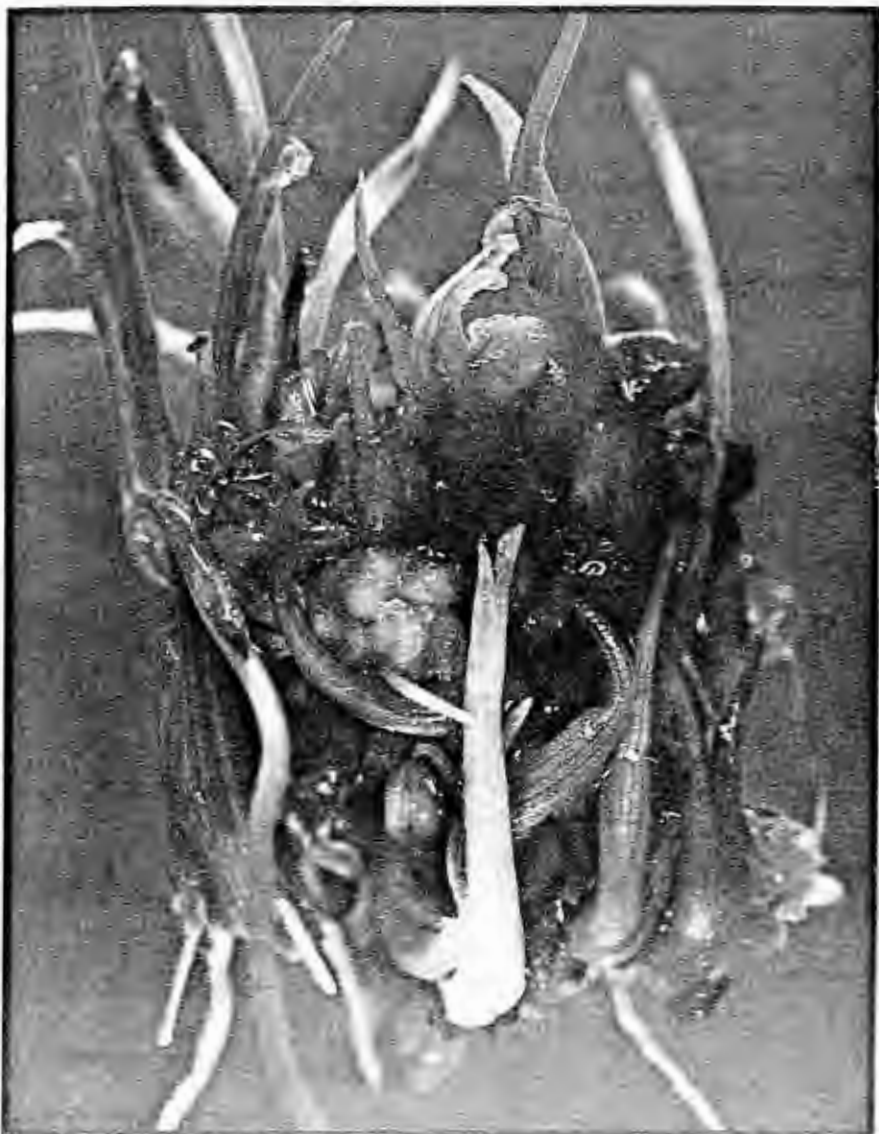


# Review of Advances in Plant Biotechnology, 1985-88

A. Mujeeb-Kazi and L.A. Sitch, technical editors



International Maize and Wheat Improvement Center  
International Rice Research Institute

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Responsibility for this publication rests solely with CIMMYT and IRRI.

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# Preface

There is great potential for biotechnology as a tool in crop breeding. Despite the risk in such upstream research, many plant breeders are becoming more involved in this work to facilitate the rapid exploitation of new techniques. Genetic manipulation of plants involving tissue culture, restriction fragment length polymorphisms, isozymes, and incorporation of genes from wild species is creating a meteoric revolution for plant breeders.

