield/Plant	372	- 43	802	335	80	0.90
25 Kernel Weight	123	-349	562	-478	332	0.79
Factor 4						
% Viable Pollen	178	117	239	842	128	0.83
Factor 5						
Seed Density	157	- 72	281	100	929	0.98

5% when they possessed tetraploid wheat cytoplasm. Furthermore plant height and 25 kernel weight were each a factor by themselves in the population with 6x cytoplasm. On the other hand, in the population possessing 4x wheat cytoplasm, 25 kernel weight was associated with yield and fertile tillers, while plant height was associated with morphological characters as well as exhibiting intermediate association with 25 kernel weight and yield. In addition the percentage of unexplained (unaccountable) variance was less than 4% in plants possessing hexaploid wheat cytoplasm and more than 11% in those possessing tetraploid wheat cytoplasm. In general, the higher the proportion of accountable variance, the better are the chances for the identification and improvement of characters.

DISCUSSION

Results of the present study indicated that interrelationships among agronomic attributes were influenced by source of wheat cytoplasm. Supporting our earlier findings, mean values over all genotypes showed that triticales possessing hexaploid wheat cytoplasm perform more efficiently than their genetically identical counterparts with cytoplasm from tetraploid wheats. The association of characters are also different to some degree between triticales synthesized in either common wheat or emmer cytoplasm, as evident from the correlation analysis. The most striking difference is the lack of association between seed density and any other plant characters in the population possessing common wheat cytoplasm, whereas significant associations with most characters including yield and kernel weight were observed in the population possessing emmer cytoplasm. Unexpectedly, in both plant populations we observed a lack of correlation between percent viable pollen and kernels per spikelet, while a significant correlation was obtained between pollen viability and either kernels per spike or yield per plant. For hexaploid triticales we found that there was a significant correlation between percent viable pollen and seed-set when assessed in terms of kernels per spikelet in the primary and secondary florets only (Hsam and Larter, 1973). Although the same association was not studied in the present investigation, it appears that the variability introduced by the number of seeds in the tertiary and quaternary florets could

offset the correlation.

A correlation between two characters however, does not always necessarily imply a cause-and-effect relationship. Factor analysis, a multivariate statistical technique, provides a useful tool in explaining the inter-correlations among a set of characters (Lawley and Maxwell, 1963; Harman, 1967). This method basically reduces a large number of correlated characters to a small number of uncorrelated main factors. In addition it also helps to identify the number and the nature of the common underlying influences which produce the inter-correlations among the set of characters. From a practical standpoint, a knowledge of the common causal influences affecting a specific character could enhance the selection and improvement of that character. This knowledge is very valuable for any crop species, since only after obtaining this information is a plant breeder able to concentrate more specifically on identifying superior genotypes.

Our findings indicated that the importance of each of the factors extracted, and the characters belonging to individual factors were, by and large, different for the two triticale populations; particularly those characters associated with the less important factors (Factors 4 to 7; Tables 3 and 4). Nevertheless, they involved characters considered important for improvement of hexaploid triticales, especially seed density. Morphological characters of spikes including the numbers of spikelets, florets and the length of spikes were included in the

most important factor (Factor 1), in both triticale populations. Therefore, the expressions of these 3 characters were simultaneously affected by some common underlying influence which appeared similar for both populations. In our earlier findings (in Section I of this thesis) only minor differences were observed in reference to these characters between the two triticale populations. It is suggested that the expressions of these 3 morphological characters, are under genetic control rather than a result of nuclear-cytoplasmic influence. In both populations, fertility, yield and seed density as well as percentage of viable pollen are being influenced by different underlying factors. A similar pattern of association was also observed earlier by Hsam and Larter (1973) for hexaploid triticale hybrids.

In reference to these characters, it appears that by improving a character in one factor would not necessarily improve some other character in a different factor. This is further supported by the prevalence of seed shrivelling in today's triticales which have undergone selection for other attributes; e.g. improved yields and for other desirable agronomic attributes. Since triticales possessing hexaploid wheat cytoplasm perform better in terms of fertility, seed development, and for other general agronomic characteristics, it is suggested that the utilization of these forms in a triticale program would in all likelihood enhance its success and development.

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SECTION IV

BIOCHEMICAL AND NUTRITIONAL PROPERTIES OF HEXAPLOID TRITICALE AS INFLUENCED BY SOURCE OF WHEAT CYTOPLASM

INTRODUCTION

The expression of a genotype depends to a certain degree on its cytoplasmic environment. If a harmonious relationship is to exist between a nucleus and its cytoplasm, it has to be both quantitative as well as qualitative in nature. This phenomenon can be expected to be more evident in an intergeneric hybrid such as triticale. As reported earlier by Larter and Hsam (1973; and in Sections I and II of thesis) we found that hexaploid triticales synthesized in Triticum aestivum's (6x wheat) cytoplasm performed more efficiently than in T. turgidum's (4x wheat) cytoplasm when assessed in terms of agronomic attributes, seed development, and cytological stability.

Chen and Bushuk (1969) using the polyacrylamide gel technique, showed that all the bands of triticale proteins were present in the patterns of either the rye or the durum parents. This advocates that the determination of protein in a durum-triticale is genotypic. However, if a differential interaction is present between an AABBRR nucleus in either hexaploid or tetraploid wheat cytoplasm, a different pattern in the synthesis of macromolecules including amino acids and proteins should be apparent.

The present study was undertaken to evaluate the extent of cytoplasmic influence on amino acid compositions and protein characteristics of triticales with specific reference to its quality and nutritional values.

MATERIALS AND METHODS

Mature seeds from reciprocal F_1 pairs and C_2 populations of hexaploid triticales as obtained in the manner previously described (Section I) were used in the study. The parentage and importance of these materials were described by Larter and Hsam (1973; and in Section I of this thesis). Approximately 10 grams of seeds were used for each of the crosses in the analysis for protein contents and amino acids compositions. Samples were analysed in duplicates for all the crosses except for a C_2 line of Manitou x T. turgidum L. var. orientale.

1. Determination of protein contents

The standard macro Kjeldahl procedure was used to determine nitrogen contents of ground seed samples. Protein contents were obtained using a conversion factor 5.7 on a 14.0% moisture basis.

2. Amino acid analysis

Ground seed samples (40 mg.) were weighed into hydrolysis tubes and hydrolyzed with 4 ml. of triple-distilled 6N hydrochloric acid under vacuum at 110°C for 24 hours. After hydrolysis, the HCl was removed by drying the samples over sodium hydroxide pellets in a vacuum desiccator. The amino acids were dissolved in 8 ml. of pH 2.2 sodium citrate buffer and an aliquot of 2.0 ml. was used for the analysis. Analyses were carried out on a Beckman Model 121 automatic amino acid analyser using the method of Spackman et al. (1958), as modified by

Tkachuk (1966). This standard procedure does not assay for tryptophan, cysteine, or cystine.

3. Extraction of seed proteins

Three protein fractions, viz. albumins and globulins, gliadin, and glutenin were obtained. Albumins and globulins were extracted first as a fraction, from 1 gram of ground grain using 0.01 M sodium pyrophosphate (at pH 7) as described by Coates and Simmonds (1961). The residual proteins were further separated into 2 fractions by a successive extraction procedure using 2M and 6M urea solution. Evidence based on gel-filtration studies indicated that the "early" and "late" urea extracts correspond closely to gliadin and glutenin respectively (Meredith and Wren, 1966; Lee, 1968; Lee and MacRitchie, 1971; and Simmonds and Wrigley, 1972). All soluble fractions were dialyzed for 3 days against distilled water and were freeze-dried.

4. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

The method of Weber and Osborn (1969) as applied by Orth and Bushuk (1973) was used to determine the number and approximate molecular weight of the subunits of each protein fraction. The same solutions and molecular weight markers as described by Orth and Bushuk (1973) were also used in this experiment (see Appendix IV-1, 2). A model E-C 470 vertical slab electrophoresis apparatus was used to perform the SDS-PAGE. The gel dimensions for this apparatus were 22 cm. (length) by

10.5 cm. (width) by 6 mm. (thickness).

a) Preparation of SDS subunits

SDS complexes of reduced glutenin were prepared by dissolving 10 mg. of each freeze-dried sample in 1 ml. of protein solvent containing 1% (w./v.) SDS and 1% (v./v.) β-mercaptoethanol. The solutions were incubated at 40°C overnight to allow for complete reaction of the β-mercaptoethanol with disulfide bonds, and for the complexing of SDS with resulting peptides. The resulting protein solutions were made 10% w./v. in sucrose to increase density, and 20 µl. of a 0.3% aqueous solution of bromophenol blue was added to each sample as the electrophoretic front marker. Similarly, gliadin (10 mg.), albumins and globulins (10 mg.) samples as well as molecular-weight markers (1 mg./ml.) were also complexed with SDS. Since reduction of these proteins were not required, 0.002M N-ethylmaleimide (NEMI) replaced β-mercaptoethanol to prevent disulfide-interchange.

b) Preparation of gels

The 5% gel was prepared by dissolving 11.0 g. of acrylamide and 0.29 g. of bisacrylamide cross-linking agent in 210 ml. of electrode buffer at pH 7.3 containing 0.33 ml. of N,N,N',N'-tetramethyl-ethylenediamine. This solution was deaerated, and 100 mg. of ammonium persulphate in 10 ml. of buffer was added. The gel slab was polymerized in the electrophoretic unit using 6-mm. spacers and an 8-slot mold.

c) Electrophoresis

The gel was conditioned in buffer for 30 minutes using the required voltage of 130V. (120mA). 50 µl. of each SDS-sample protein solution, or 10 µl. of the standard protein solution was allowed to run until the marker dye had migrated 7 cm. (approximately 3 hours). The gel was stained with Coomassie Brilliant Blue (see Appendix IV-3) according to the method of Koenig, et al. (1970). A plot of log molecular weight versus mobility for the standard proteins gave a calibration curve that was used to determine the molecular weights of the sample protein subunits. The gels were also scanned with a Joyce-Loebl Chromoscan Scanning Densitometer, using visible reflectance. The profiles obtained in this manner were both quantitatively and qualitatively accurate representations of the gel patterns (Wasik, 1973).

RESULTS

1. Protein contents

Protein contents for the various C_2 and F_1 seed samples were relatively high for both groups of triticales either in 6x or 4x wheat cytoplasm. The coefficients of variability for C_2 and F_1 populations were 2.17 and 2.28% respectively (Table 3). Protein values of C_2 population ranged from 16.75% for Pitic x T. turgidum L. var. turgidum to 19.1% for Manitou x T. turgidum L. var. orientale (Table 1). In the F_1 population, the F_1 of the cross involving Pitic x T. turgidum L. var. turgidum, and its reciprocal showed the lowest protein content (15.55%); while the F_1 of the cross involving T. turgidum L. var. orientale x Manitou, and its reciprocal exhibited the highest content of 18.8% (Table 2). In general, C_2's had higher levels of protein than F_1's.