To keep abreast of the rapid developments in this field, nearly 50 participants from developed and developing countries attended the Second International Symposium on Genetic Manipulation in Crops at the International Maize and Wheat Improvement Center (CIMMYT), El Batán, Mexico, August 29-31, 1988. This meeting was a follow-up to the First International Symposium on Genetic Manipulation in Crops held Oct. 22-26, 1984, in Beijing, China.

The first symposium, which essentially reviewed genetic manipulation work up through 1984, was sponsored by Academia Sinica and the International Rice Research Institute (IRRI). The first meeting was so successful that it prompted CIMMYT to join Academia Sinica and IRRI in co-sponsoring the second symposium.

The second symposium provided the opportunity to analyze research achievements since 1984—hence the title of these proceedings, *Review of Advances in Plant Biotechnology, 1985-88*. The relatively small number of participants (compared to the first symposium) permitted conferees to focus more sharply on research needs and develop the collaborative mechanisms necessary for charting broad pathways for work in this field into the future.

These proceedings start off with a keynote address that looks into the scientific, social, economic, and ethical implication of genetic manipulation in crops. Following this are a selected collection of 27 papers and 6 posters presented during the symposium that update current work in the discipline and review the literature in four specific areas: 1) anther culture and haploid breeding, 2) protoplast culture, somatic hybridization, and transformation systems, 3) distant hybridization; and 4) somatic embryogenesis

and somaclonal variation. A fifth session addressed international collaboration in genetic manipulation of crop plants. Crops covered in these presentations include rice, wheat, maize, barley, triticale, citrus, sugar beet, brassicas, tropical forage legumes, cassava, and cotton.

It is the consensus of plant breeders, geneticists, and other biologists working in crop plant improvement that biotechnology holds the most hope for rapid improvement of crop plants, and for achieving the kinds of advances required for the sustained yield increases demanded in the face of an expanding world population and shrinking land resources. Both CIMMYT and IRRI, currently developing their research strategies towards the year 2000, found the ideas that emerged from this second symposium to be very useful in their program development.

It is now apparent that additional symposia on the subject of Genetic Manipulation in Crops will be held. At this meeting, an organizing committee was formed to start planning for the third symposium, tentatively set for 1991 in Africa.

We take this opportunity to recognize other members of the Second Symposium Organizing Committee, namely: Z.S. Li, H. Hu, and Q. Q. Shao from Academia Sinica; and G.S. Khush from IRRI. We express gratitude to the staff of CIMMYT for being our gracious hosts, and to the United Nations Development Programme, the Rockefeller Foundation, the U.S. Agency for International Development, and the Third World Academy of Sciences for their financial assistance. We thank Gene P. Hettel, science writer/editor for CIMMYT Information Services, for editing these proceedings and coordinating their publication.

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# Diagnostic markers in wheat wide crosses

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It is important to identify alien chromatin accurately and rapidly in a wide hybridization program. Markers are heritable characteristics associated with and useful for the identification of specific genotypes. Markers of significance usually show high polymorphism, few epistatic or pleiotropic effects, exhibit co-dominant inheritance, and are free from environmental and developmental influence.

Markers are heritable characteristics associated with specific genotypes and hence often used for genotypic characterization. Among other things, markers are used to describe parental lines and populations, assess genetic variability and phylogenetic relationships, identify gene blocks contributing to important agronomic characters, tag genes of agronomic traits, test for normality of gene flow in interspecific or intergeneric crosses, confirm hybrids, identify aneuploids, establish wheat-alien chromosome homoeologies, and track alien chromosome segments in wheat backgrounds. Important features of a useful marker include a high level of polymorphism, speed of assay, few epistatic or pleiotropic effects and a co-dominant mode of inheritance. Markers may be categorized as morphological, cytological, or biochemical.

## **MORPHOLOGICAL MARKERS**

Morphological markers are often easily identified but are limited by environmental influences, exhibit dominant/recessive inheritance and relatively low polymorphism. Characters such as pubescence, presence of anthocyanin pigmentation (Fig. 1a), grain color (Fig. 1b), stem solidity (Fig. 1c), and spike shape (Fig. 1d) have been often used in the Triticeae to indicate the presence of their respective genes. Wheat plants are often modified in terms of such characters upon introgression of alien chromatin, thus assisting characterization of the introduced segments since genetic control for most of these characters has already been attributed to specific homoeologous groups.

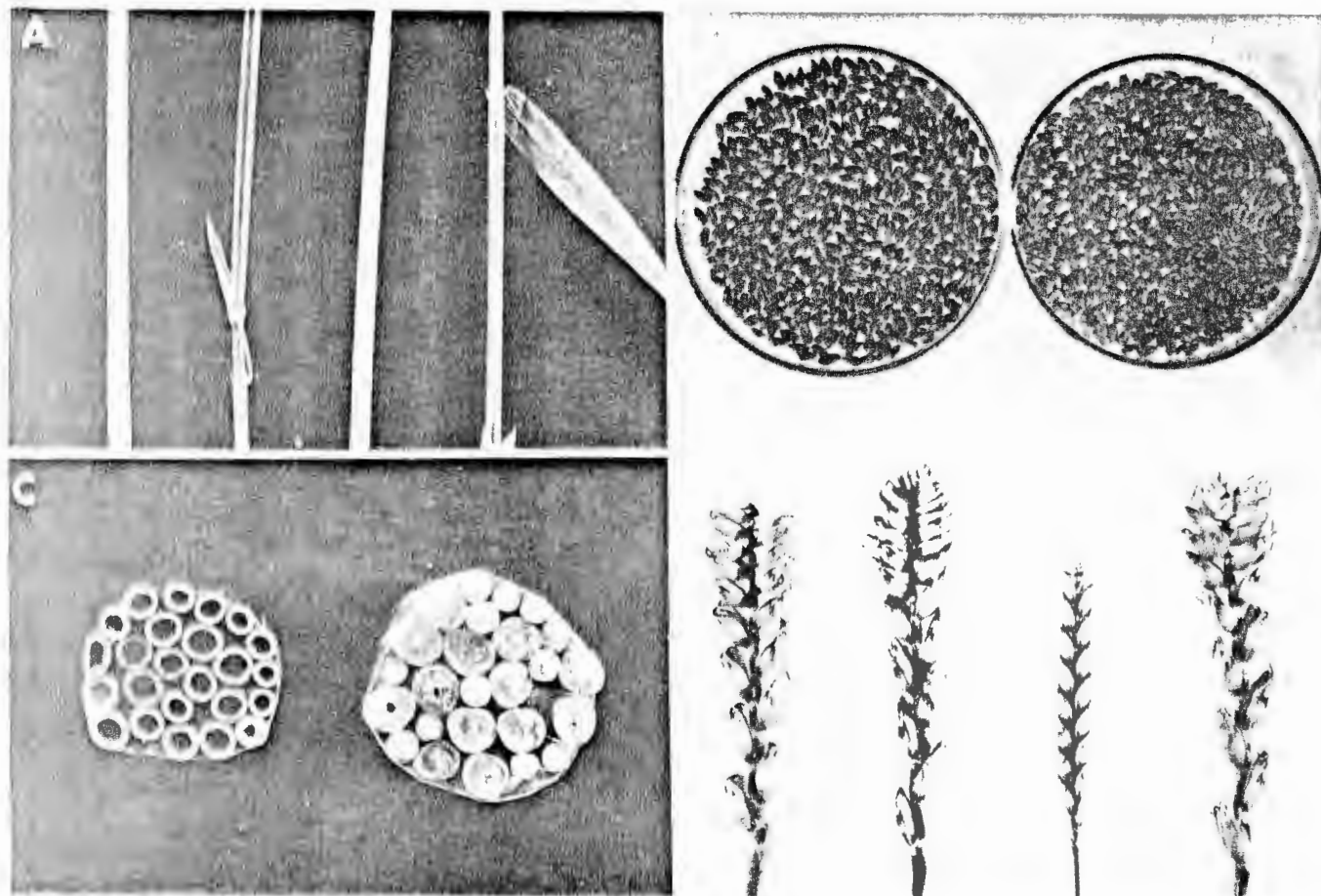


Figure 1. Morphological markers showing in: A) presence of anthocyanin (segregation for color); B) seed color (blue aleurone versus normal color); C) stem solidity (normal versus solid stem); and D) spike morphology, left to right, 'Chinese Spring' (CS) + 5R (from *Secale cereale*), CS + 5J (*Thinopyrum bessarabicum*, *Agropyron junceum*), CS, CS + 5 UM (*Aegilops umbellulata*).