An overall mean value showed that triticales with 6x wheat cytoplasm possessed a higher protein content in C_2's, and a lower content in F_1's (Table 3). A detailed analysis showed that C_2 triticales with 6x wheat cytoplasm had a mean value of 18.10±0.36 compared to 17.25±0.30 for those in 4x wheat cytoplasm. A factorial analysis of variance indicated both main effects, genotypic as well as cytoplasmic were significant at the 1% level (Table 4). However, when tested against their interaction which was also significant at the 10% level, only the genotypic variance remained significant ($P \leqslant 0.10$). In the F_1 population however, triticales in 6x wheat cytoplasm had a lower mean protein value of 16.95±0.41

compared to 17.54±0.30 for triticales in 4x wheat cytoplasm. Both main effects were significant ($P < 0.05$) with their interaction non-significant (Table 3, 4).

2. Amino acid compositions

The percentage of nitrogen recovery was high ($>$ 90%) for all the amino acids determined. An interesting pattern was evident between the two types of triticales (either in 6x or 4x wheat cytoplasm) for both C_2 and F_1 populations (Table 1, 2). Tables 3 and 4 show the overall mean and the mean squares respectively, obtained from the factorial analysis of variance.

Lysine content was higher in all triticales with 6x wheat cytoplasm (both C_2's and F_1's) except for a C_2 line involving Pitic x T. turgidum L. var. turgidum. It should be noted that this was also the line lowest in protein content. Due to this fact an analysis of the C_2 population gave a significant G x C interaction value ($P < 0.05$), which now rendered the previously significant main effects ($P < 0.05$) non-significant. F_1's showed both genotype and cytoplasm were significant at the 5% level, with the interaction non-significant at this level. Of the other 8 essential amino acids studied, overall mean values indicated that except for methionine and phenylalanine, triticales in 6x wheat cytoplasm possessed a higher beneficial level in both C_2 and F_1 populations. However, a more detailed analysis showed cytoplasmic effects were significant only for histidine, arginine, threonine (all C_2's); and for valine of

Table 1. Seed protein and amino acid compositions of C_2 populations as influenced by source of wheat cytoplasm

Amino Acid[a]	A	A^1	B	B^1	C	C^1	^+D	D^1
Lysine	[b]2.98	3.13	3.31	3.13	3.27	3.12	3.18	2.92
Histidine	2.40	2.80	2.46	2.33	2.43	2.29	2.55	2.39
Ammonia	3.80	3.78	3.82	3.86	3.73	3.85	3.78	4.02
Arginine	5.19	5.25	5.34	5.33	5.38	5.24	5.51	4.98
Aspartic Acid	6.24	6.62	6.26	6.44	6.78	6.60	6.50	5.98
Threonine	3.06	2.91	3.17	2.93	3.19	2.99	3.15	2.96
Serine	4.59	4.15	4.81	4.34	4.62	4.56	4.80	4.57
Glutamic Acid	34.73	34.00	33.49	35.80	35.59	36.31	35.68	39.16
Proline	11.69	11.47	11.48	11.50	11.52	12.20	11.66	12.17
Glycine	4.20	4.02	4.11	4.04	4.15	4.18	4.26	3.89
Alanine	3.75	3.85	3.98	3.83	4.03	3.85	3.88	3.63
Valine	4.60	4.63	4.78	4.68	4.71	4.55	4.73	4.57
Methionine	1.03	1.44	1.26	1.16	1.24	1.35	1.09	1.16
Isoleucine	3.74	3.62	3.75	3.59	3.70	3.63	3.63	3.74
Leucine	7.04	6.84	7.34	6.91	6.95	6.97	7.05	7.13
Tyrosine	2.71	2.71	2.74	2.66	2.59	2.92	2.82	2.68
Phenylalanine	5.25	5.06	5.24	5.14	5.25	5.49	5.11	5.45
N, % Recovery	92.34	91.45	93.14	93.00	93.73	94.29	94.38	95.12
Protein (N x 5.7; 14% m.b.)	16.75	16.80	18.50	17.75	18.05	16.25	19.10	18.20

Each value is a mean of two samples (+ value of one sample).

[a] Tryptophan, cysteine (and cystine) were not determined.

[b] g amino acid per 100 g protein.

Table 2. Seed protein and amino acid compositions of reciprocal F_1 populations in either common wheat or emmer cytoplasm

Amino Acid[a]	A x A^1	A^1 x A	B x B^1	B^1 x B	C x C^1	C^1 x C	D x D^1	D^1 x D
	[b]							
Lysine	3.44	3.11	3.41	3.38	3.21	3.20	3.18	3.09
Histidine	2.39	2.35	2.35	2.37	2.29	2.24	2.38	2.35
Ammonia	3.55	3.89	3.72	3.69	3.76	3.74	3.82	3.95
Arginine	5.93	5.47	5.54	5.63	5.25	5.32	5.28	5.17
Aspartic Acid	7.18	6.63	6.70	6.44	6.32	6.42	6.51	6.49
Threonine	3.24	2.99	3.14	3.17	3.03	3.06	3.10	3.08
Serine	4.49	4.69	4.53	4.81	4.66	4.61	4.72	4.59
Glutamic Acid	31.96	35.59	34.04	34.22	34.27	34.17	36.34	39.45
Proline	10.47	11.48	11.61	11.39	11.69	11.78	11.30	12.03
Glycine	4.48	4.06	4.09	4.09	4.06	4.08	4.00	3.96
Alanine	4.19	3.83	3.99	3.95	3.82	3.88	3.90	3.95
Valine	4.84	4.60	4.82	4.75	4.57	4.57	4.71	4.71
Methionine	1.45	1.23	1.39	1.28	1.20	1.27	1.26	1.53
Isoleucine	3.65	3.63	3.74	3.67	3.63	3.61	3.64	3.68
Leucine	6.89	6.87	7.23	7.13	6.87	6.84	6.98	7.10
Tyrosine	2.76	2.79	2.73	2.64	2.67	2.69	2.55	2.71
Phenylalanine	4.86	5.06	5.11	5.09	5.29	5.22	5.11	5.28
N, % Recovery	91.98	93.69	93.31	93.04	91.94	92.03	93.52	96.18
Protein (N x 5.7; 14% m.b.)	15.55	17.10	17.05	17.20	16.70	17.05	18.50	18.80

Each value is a mean of 2 samples.

[a] Tryptophan, cysteine (and cystine) were not determined.

[b] g amino acid per 100 g protein.

Table 3. Overall mean for seed protein and amino acids of C_2 and reciprocal F_1 populations in either common wheat (6 x) or emmer (4 x) cytoplasm

Variable	C_2			F_1		
	6 x CYTO	4 x CYTO	CV(%)+	6 x CYTO	4 x CYTO	CV(%)+
Lysine	3.18	3.07	2.45	3.31	3.19	2.61
Histidine	2.46	2.32	1.48	2.35	2.33	1.64
Ammonia	3.78	3.88	3.26	3.71	3.81	2.12
Arginine	5.35	5.30	2.73	5.49	5.39	3.25
Aspartic Acid	6.44	6.40	2.79	6.68	6.49	2.62
Threonine	3.14	2.94	2.40	3.12	3.07	2.03
Serine	4.70	4.40	3.09	4.60	4.67	2.65
Glutamic Acid	34.87	36.31	3.95	34.15	35.85	3.61
Proline	11.59	11.83	2.39	11.26	11.67	2.76
Glycine	4.18	4.03	2.13	4.15	4.04	2.13
Alanine	3.91	3.79	2.49	3.97	3.87	1.79
Valine	4.70	4.60	2.27	4.73	4.65	1.71
Methionine	1.15	1.27	12.43	1.32	1.33	9.76
Isoleucine	3.70	3.64	2.22	3.66	3.64	2.09
Leucine	7.09	6.96	2.36	6.99	6.98	2.17
Tyrosine	2.71	2.74	3.01	2.67	2.70	3.07
Phenylalanine	5.21	5.28	2.66	5.09	5.16	2.30
Seed Protein	18.10	17.25	2.17	16.95	17.54	2.28

+ Total coefficient of variation in the analysis of variance.

Table 4. Mean squares and their level of significance for amino acids and seed protein contents of C_2 and reciprocal F_1 populations

Variable	Mean Squares (C_2)				Mean Squares (F_1)			
	Genotype (G)	Cytoplasm (C)	G x C	Error	Genotype (G)	Cytoplasm (C)	G x C	Error
Lysine	0.0290*	0.0473*	0.0283*	0.0059	0.0489*	0.0518*	0.0228	0.0072
Histidine	0.0107**	0.0693**	0.0007	0.0012	0.0104*	0.0020	0.0008	0.0015
Ammonia	0.0101	0.0337	0.0112	0.0157	0.0262*	0.0452*	0.0307*	0.0063
Arginine	0.0111	0.0883+	0.0623	0.0206	0.2099*	0.0410	0.0649	0.0313
Aspartic Acid	0.1360	0.0056	0.1413*	0.0320	0.2102*	0.1351+	0.0807	0.0299
Threonine	0.0069	0.1401**	0.0014	0.0053	0.0085	0.0121	0.0184*	0.0040
Serine	0.0661	0.3332**	0.0325	0.0197	0.0047	0.0210	0.0395	0.0151
Glutamic Acid	7.2405+	7.7953+	2.9980	1.9780	14.9905**	11.6111*	3.7383	1.5940
Proline	0.1615	0.2241	0.1558	0.0781	0.4694*	0.6521*	0.3184	0.1003
Glycine	0.0063	0.0826*	0.0271+	0.0076	0.0583**	0.0484*	0.0419*	0.0076
Alanine	0.0275	0.0526*	0.0206	0.0092	0.0235*	0.0390*	0.0315*	0.0050
Valine	0.0099	0.0383	0.0070	0.0112	0.0323*	0.0240+	0.0136	0.0064
Methionine	0.0179	0.0549	0.0417	0.0230	0.0182	0.0001	0.0460	0.0167
Isoleucine	0.0003	0.0146	0.0124	0.0067	0.0055	0.0018	0.0020	0.0058
Leucine	0.0325	0.0655	0.0492	0.0274	0.0917+	0.0002	0.0084	0.0229
Tyrosine	0.0026	0.0028	0.0393*	0.0067	0.0153	0.0039	0.0109	0.0068
Phenylalanine	0.0338	0.0176	0.0580	0.0195	0.0647*	0.0203	0.0186	0.0139
Seed Protein	2.7554**	2.6973**	0.5111+	0.1457	3.9623**	1.3806*	0.4190	0.1544

Degrees of freedom: G = 3, C = 1, G x C = 3, Error = 7 for C_2, and 8 for F_1.

+ ($P \leqslant 0.10$), * ($P \leqslant 0.05$), ** ($P \leqslant 0.01$).

the F_1 populations. No statistical significance was obtained for differences of both methionine and phenylalanine. Apparently, these were due to the facts that methionine had the highest variability (C.V. = 10%) in comparison to the other amino acids (C.V. $<$ 4%) and the differences observed in phenylalanine was genotypic rather than cytoplasmic.

Of the non-essential amino acids, triticales with 6x wheat cytoplasm had higher levels of aspartic acid, glycine, and alanine in both the C_2 and F_1 populations. Triticales with 4x wheat cytoplasm possessed more ammonia, glutamic acid, proline, and tryosine in both C_2's and F_1's. Serine was the only amino acid that was found to be higher in 6x's C_2, and lower in 6x's F_1. However, when tested for statistical significance (after removing the G x C interaction) cytoplasmic effects were found to be significant only for aspartic acid (F_1), serine (C_2), glycine (both C_2 and F_1), alanine (C_2), glutamic acid (both C_2 and F_1) and proline (F_1).