## CYTOLOGICAL MARKERS

Cytological markers include features such as chromosomal size/arm ratio (Fig. 2a; Mujeeb-Kazi and Miranda 1985), satellites/secondary constrictions (Fig. 2b; Mujeeb-Kazi and Miranda 1985), and differential C- and N-banding staining patterns (Figs. 2c and d; Mujeeb-Kazi 1982; Mujeeb-Kazi *et al.* 1989) that are useful in identification of wheat and alien chromosomes. Many different stains have been used to facilitate such identification, the more common being feulgen, acetocarmine, aceto-orcein, giemsa, and Leishman. Recently radioactive- and biotin-labelled DNA sequences have been introduced as markers through their *in situ* hybridization to complementary DNA sequences in cytological preparations and the detection of the resulting duplexes by autoradiography, fluorescence, or through enzymatic procedures. The application of biotin labelled probes is projected to detect D genome/alien genome chromosome translocations; stocks that are being developed at CIMMYT (Mujeeb-Kazi 1984).

## BIOCHEMICAL MARKERS

Biochemical markers may be subdivided on the basis of whether they are the gene products (proteins) or DNA sequences. Differential characteristics of proteins and DNA have been used to distinguish among members of the Triticeae and their progenies. Protein markers have been used more extensively than DNA markers and involve analyses of isozymes and/or storage proteins. There are currently over 100 structural gene loci in wheat identifiable by protein markers. Homoeoallelic loci have been identified in related species. Only the short arms of homoeologous group 2 chromosomes have not been reliably marked so far in this way. The protein separation techniques used in the wheat wide crosses program at CIMMYT are: cellulose acetate electrophoresis, polyacrylamide gel electrophoresis (PAGE) under native conditions, sodium dodecyl sulfate (PAGE), and polyacrylamide gel isoelectric focussing (PAG.IEF).

Characteristic separation patterns have been established of many isoenzymes and storage proteins for many spring wheats and alien species. Consistent differences between the patterns of the alien species as compared to those of wheat, especially as confirmed in the wheat-alien species hybrid, serve as useful markers. The enzymes analyzed most often in our program are acid phosphatase, acõnitate hydratase, aliphatic alcohol dehydrogenase, esterases (Figs. 3a and b), glucose phosphate isomerase, glutamate oxaloacetate transaminase, isocitrate dehydrogenase, malate dehydrogenase, phosphoglucomutase (Fig. 3c), aspartate aminotransferase (Fig. 4a), 6-phosphogluconate dehydrogenase (Fig. 4b), shikimate dehydrogenase and superoxide dismutase. DNA markers termed restriction fragment length polymorphisms (RFLPs) have the potential to saturate the genetic maps of most crop plants as they have the capacity to detect differences in the DNA sequence which may not result in detectable gene products or changes in gross morphology. RFLP technology is yet to be extensively employed in wheat wide hybridization owing to the lack of suitable probes in the public sector.