3. Protein characteristics obtained by SDS-PAGE

The electrophoretic profiles of the triticales were separately grouped according to genotypes. For each of the 4 genotypes, densitometer recordings of the electrophoretic profiles for the 2 C_2's and their reciprocal F_1 pairs were further represented in a common graph to facilitate the identification of similar protein subunits. The profiles can be divided approximately into 4 regions, with molecular weight (MW) of less than 21,600; between 21,600 to 66,000; between 66,000 to

160,000, and higher than 160,000 daltons. No protein subunit was detected above MW 160,000 in any of the triticales studied.

a) Albumins and globulins

The electrophoretic profiles and their densitometer recordings are presented in Fig. 1, and 2 respectively. It was observed that there were some distinct differences as well as similarities between the genotypes. The densitometer recordings showed that differences between genotypes were both quantitative as well as qualitative, and was more obvious in triticales with 4x wheat cytoplasm. Within a genotype, the most striking difference was observed between crosses involving Pitic x T. turgidum L. var. turgidum triticales in either 6x or 4x wheat cytoplasm.

The two reciprocal F_1 pairs differed markedly while showing similarity to their corresponding C_2's of the same cytoplasm type. Triticales in 4x wheat cytoplasm lacked subunits above 30,000 daltons. On the other hand, 5 distinct bands could be observed in those with 6x wheat cytoplasm. However, there was a fast moving band of MW less than 14,000 in the 4x wheat cytoplasm triticales. This band was lacking in triticales with 6x wheat cytoplasm. Differences in other genotypes were not as striking. However, all profiles quantitatively showed that triticales with 4x wheat cytoplasm appeared to possess more fast moving protein of subunits less than 14,000, at the expense of higher MW subunits.

Figure 1

SDS-PAGE profiles of albumins and globulins.

(A) Pitic x T. turgidum F_3/Centeno

(A^1) T. turgidum x Pitic F_3/ "

(B) Pitic x T. durum F_3/ "

(B^1) T. durum x Pitic F_3/ "

(C) Manitou x T. turgidum F_3/Centeno

(C^1) T. turgidum x Manitou F_3/ "

(D) Manitou x T. orientale F_3/Centeno

(D^1) T. orientale x Manitou F_3/ "

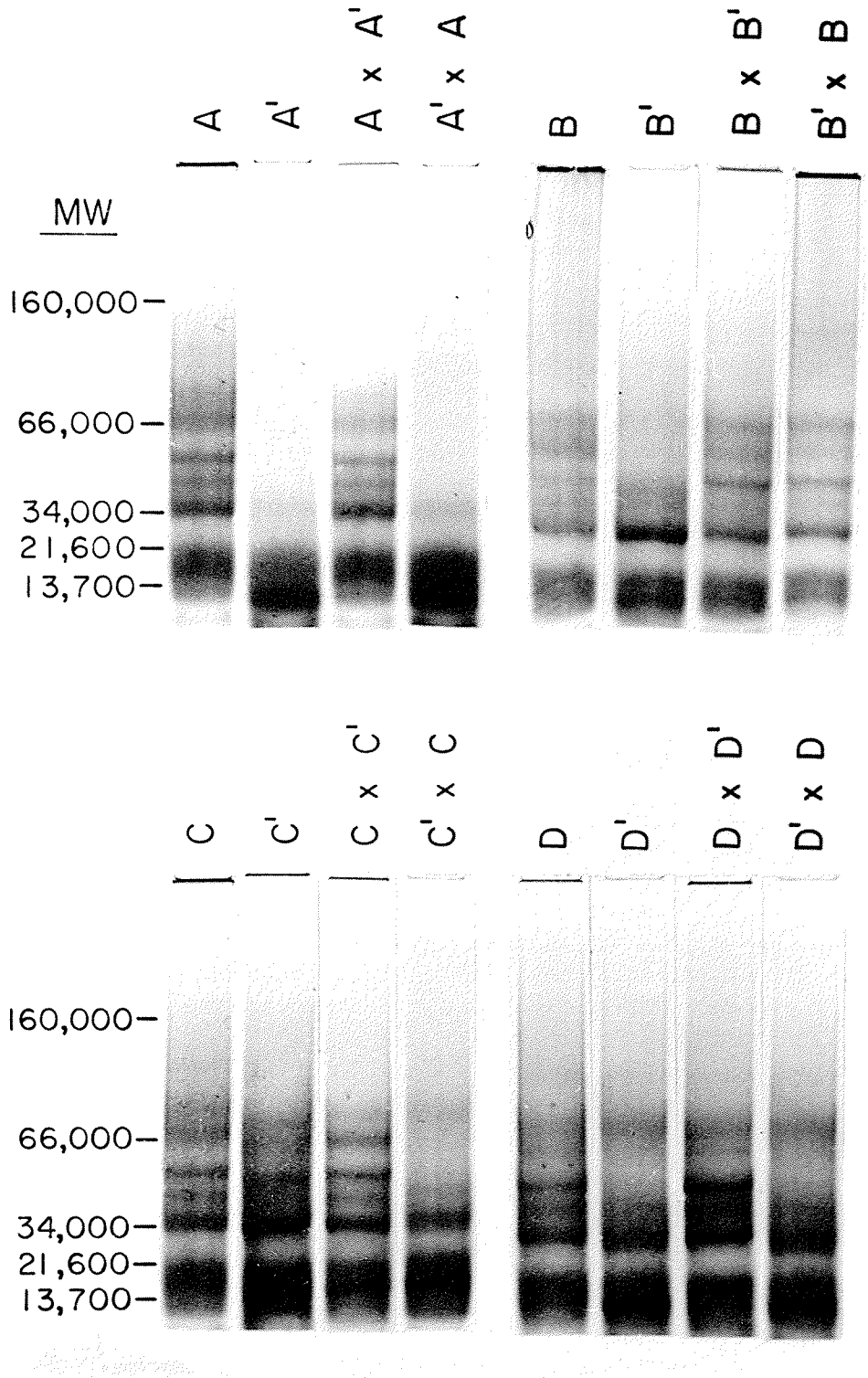

A
A'
A x A'
A' x A
B
B'
B x B'
B' x B
MW
160,000
66,000
34,000
21,600
13,700
C
C'
C x C'
C' x C
D
D'
D x D'
D' x D
160,000
66,000
34,000
21,600
13,700

Figure 2

Densitometer recordings of SDS-PAGE profiles of albumins and globulins.

(A) Pitic x T. turgidum F_3/Centeno

(A^1) T. turgidum x Pitic F_3/ "

(B) Pitic x T. durum F_3/ "

(B^1) T. durum x Pitic F_3/ "

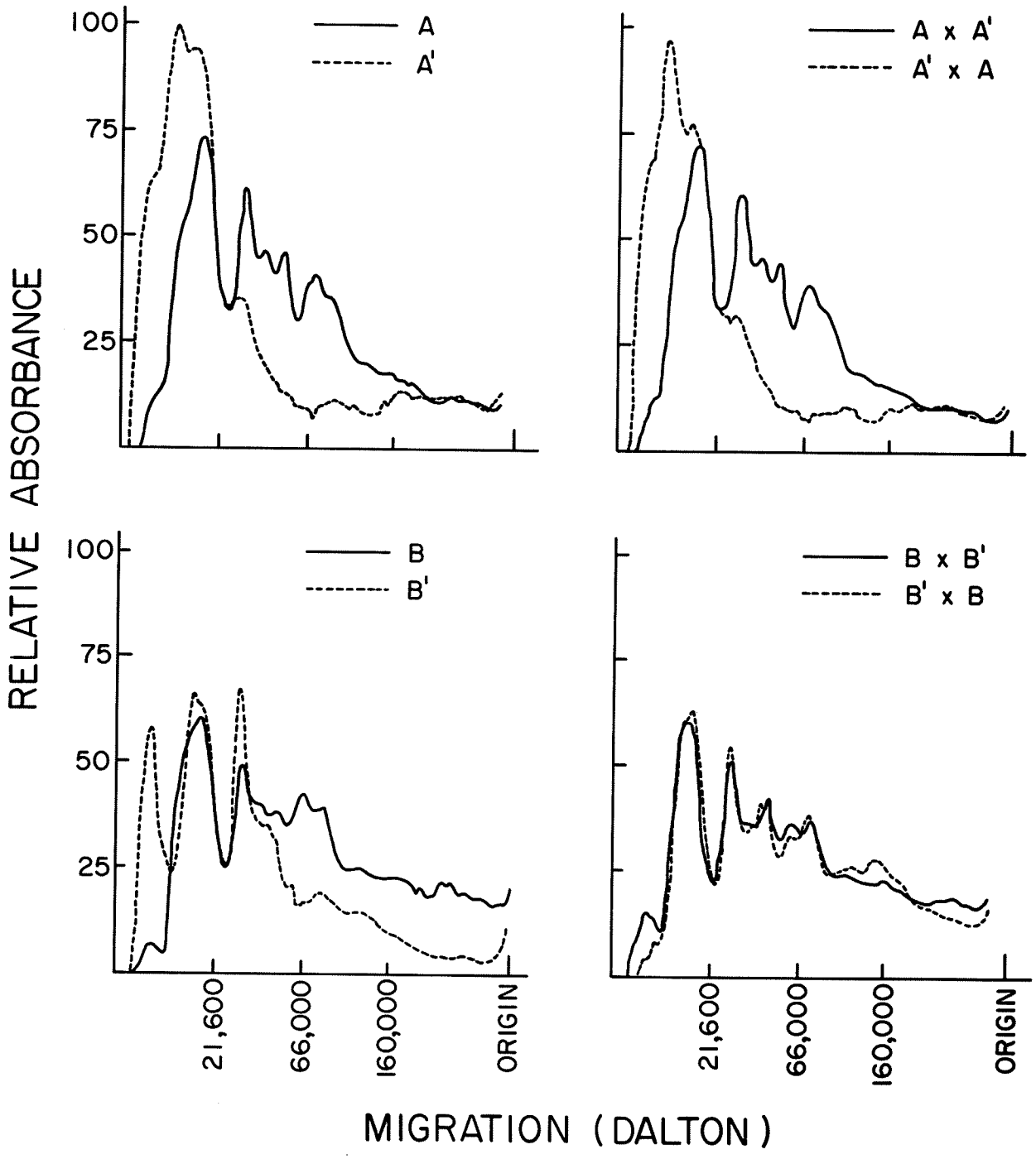

RELATIVE ABSORBANCE
100
75
50
25
A
A'
A x A'
A' x A
B
B'
B x B'
B' x B
21,600
66,000
160,000
ORIGIN
MIGRATION (DALTON)