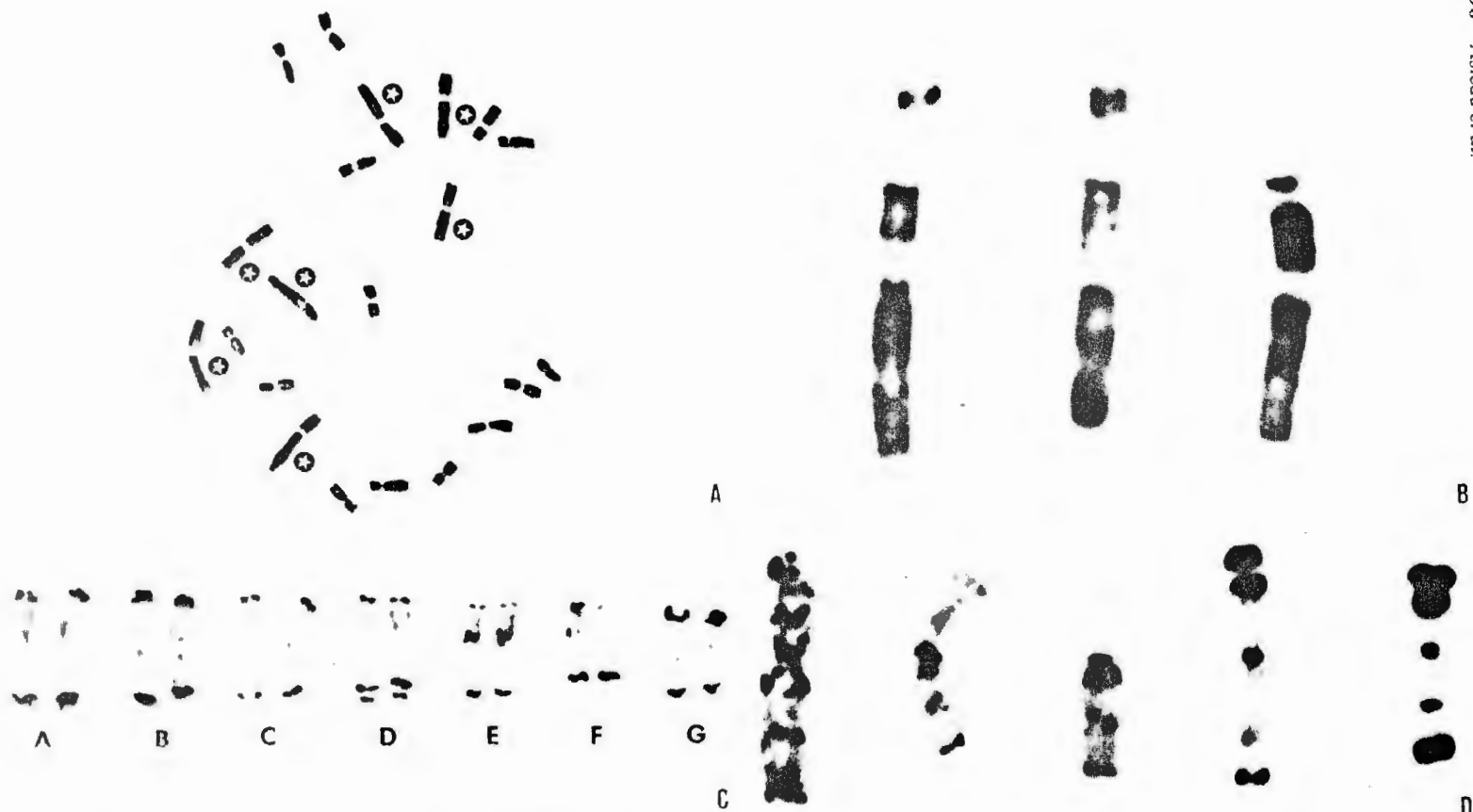


Figure 2. Chromosome structure showing size/arm ratio, satellites/secondary constrictions, differential staining (C and N banding) as follows: A) chromosomes of *Agropyron elongatum/Secale cereale* F<sub>1</sub> hybrid (\* = *S. cereale*) (Mujeeb-Kazi and Miranda 1985), B) left to right: satellited chromosomes 1B, 6B, and 5D of *Triticum aestivum* (Mujeeb-Kazi and Miranda 1985); C) C-banded karyotype of *Haynaldia villosa* (Mujeeb-Kazi 1982); and D) left to right: N-banded chromosomes of 1B, 1BL/1RS, 1R, and C-banded 1BL/1RS, 1R. (Mujeeb-Kazi 1982, Mujeeb-Kazi *et al.* 1989).