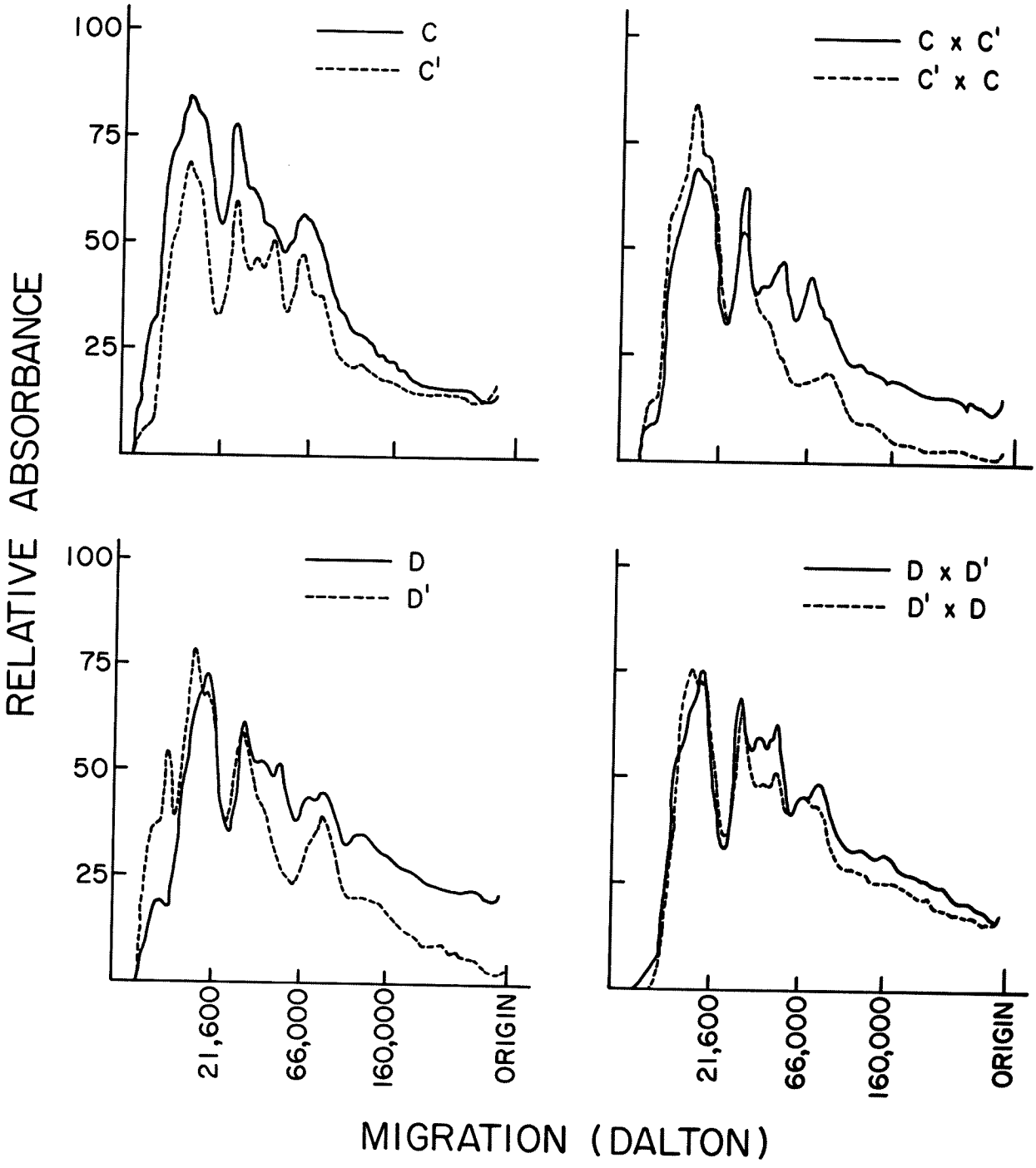
Figure 2 - continued

(C) Manitou x T. turgidum F_3/Centeno

(C^1) T. turgidum x Manitou F_3/ "

(D) Manitou x T. orientale F_3/Centeno

(D^1) T. orientale x Manitou F_3/ "


RELATIVE ABSORBANCE
100
75
50
25
C
C'
C x C'
C' x C
D
D'
D x D'
D' x D
21,600
66,000
160,000
ORIGIN
MIGRATION (DALTON)

b) Gliadin

The electrophoretic profiles and their densitometer recordings are presented in Fig. 3 and 4 respectively. In this protein fraction the most obvious qualitative difference was between genotypes. Except for crosses involving Manitou x T. turgidum L. var. turgidum which contained polypeptides of MW of approximately 77,000 daltons, all other genotypes possessed subunits with a MW less than 66,000 daltons. A strong banding was observed at about 35,000 daltons. In both C_2's and F_1's of all genotypes, differences due to cytoplasm were found to be quantitative rather than qualitative in nature as was apparent from the densitometer recordings. In general, triticales with 6x wheat cytoplasm possessed a larger amount of main protein subunits than those with 4x wheat cytoplasm and was characteristic of both C_2 and reciprocal F_1 populations.

c) Reduced glutenin

Electrophoretic profiles and their densitometer recordings of reduced glutenin are presented in Fig. 5, and 6 respectively. There was no obvious qualitative differences either between genotypes or within genotypes. Quantitative differences however, were observed between the genotypes as well as between C_2 pairs and their reciprocal F_1's. Electrophoretic patterns suggest however, that insofar as reduced glutenins were concerned, differences were not sufficiently great to establish a specific pattern for triticales of either cytoplasm type.

Figure 3

SDS-PAGE profiles of gliadin.

(A) Pitic x T. turgidum F_3/Centeno

(A^1) T. turgidum x Pitic F_3/ "

(B) Pitic x T. durum F_3/ "

(B^1) T. durum x Pitic F_3/ "

(C) Manitou x T. turgidum F_3/Centeno

(C^1) T. turgidum x Manitou F_3/ "

(D) Manitou x T. orientale F_3/ "

(D^1) T. orientale x Manitou F_3/ "

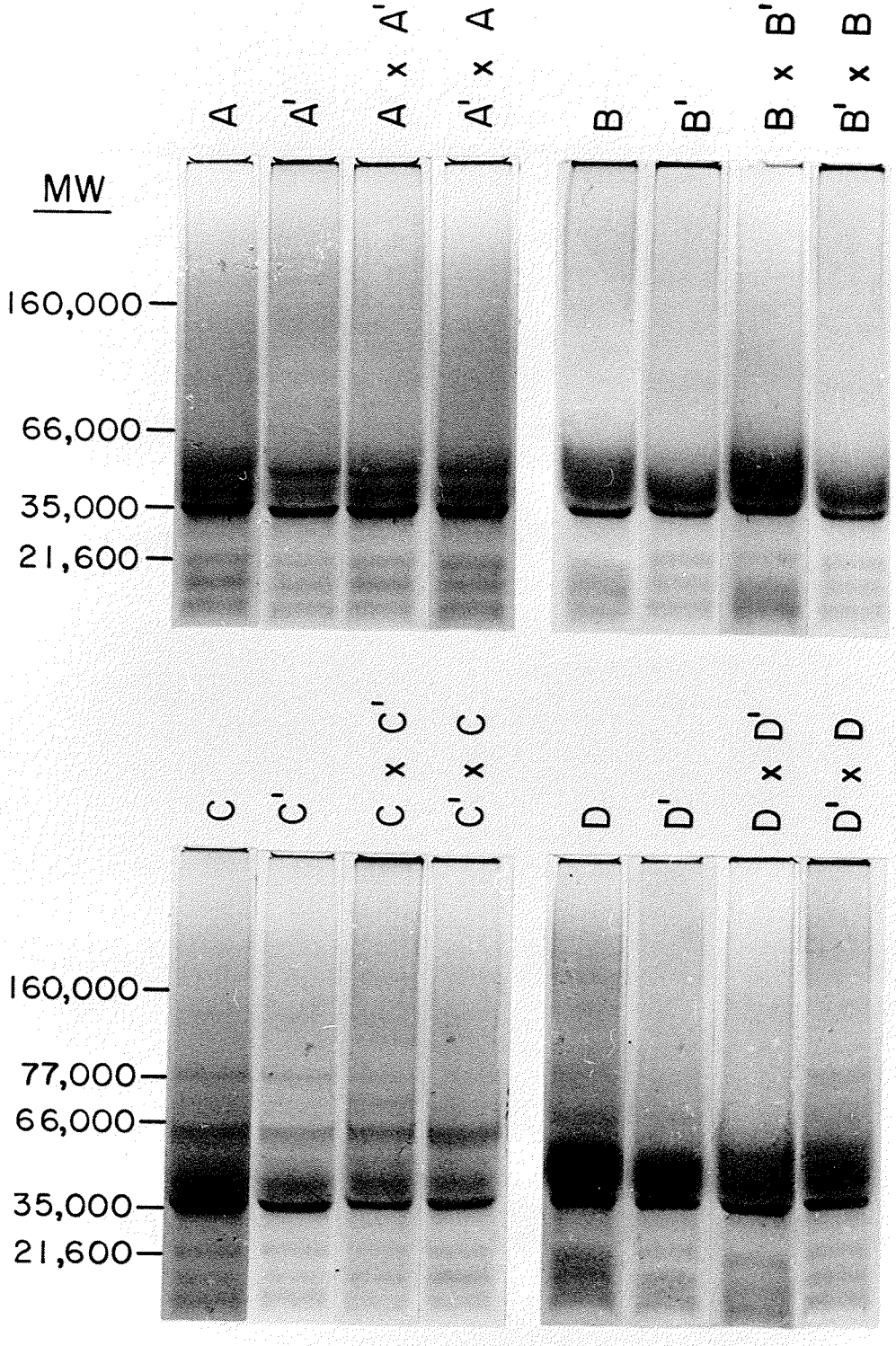

A
A'
A x A'
A' x A
B
B'
B x B'
B' x B
MW
160,000
66,000
35,000
21,600
C
C'
C x C'
C' x C
D
D'
D x D'
D' x D
160,000
77,000
66,000
35,000
21,600

Figure 4

Densitometer recordings of SDS-PAGE profiles of gliadin.

(A) Pitic x T. turgidum F_3/Centeno

(A^1) T. turgidum x Pitic F_3/ "

(B) Pitic x T. durum F_3/ "

(B^1) T. durum x Pitic F_3/ "

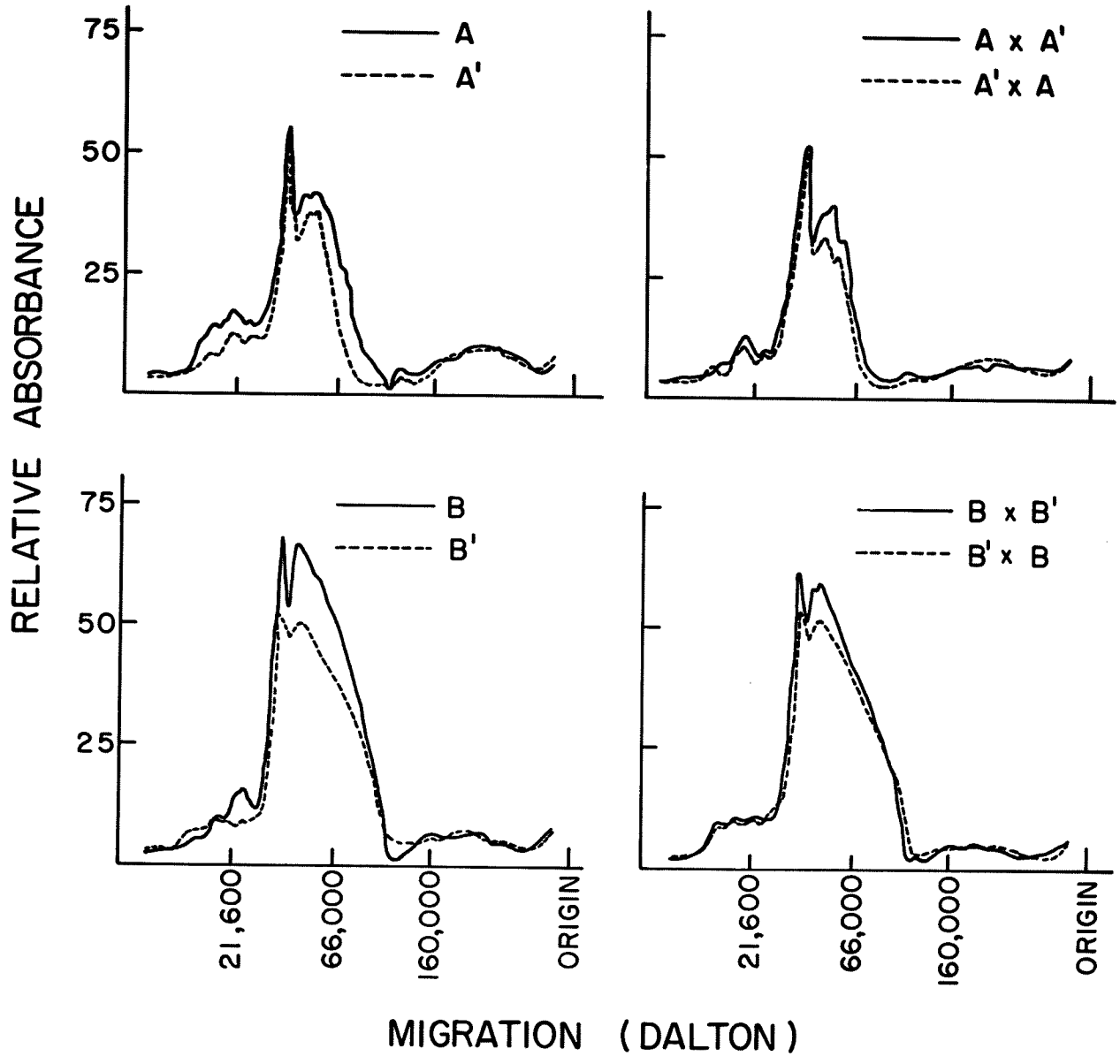

RELATIVE ABSORBANCE
75
50
25
A
A'
A x A'
A' x A
B
B'
B x B'
B' x B
21,600
66,000
160,000
ORIGIN
MIGRATION (DALTON)

Figure 4 - continued

(C) Manitou x T. turgidum F_3/Centeno

(C^1) T. turgidum x Manitou F_3/ "

(D) Manitou x T. orientale F_3/Centeno

(D^1) T. orientale x Manitou F_3/ "

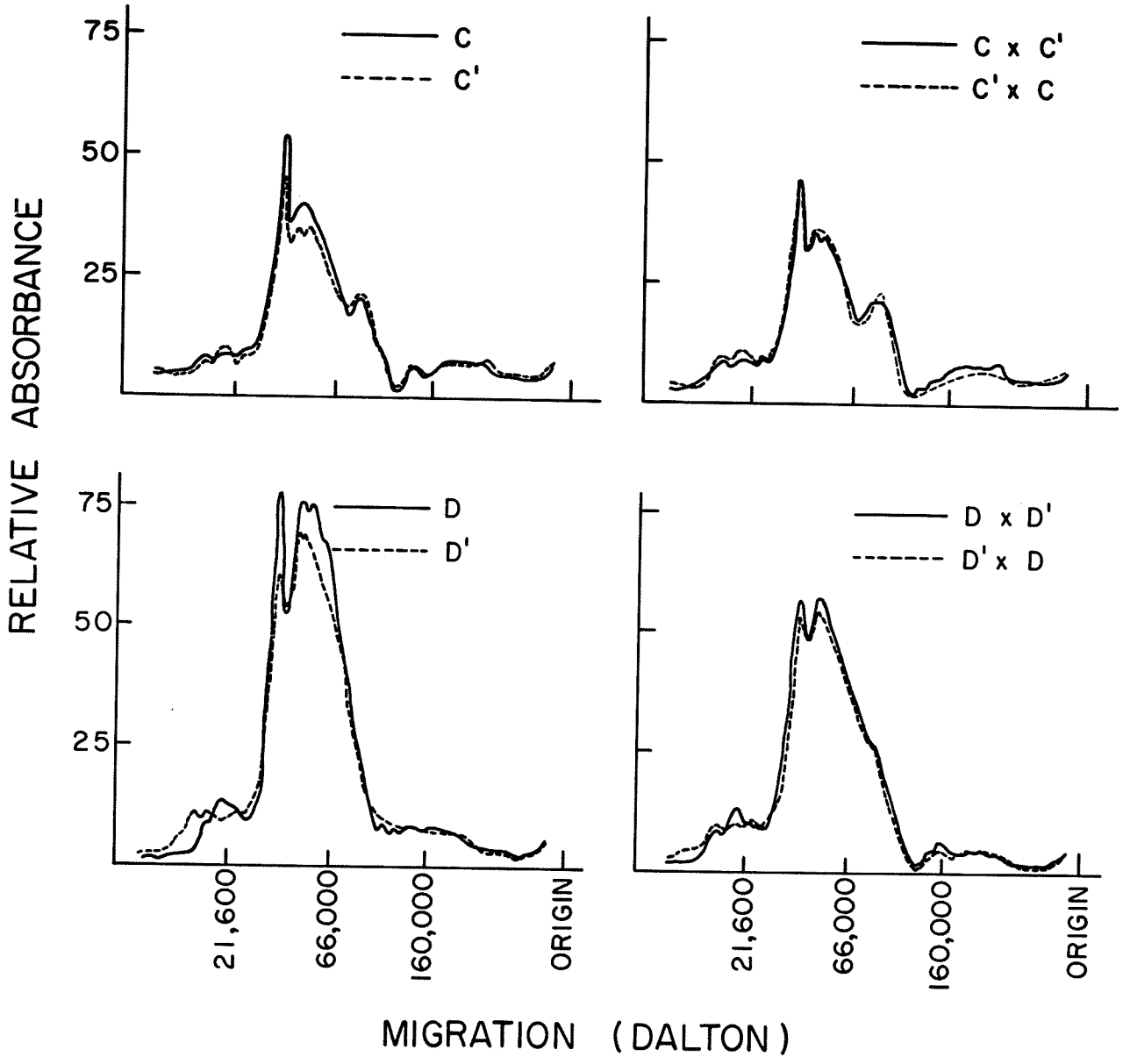

RELATIVE ABSORBANCE
75
50
25
C
C'
C x C'
C' x C
D
D'
D x D'
D' x D
21,600
66,000
160,000
ORIGIN
MIGRATION (DALTON)

Figure 5

SDS-PAGE profiles of reduced glutenin.

(A) Pitic x *T. turgidum* F_3/Centeno

(A^1) *T. turgidum* x Pitic F_3/ "

(B) Pitic x *T. durum* F_3/ "

(B^1) *T. durum* x Pitic F_3/ "

(C) Manitou x *T. turgidum* F_3/Centeno

(C^1) *T. turgidum* x Manitou F_3/ "

(D) Manitou x *T. orientale* F_3/ "

(D^1) *T. orientale* x Manitou F_3/ "

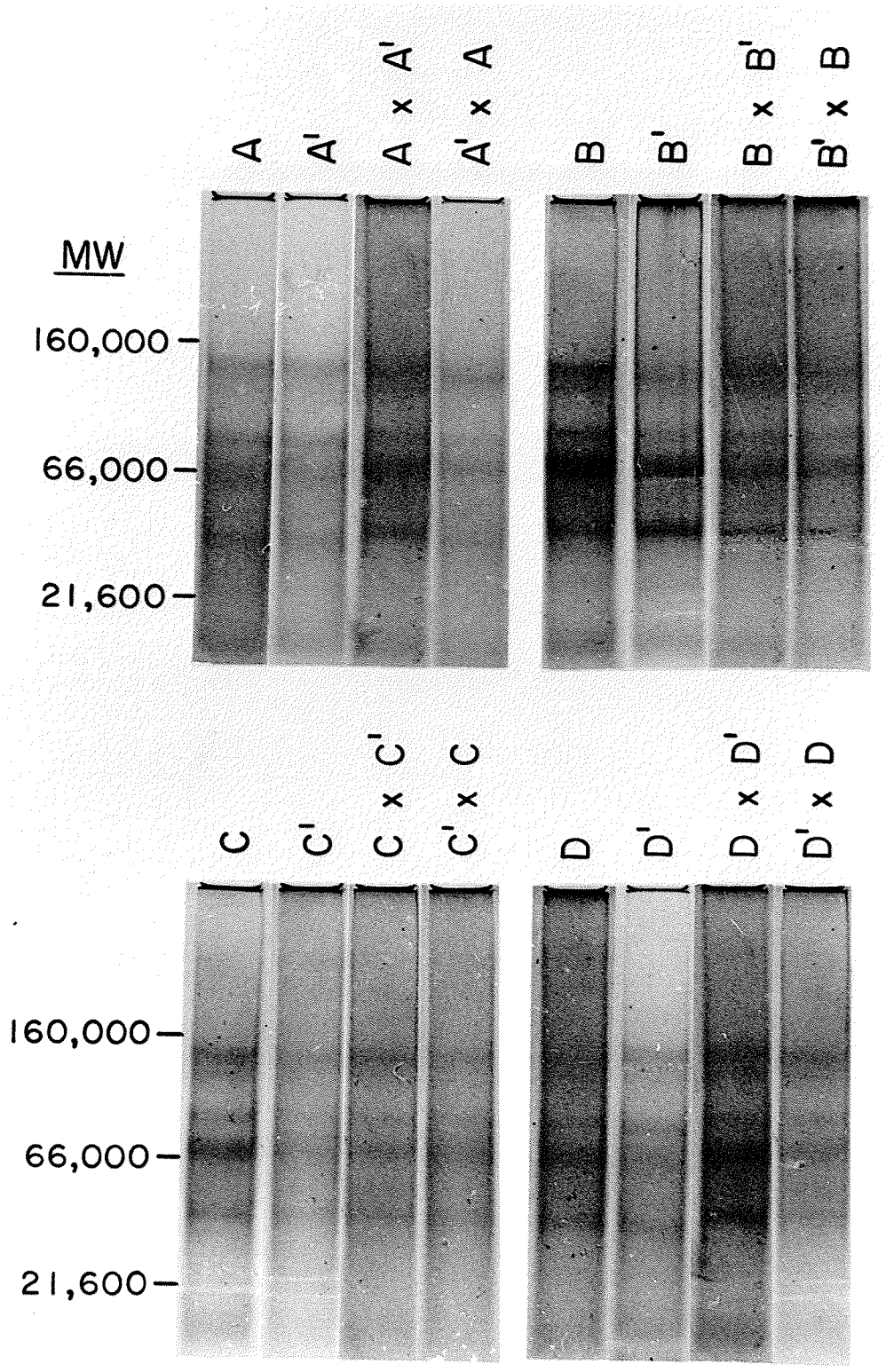

A
A'
A x A'
A' x A
B
B'
B x B'
B' x B
MW
160,000
66,000
21,600
C
C'
C x C'
C' x C
D
D'
D x D'
D' x D
160,000
66,000
21,600

Figure 6

Densitometer recordings of SDS-PAGE profiles of reduced glutenin.