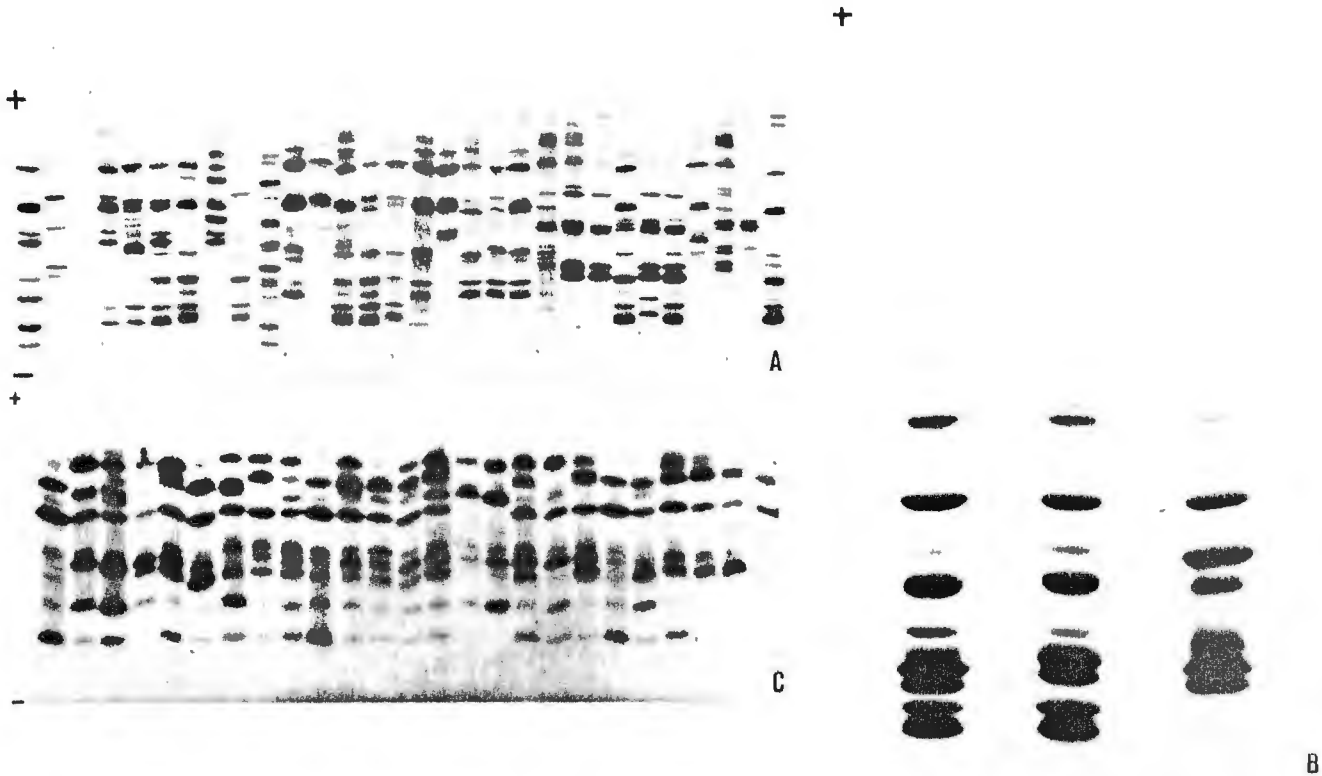
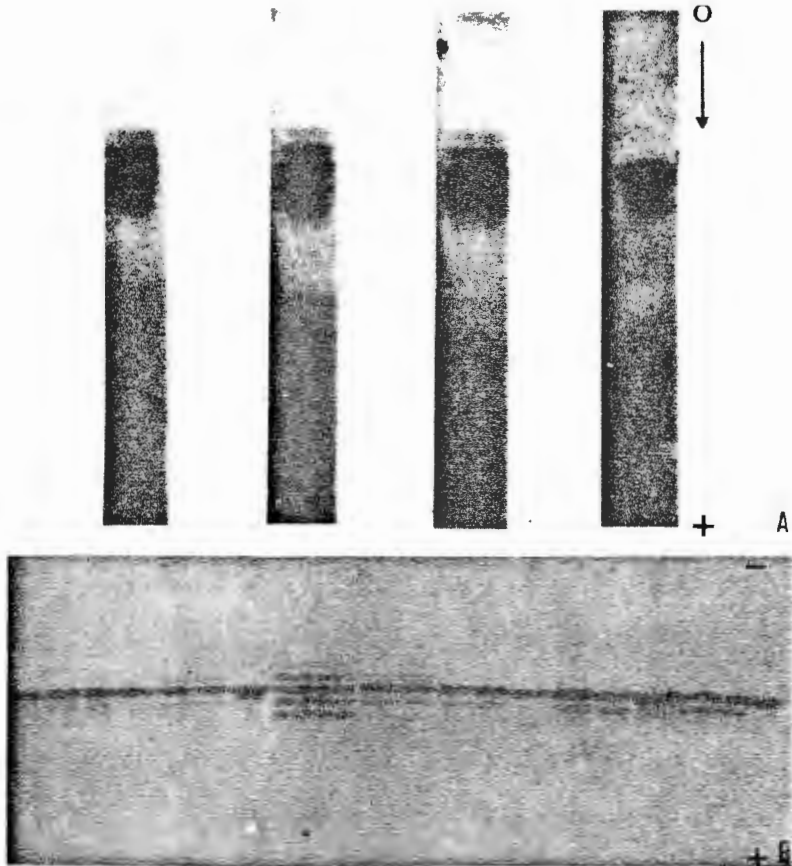