(A) Pitic x *T. turgidum* F_3/Centeno

(A^1) *T. turgidum* x Pitic F_3/ "

(B) Pitic x *T. durum* F_3/ "

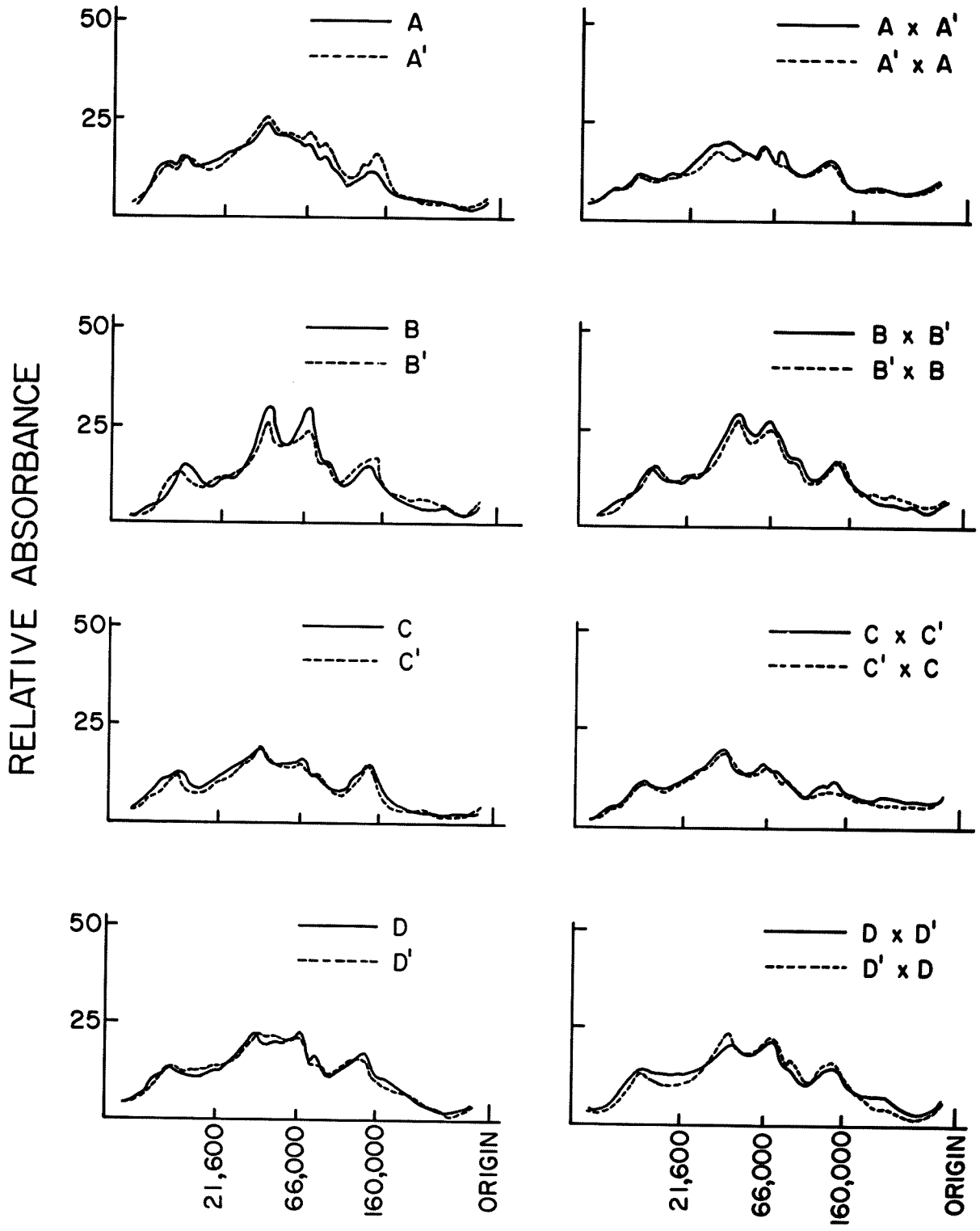
(B^1) *T. durum* x Pitic F_3/ "

(C) Manitou x *T. turgidum* F_3/Centeno

(C^1) *T. turgidum* x Manitou F_3/ "

(D) Manitou x *T. orientale* F_3/ "

(D^1) *T. orientale* x Manitou F_3/ "


50
25
A
A'
A x A'
A' x A
B
B'
B x B'
B' x B
C
C'
C x C'
C' x C
D
D'
D x D'
D' x D
RELATIVE ABSORBANCE
21,600
66,000
160,000
ORIGIN
MIGRATION (DALTON)

DISCUSSION

The development of the wheat-rye hybrid, Triticale has progressed to a stage where it is now recognized as a potential crop of commerce in Canada, U.S.A., and parts of Europe. If triticale is to compete successfully with other cereals as a food for humans or as feed for animals, it must also be nutritious in addition to palatable and productive. In the present study, differential interaction of an AABBRR triticale nucleus in hexaploid or tetraploid wheat cytoplasm was further demonstrated. However quantitatively speaking, an obvious trend was not evident in protein content. Triticales with 6x wheat cytoplasm had a higher level of protein in C_2, and lower in F_1 when compared to triticales with 4x wheat cytoplasm. The latter however, possessed approximately the same levels of protein in both C_2 and F_1 populations. This was influenced by other factors such as the degree of seed shrivelling in the samples analysed, in addition to genotypic and cytoplasmic effects. Villegas (as cited by Zillinsky and Borlaug, 1971) had reported that there was a tendency for triticales with shrivelled seeds to be higher in protein content. The above author also stated that there was an inverse relationship between protein content and the level of lysine in the protein. However, it was later shown that these associations were rather loose and were influenced considerably by environment and fertility of the soil (Zillinsky and Borlaug, 1971). This same correlation was also lacking in the present study.

In recent years triticales have received considerable attention because of their relatively higher lysine content than bread wheat. For human consumption however, subsequent studies have shown lysine to be still the first limiting amino acid (Kies and Fox, 1970a, 1970b). In view of this, it was very encouraging to observe that triticales with 6x wheat cytoplasm possessed higher levels of lysine than those with 4x wheat cytoplasm. In addition, it was also observed to be the same for other essential amino acids including histidine, arginine, threonine and valine. Of the non-essential amino acids analysed, triticales with 4x wheat cytoplasm were higher only in glutamic acid and proline levels. However, as also reported by Sauer (1972), the non-essential amino acids, glutamic acid, proline and serine were the most available in triticales. Therefore, the levels of these amino acids would not affect the nutritional properties of triticales possessing hexaploid wheat cytoplasm. Furthermore, the least available non-essential amino acid, alanine (Sauer, 1972), was also higher in triticales with 6x wheat cytoplasm. This clearly supports the hypothesis that triticales with 6x cytoplasm would be more nutritious and feeding trials with either rats or mice are planned for the future.

Comparative studies of protein characteristics, and isoenzymes behaviour by gel electrophoresis provide a rapid method of ascertaining genetic homologies. In the past few years this criterion has been used by several workers to investigate phylogenetic relationships between

species (Cherry, Katterman and Endrizzi, 1970; Ladizinsky and Johnson, 1972); as well as to identify probable genome donors and additivity activities in amphiploid species including wheat (Johnson, 1972a, 1972b) and triticales (Barber, Driscoll and Vickery, 1968; Chen and Bushuk, 1969).

In the present study SDS-PAGE has been used to study protein characteristics between reciprocal F_1 and C_2 pairs, which had been shown to be free of genetic influence (Larter and Hsam, 1973). Furthermore as protein had been extracted according to their solubility characteristics, the accuracy of determining specific properties has also been enhanced. Generally, the albumin and globulin classes include the enzyme proteins, and the gliadin and glutenin (gluten) classes the main proteinaceous materials responsible for the cohesive protein network of doughs. In the present study we observed that qualitative as well as quantitative differences were more evident in the enzyme protein fractions than in the gluten or structural protein fraction.

In higher organisms, control of protein synthesis is complex, and regulation can operate at several levels (Lewin, 1970). However in any of the F_1 or C_2 pairs of the present study, genetic influences were similar, and the differential phenotypic expressions were attributed to both cytoplasm and nuclear-cytoplasmic influences. Cytoplasmic influences were apparent when a consistent beneficial relationship existed in favour of triticales possessing 6x wheat cytoplasm. On the other

hand as there was also a certain degree of differential response for each genotype studied to the same cytoplasm type, a nuclear-cytoplasmic interaction was suspected.

In the present study, it was observed that an AABBRR nucleus synthesizes more structural protein in 6x wheat cytoplasm. In addition, in the albumin and globulin class, higher molecular weight protein subunits were preferentially synthesized in 6x wheat cytoplasm compared to 4x wheat cytoplasm. In contrast, triticales in 4x wheat cytoplasm synthesized more protein subunits possessing a molecular weight less than 34,000. This could be a differential synthesis of certain enzymes. Although it is still a conjecture at this point in time (since the exact nature of these molecules has yet to be identified), our earlier findings (Section II of this thesis) that α-amylase enzyme levels were significantly higher in triticales with 4x wheat cytoplasm appears to support this hypothesis. As dry seeds were used in all our analyses, all life processes were at a very low metabolic level and probably no new proteins were synthesized. Hence the influence of both cytoplasm and nuclear-cytoplasmic interactions were presumed to be effective from the time of early embryo formation and development. Whether this is due to a larger number of polypeptide molecules initially synthesized or is a result of regulation remains to be established.

Available evidence on the cytoplasmic relationships in the Triticinae suggests that both tetraploid and hexaploid wheat have the same

source of cytoplasm (Kihara, 1968; Suemoto, 1968). Nevertheless it remains clear that bread wheat cytoplasm has become modified through evolution to co-exist harmoniously with a hexaploid nucleus (see Larter and Hsam, 1973). Thus, findings from the present study further strengthens the fact that synthesis and utilization of triticales in 6x wheat cytoplasm should receive serious consideration as being part of any triticale breeding program.

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SECTION V

QUANTITATIVE RELATIONSHIPS OF CELLULAR PROTEIN, RNA AND NUCLEAR HISTONE IN HEXAPLOID TRITICALE AS INFLUENCED BY SOURCE OF WHEAT CYTOPLASM

INTRODUCTION

It is now well documented that genetic information is conserved in DNA base sequences mainly in the nucleus. Expression of this genetic information, on the other hand is primarily in the cytoplasm (see Watson, 1970). Since the pioneer work of Hämmerling with Acetabularia in the mid 1930's led to our early understanding of nuclear-cytoplasmic relationships, evidence has accumulated to support these relationships in higher organisms as well (Harris, 1970). It has been rewarding for plant breeders to manipulate these nuclear-cytoplasmic relationships toward a practical application. Cytoplasmic pollen sterility and fertility restoration mainly geared towards hybrid-seed production, has been successfully incorporated into several crop species including wheat (Schmidt and Johnson, 1966; Wilson, 1968; Maan and Lucken, 1972), and corn (see Hooker, 1972). Recently, nuclear-cytoplasmic relationships have also become of importance in triticale breeding.

Triticale is an intergeneric hybrid derived from crosses between wheat (Triticum sp. L.) and rye (Secale sp. L.). Hexaploid triticale derivatives ($2n = 6x = 42$), depending upon the species of wheat employed as the female parent, possessed either T. aestivum L. em. Thell. ($6x$) cytoplasm, or T. turgidum L. ($4x$) cytoplasm. In our own work we observed that identical hexaploid triticale genotypes performed more efficiently in $6x$ wheat cytoplasm than in $4x$'s, with references to cytology, agronomic attributes as well as nutritional properties (Larter

and Hsam, 1973; Section I to IV of this thesis).

The present work is concerned with an attempt, using microphotometric methods, to characterize more specifically some of the components of the cell thought most likely to contribute to the established pattern of variation in nuclear-cytoplasmic compatibility; viz. total cellular protein, cellular RNA, and nuclear histone.

MATERIALS AND METHODS

1. Cultivation and fixation

Seeds obtained from the two triticale reciprocal F_1 populations (previously described by Larter and Hsam, 1973; Section I of this thesis) were germinated on blotting papers soaked in distilled water. There was no addition of mineral nutrients. Seedlings were kept at about 21°C and when roots were approximately 1½ cm long, the meristematic region was fixed. For the estimation of nucleic acids and protein, root-tips were fixed in 4% aqueous formaldehyde in M/15 phosphate buffer at pH 7 for 3 hours, washed free of fixative and refrigerated in 70% alcohol (see Mitchell, 1967). For the determination of nuclear histone content, nuclei were extracted by tapping root-tips after fixation for 15 minutes in 2% formaldehyde in ice cold M/30 phosphate buffer at pH 7 (McCleish, 1963). The slides with isolated nuclei were flooded with absolute alcohol for 2 minutes (Bennett, 1970), air dried and stored.

2. Staining and photometry

A GN2 integrating microdensitometer (Barr and Stroud Ltd., Glasgow, Scotland) was employed in the estimation of nucleic acids, protein and nuclear histone. This instrument incorporates a scanning device that minimizes distributional error. Extinction is integrated as the scanning progresses, so that a direct measurement of total extinction is provided in arbitrary units (Deeley, 1954). A total of fifty 4C

(prophase) cells, i.e. 10 from each of 5 seedlings, were measured for each cross.

a) Estimation of nucleic acids

DNA was estimated from gallocyanin stained cells after RNase treatment. Gallocyanin-chromalum has been shown to be a reliable stain for nucleic acids in animal as well as plant cells (Sandritter, et al., 1966; Kiefer, et al., 1966; Chen, 1966; and Kiefer, 1970; Fig. 1b).

A mean total cellular RNA content for each seedling was derived as the difference in mean absorption at 570 mμ between samples of gallocyanin-chromalum stained cells prior to RNase treatment (i.e. total nucleic acids, Fig. 1a) and the mean DNA content obtained for each respective cross (Chen, 1966; Bennett, 1970).

b) Total cellular protein

Root-tips were stained overnight with 2:4-Dinitro-fluorobenzene (DNFB) in 0.01 N alcoholic NaOH at 70°C. The method is given by Mitchell (1967) and provides a quantitative assessment of total protein. DNFB stains the protein by forming a yellow complex with the free $-NH_2$ groups of proteins (Sanger, 1949; Maddy, 1961; Fig. 1c), and has a maximum absorption peak at 400 mμ. Since single cells were needed for the measurements, roots were treated with a dilute solution of diaminoethane tetra-acetic acid (EDTA) to separate the cells by the method of Ginzburg (1958) as modified by Mitchell (1967).

c) Nuclear histone content

Alfert and Geschwind (1953) reported a selective staining for histone (basic chromosome protein) with Fast Green. It depends on the fact that histones have an isoelectric point which is much more alkaline (pH 10-11) than other proteins. At pH 8-8.1 most proteins are near or above their isoelectric points whereas the histones are well below. By staining nuclei at pH 8.04 in Fast Green according to Bennett (1970), only the histone protein will bind this acid dye. Light absorption was measured at 655 mμ (Fig. 1d).

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Figure 1

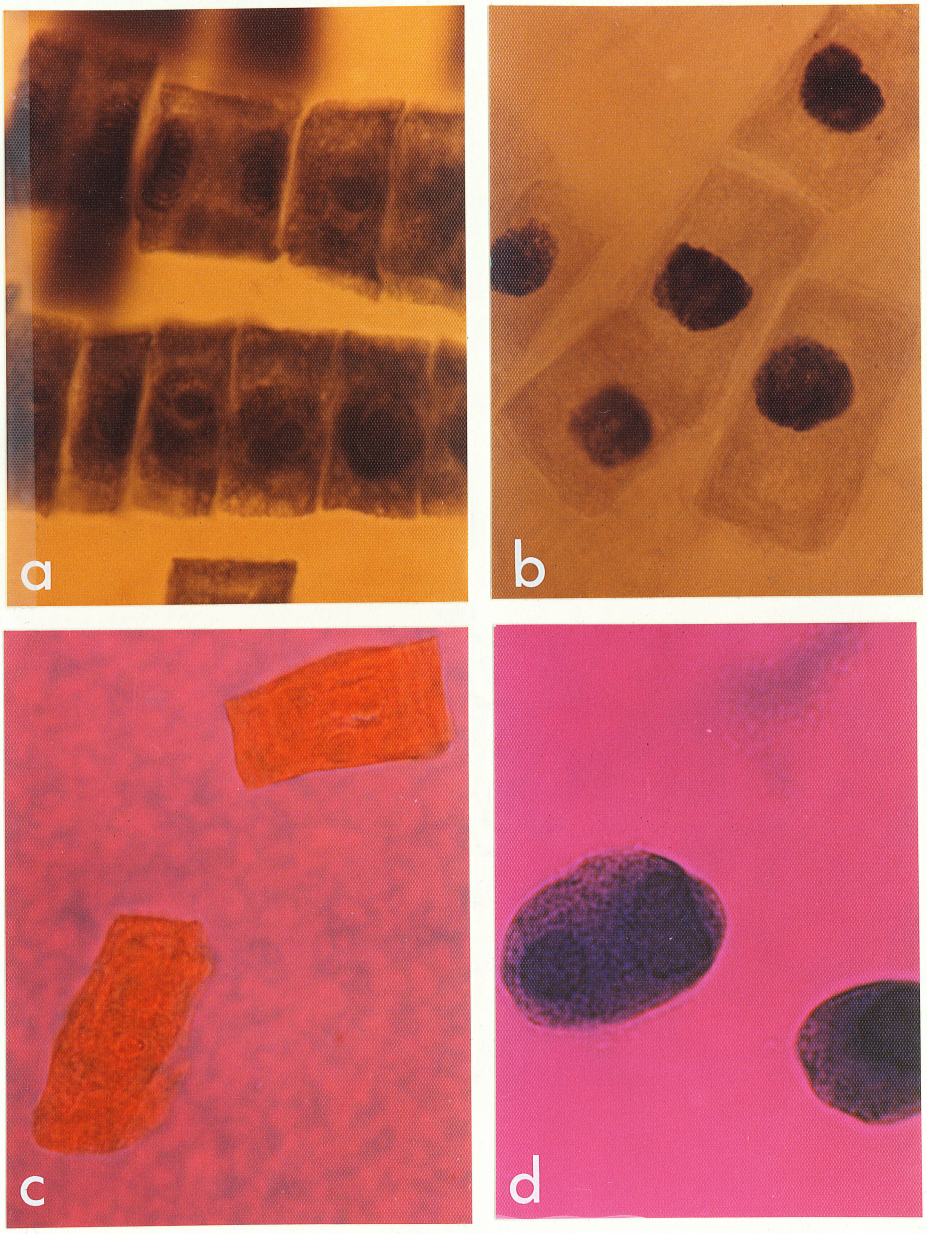
Types of stains employed on root meristematic cells for microphotometric determination.

(a) Gallocyanin chromalum stain for total nucleic acids (x 700).

(b) Gallocyanin chromalum stain for DNA (x 730).

(c) DNFB stain for total protein (x 730).

(d) Fast Green stain for nuclear histone (x 1450).


a
b
c
d

RESULTS

1. Total cellular DNA

The mean cellular DNA content is shown in Table 1. In 3 of the 4 genotypes studied, the total DNA content was slightly higher when the female involved in the cross carried hexaploid wheat cytoplasm. However, a *t*-test between the means of each reciprocal pair, as well as a factorial analysis of variance of the population indicated that differences were not significant at the 5% level.

2. Total cellular RNA

As shown in Table 1, the mean total RNA content was higher when the female involved in the cross carried hexaploid wheat cytoplasm. This was true for all the 4 genotypes employed in the comparisons. A factorial analysis of variance (Table 4) indicated both main effects, viz. genotypic and cytoplasmic, were significant at the 1% level.

As was true with the quantity of total cellular RNA, the mean RNA/DNA ratio also indicated that more RNA was present when hexaploid wheat cytoplasm was involved (Table 1). A comparison of each reciprocal pair showed one exception to this trend, viz. the reciprocal cross involving Pitic x *T. turgidum* L. var. turgidum, in which the cross with 4*x* wheat cytoplasm had more RNA per unit DNA. The difference was not statistically significant however. A factorial analysis of variance indicates that genotypic and cytoplasmic effects were both significant at the 1

Table 1. Total cellular DNA and RNA values (arbitrary units) of reciprocal F_1 populations

Designation	No. of cells measured	DNA	RNA	RNA/DNA ratio
A x A^1	[a]50	[b]14.88 ± 0.50	17.84 ± 1.26	1.21 ± 0.08
A^1 x A	50	13.01 ± 0.68	16.58 ± 0.92	1.27 ± 0.07
B x B^1	50	11.02 ± 0.46	13.44 ± 0.76	1.22 ± 0.07
B^1 x B	50	11.75 ± 0.15	13.16 ± 0.45	1.12 ± 0.04
C x C^1	50	13.98 ± 0.89	14.43 ± 0.95	1.03 ± 0.07
C^1 x C	50	13.29 ± 0.74	10.06 ± 0.70	0.76 ± 0.05
D x D^1	50	17.21 ± 0.37	10.14 ± 0.89	0.59 ± 0.05
D^1 x D	50	15.50 ± 0.62	7.93 ± 0.69	0.51 ± 0.04

[a] 10 cells from each of 5 seedlings were measured.

[b] Overall mean and standard error of 5 seedlings.

and 10% level respectively (Table 4).