Figure 3. Polyacrylamide gel isoelectric focussing (PAG.IEF) patterns of the following: A) Esterase (E.C.3.1.1.2) for three accessions of *Thinopyrum (Agropyron) intermedium* (10 tracks/accession); B) Esterase showing polymorphism for different CIMMYT spring wheat (*Triticum aestivum*); C) Phosphoglucomutase (E.C.2.7.5.1) for 25 accessions of *Th. (A.) intermedium*.



**Figure 4.** Polyacrylamide gel electrophoresis (PAGE) patterns of: **A)** aspartate amino transferase (E.C.2.6.1.1) for, left to right, *Thinopyrum (Agropyron) acutum*, *Triticum turgidum* L. cv. Yavaros/*Th. (A.) acutum* (2 tracks) and *T. turgidum* L. cv. Yavaros; **B)** 6-phosphogluconate dehydrogenase (E.C.1.1.1.44) for backcross derivatives of *T. aestivum* L./*Aegilops variabilis*.

## CONCLUSION

All classes of markers have their advantages and limitations but very often marker combinations from different categories are beneficial. Naturally, the relative emphasis on the different classes depends on the individual project, stages of the project and/or the resources available. The biochemical markers, in particular, are very versatile. They have an abundance of polymorphism, exhibit little to no pleiotropic or epistatic effects, are less affected by environmental influence, are subject to rapid detection, and are inherited co-dominantly. Furthermore the DNA markers are detectable at all plant developmental stages and all the polymorphisms at all marker loci can be identified by a single technique.

## ACKNOWLEDGMENT

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## REFERENCES CITED

- Mujeeb-Kazi, A. 1982. Wide Crosses. *In: CIMMYT Report on Wheat Improvement*, pp. 78-85.
- Mujeeb-Kazi, A. 1984. Wide Crosses. *In: CIMMYT Report on Wheat Improvement*, pp. 54-65.
- Mujeeb-Kazi, A., J.L. Miranda. 1985. Enhanced resolution of somatic chromosome constrictions as an aid to identifying intergeneric hybrids among some Triticeae. *Cytologia* **50**:701-709.
- Mujeeb-Kazi, A., R. Asiedu, S. Rajaram, R.J. Peña, and A. Amaya. 1989. Some spring wheat varieties of *Triticum aestivum* with the 1B/1R chromosome translocation. *Theor. Appl. Genet.* (submitted).

## RESUMEN

En un programa de hibridización amplia, es de vital importancia identificar con precisión y rapidez la cromatina extraña. Los marcadores son características hereditarias que son de gran utilidad en la identificación de genotipos específicos. Los marcadores de mayor importancia suelen presentar un alto nivel de polimorfismo, escasos efectos epistáticos o pleiotrópicos, muestran herencia codominante y no se ven afectados por las influencias ambientales y del desarrollo.