3. Total cellular protein

As was true with the total RNA content, the quantity of total protein was higher in all of the 4 comparisons made when the female involved carried hexaploid wheat cytoplasm (Table 2). A factorial analysis of variance indicated that both the main effects viz. genotype and cytoplasm as well as their interaction (G x C) were significant at the 1% level (Table 4). When tested against the significant interaction, genotypic effect was reduced to a level of non-significance, and the cytoplasmic effect remained significant only at the 20% level. It was also observed that the genotype x cytoplasm interaction was mainly due to a change-in-rate (degree) interaction rather than to a complete reversal interaction (see Steel and Torrie, 1960). Hence, this fact should be taken into consideration when evaluating the level of significance for cytoplasmic as well as genotypic effect using the significant interaction mean square.

The mean protein/DNA ratio indicated that except for 1 out of 4 comparisons, more protein was synthesized per unit DNA (Table 2) when the female involved in the cross carried hexaploid wheat cytoplasm. This exception involved the reciprocal cross of Pitic x T. turgidum L. var. turgidum, but the difference was not statistically significant. A factorial analysis of variance showed that both the main effects (genotype and cytoplasm) as well as their interaction were significant at the 1% level (Table 4). In this case the differences due to main effects became

Table 2. Total cellular protein (arbitrary units) of reciprocal F_1 populations

Designation	No. of cells measured	Protein	Protein/DNA ratio
A x A^1	[a]50	[b]38.29 ± 1.14	2.57 ± 0.08
A^1 x A	50	35.14 ± 0.53	2.70 ± 0.05
B x B^1	50	43.62 ± 1.24	3.96 ± 0.11
B^1 x B	50	29.89 ± 1.07	2.54 ± 0.09
C x C^1	50	33.26 ± 1.50	2.38 ± 0.11
C^1 x C	50	30.38 ± 1.00	2.29 ± 0.08
D x D^1	50	32.28 ± 1.13	1.88 ± 0.07
D^1 x D	50	29.74 ± 0.81	1.92 ± 0.05

[a] 10 cells from each of 5 seedlings were measured.

[b] Overall mean and standard error of 5 seedlings.

non-significant when tested against the interaction.

4. Nuclear histone protein

As shown in Table 3, the quantity of nuclear histone in triticale carrying hexaploid wheat cytoplasm was less than those with tetraploid wheat cytoplasm. This was observed in all of the 4 comparisons made. Overall mean values were 3.45 $\pm$ 0.12 and 3.87 $\pm$ 0.09 (arbitrary units) for hexaploid and tetraploid cytoplasms respectively. A factorial analysis of variance indicated that both genotypic and cytoplasmic effects were significant ($P \leqslant 0.01$, Table 4). However when tested against the genotype x cytoplasm interaction, cytoplasmic effect was significant at the 20% level, while the genotypic effect was reduced to the level of non-significance.

As was true with nuclear histone content per se, the histone/DNA ratio was also lower when the female involved in the cross carried hexaploid wheat cytoplasm (Table 3). A factorial analysis of variance indicated that both genotypic and cytoplasmic effects as well as their interaction were significant at the 1% level (Table 4). However, when tested against the interaction the main effects became significant at the 20% level. As was observed for total cellular protein, the interaction of genotype x cytoplasm was not due to a complete reversal interaction.

Table 3. Nuclear histone protein (arbitrary units) from isolated nuclei of reciprocal F_1 populations

Designation	No. of nuclei measured	Histone	Histone/DNA ratio
A x A^1	[a]50	[b]2.96 ± 0.17	0.199 ± 0.01
A^1 x A	50	4.01 ± 0.07	0.308 ± 0.01
B x B^1	50	3.69 ± 0.11	0.335 ± 0.01
B^1 x B	50	3.99 ± 0.15	0.340 ± 0.01
C x C^1	50	3.16 ± 0.18	0.226 ± 0.01
C^1 x C	50	3.39 ± 0.14	0.255 ± 0.01
D x D^1	50	4.00 ± 0.11	0.232 ± 0.01
D^1 x D	50	4.09 ± 0.14	0.264 ± 0.01

[a] 10 cells from each of 5 seedlings were measured.

[b] Overall mean and standard error of 5 seedlings.

Table 4. Mean square values for the total cellular RNA, cellular protein, and nuclear histone of reciprocal F_1 populations

Source of variation	RNA	RNA/DNA ratio	Protein	Protein/DNA ratio[a]	Histone	Histone/DNA ratio	Test against G x C		
							Protein	Histone	Histone/DNA ratio
Genotype (G)	113.7**	0.98**	95.3**	3.2**	1.20**	0.02**	n.s.	n.s.	$P \leqslant 0.20$
Cytoplasm (C)	41.2**	0.09+	311.0**	1.1**	1.77**	0.02**	$P \leqslant 0.20$	$P \leqslant 0.20$	$P \leqslant 0.20$
G x C	7.6	0.05	74.0**	1.3**	0.46*	0.005**			
Error	4.6	0.02	7.3	0.04	0.12	0.0006			

[a] Genotypic as well as cytoplasmic effects were not significant when tested against G x C interaction.

Degrees of freedom, G = 3; C = 1; G x C = 3; and error = 32.

+ ($P \leqslant 0.10$), * ($P \leqslant 0.05$), ** ($P \leqslant 0.01$).

DISCUSSION

A differential interaction of a hexaploid triticale nucleus (AABBRR) in either hexaploid or tetraploid wheat cytoplasm is further demonstrated at the cellular level. Our results indicated that relative to triticale carrying tetraploid cytoplasm, AABBRR nuclei in a hexaploid wheat cytoplasm perform more beneficially in that more RNA, as well as protein quantity was obtained. The theory that the transfer of genetic information encoded in a gene into protein is through an RNA intermediate as formulated by Crick (1958), has been widely accepted. Likewise, it is also recognized that several species of RNA exist (Watson, 1970). In higher organisms, messenger RNA is transcribed from DNA in the nucleus and subsequently moves through the nuclear membrane into the cytoplasm (Georgiev, 1967). Furthermore, evidence is also accumulating that chloroplasts and mitochondrial DNA contributes a certain portion to the total cellular RNA (see Sager, 1972). However, a large part of the total RNA in a growing cell is ribosomal RNA, and most of the remainder is transfer RNA (Lewin, 1970). Although the function of ribosomal RNA is not definitely known, it may be involved in the binding of transfer RNA and messenger RNA to ribosomes (Watson, 1970); and also may play some part in plant development (Ingle and Sinclair, 1972).

In the present study, the total RNA per cell was measured, therefore it would be a matter of conjecture at this point in time to specify the species of RNA which might contribute to the observed difference between

the reciprocal populations. Nevertheless, if it is accepted that the quantity of protein and of RNA reflects the rate and/or the amount of total genetic activity in a cell, it provides some evidence which indicates a differential genetic activity due to nuclear-cytoplasmic interaction.

In the present study, a significant quantitative difference in the nuclear histone content as well as in the histone/DNA ratio between the two reciprocal populations were observed. Since Stedman and Stedman (1950) first suggested that histones might function as gene regulators, considerable information has accumulated to suggest that histones do indeed play an important role in the regulation of chromosome function. Spelsberg et al. (1972) provides evidence that the fraction of genetically active DNA varies with the cellular phenotype, both quantitatively and qualitatively. If the DNA in chromatin is freed of its associated protein species (histones and non-histone proteins), it becomes a much more effective primer for RNA synthesis in vitro (Huang and Bonner, 1962; Paul and Gilmour, 1968; Georgiev, 1969; Wilhelm, et al., 1971). Paul and Gilmour (1968) concluded that histones are necessary for quantitative masking of DNA in chromatin, but that their effect is non-specific. Specificity of gene repression is thought to be mediated either through chromosomal RNA (Beckor, et al., 1969) or through the acidic non-histone proteins (Spelsberg, et al., 1972).

If it is accepted that histones function as repressors of gene

action, then it follows that genetic expression will be negatively correlated with amount of histone. In our results a significant negative regression value of mean histone content on mean RNA content was observed for the cell population in hexaploid wheat cytoplasm ($b = -6.23$, $r = -0.94$; $P \leqslant 0.10$). However a significant regression value of histone on RNA was not obtained for the cell population in tetraploid wheat cytoplasm

Perhaps, insofar as our results are concerned, nuclear-cytoplasmic interactions *per se* in hexaploid triticale are initially being expressed both in the level of nuclear histone and in the quantity of total RNA and subsequently, in the synthesis of proteins. The quantity of total RNA will depend on both nuclear and cytoplasmic DNA. It is not yet known whether the DNA of chloroplasts and mitochondria of hexaploid and tetraploid wheat *per se* differ in their transcribing abilities. However, that mutation can occur during the process of evolution in chloroplast DNA has recently been demonstrated in *Nicotiana*. The ancestors of *Nicotiana* species indigenous to Australia had been separated from those of the Western Hemisphere species for more than 150 million years (Goodspeed, 1954). Chen and Wildman (1972) studying the large subunit of Fraction I proteins revealed that *Nicotiana* species (Australian) possessed one tryptic peptide that was absent in those species indigenous to the western Hemisphere. The extra peptide appeared in the reciprocal F_1 hybrids of *N. gossei* (Aust.) x *N. tabacum* (W. Hemisphere), only when

N. gossei was the female parent. The authors attributed this to a mutation in the cistron of chloroplast DNA which has survived during years of evolution separating the Australian from the western Hemisphere Nicotiana species.

In wheat, available evidence suggests that the cytoplasm of hexaploid wheat has become modified through evolution to co-exist harmoniously with the additional genome contributed by Ae. squarrosa (see Larter and Hsam, 1973; and Section II of this Thesis). The synthesis and utilization of secondary hexaploid triticales would be making use of the evolutionary advantages of hexaploid wheat cytoplasm, which in turn should be reflected in their improved performance relative to primary hexaploid triticales.

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APPENDIX IV - 1

SDS-PAGE molecular weight markers

Protein	Molecular weight (daltons)	Source
γ -globulins	160,000	Sigma
Bovine serum albumin-dimer	132,000	Calbiochem.
Bovine serum albumin-monomer	66,000	Calbiochem.
Ovalbumin	45,000	Sigma
Pepsin	35,000	Nutritional-Biochem.
α -Chymotrypsin	21,600	Calbiochem.
Myoglobin	17,000	Calbiochem.
Ribonuclease	13,700	Dickinson
Cytochrome c	12,400	Calbiochem.

APPENDIX IV - 2

Solutions for SDS-PAGE

Stock Buffer: 1 liter containing

7.8 g. $NaH_2PO_4.H_2O$

20.4 g. Na_2HPO_4

10.0 g. Sodium dodecyl sulfate (SDS)

Electrode buffer:

Dilute the stock buffer to 1/10 original strength. Final pH 7.3.

Protein solvent:

The electrode buffer containing 1% (w./v.) of SDS and either 1% (v./v.) of β-mercaptoethanol or 0.002M NEMI.

APPENDIX IV - 3

Staining and destaining solutions

Solution 1:

Isopropyl alcohol	25%
Acetic acid	10%
Coomassie Brilliant Blue	0.05%

Solution 2:

Isopropyl alcohol	10%
Acetic acid	10%
Coomassie Brilliant Blue	0.005%

Solution 3:

Acetic acid	10%
Coomassie Brilliant Blue	0.0025%

Solution 4:

Acetic acid	10%

The periods were as follows:

Solution 1 - 3 - overnight with shaking

" 4 - several hours (until background is clear) with shaking.

All staining and destaining was done at room temperature.