

WAYTE INSTITUTE
19.3.84
LIBRARY

A STUDY OF TRITICALE - WHEAT CROSSES

by

RAYMOND LEE BRENGMAN

B.Sc.

Kansas State University

Submitted in partial fulfilment of the
requirements necessary to complete the
Degree of Master of Agricultural Science
Department of Agronomy
University of Adelaide, S.A.

Submitted May, 1983

TABLE OF CONTENTS

	<u>Page no.</u>
SUMMARY	vii
AUTHOR'S DECLARATION	xii
ACKNOWLEDGEMENTS	xiii
<u>INTRODUCTION</u>	1
<u>LITERATURE REVIEW</u>	3
1. Triticale - Historical Background	3
2. Breeding and Improvement	4
(A) Direct synthesis of primary triticales	8
(B) Production of secondary hexaploids	14
(C) Crossing of predoubled parental stocks	15
(D) Hexaploid triticale X hexaploid wheat	15
(E) Hexaploid triticale X rye (diploid and tetraploid)	19
(F) (Hexaploid wheat X rye) F ₁ X hexaploid triticale	19
3. Cytoplasm	20
4. Cytology	22
5. Cytology of Seed Shrivelling	28
6. Trigeneric Hybrids and Three-way Crosses	29
7. Embryo Culture	29
8. Conclusions	34
<u>FORMAT</u>	38
<u>CHAPTER 1</u> <u>CROSSES OF HEXAPLOID WHEAT BY HEXAPLOID TRITICALE</u> <u>AND THE EFFICIENCY OF EMBRYO CULTURING</u>	39
1.1 INTRODUCTION	39
1.2 MATERIALS AND METHODS	39
1.3 RESULTS	41

	<u>Page no.</u>
1.4 DISCUSSION OF RESULTS	42
1.4a Experiment I	42
1.4b Experiment II	43
1.4c Experiment III	44
1.4d Embryo Culturing Efficiency	44
<u>CHAPTER 2</u> <u>A STUDY OF THE INTROGRESSION OF TRITICUM</u> <u>AESTIVUM GERMPLASM INTO HEXAPLOID TRITICALE</u> <u>THROUGH DIRECT CROSSES</u>	46
2.1 INTRODUCTION	46
2.2 MATERIALS AND METHODS	48
2.2a Group 1	49
2.2b Group 2	53
2.2c General	55
2.3 RESULTS AND DISCUSSION	58
2.3a Parents and the F ₁ Plants	62
i) Parents	62
ii) F ₁ Plants	63
iii) Cytology of F ₁ Plants	63
iv) Fertility of F ₁ Plants	63
v) BC ₁ Seed	67
vi) Statistical Analysis of Contribution of Nuclear Genotype to Seed Set on F ₁ Plants	67
2.3b BC ₁ Generation	68
i) Germination and Viability of the BC ₁	68
ii) BC ₁ Fertility and Grain Quality	69
iii) Univalent Transmission to, and <u>H_p</u> Frequency in BC ₁ Plants	69
iv) Derivation of a Model of Univalent Transmission in F ₁ Plants	74
v) Correlation Between BC ₁ Data Collected	81
2.3c BC ₁ F ₂	82
2.3d BC ₁ F ₃	85
<u>CHAPTER 3</u> <u>INTEGRATED (MIXED R AND D GENOMES) TRITICALE-</u> <u>WHEAT CROSSES</u>	92
3.1 INTRODUCTION	92
3.2 MATERIALS AND METHODS	93

	<u>Page no.</u>
3.3 RESULTS AND DISCUSSION	96
3.3a Results of Primary Crosses	96
3.3b Results of Backcrossing of F_1 Hybrid	99
3.3c Results of Selfing BC_1F_1 Plants	99
3.3d Use of Genetic Male Sterile Wheat as the Female Parent of Hybrids	103
 <u>CONCLUSIONS</u>	 105
a) Embryo Culture	105
b) Genotypes	106
c) Direction of Primary Cross	107
d) Cytoplasm	108
 <u>PROPOSED METHOD OF OBTAINING MAXIMUM GENETIC AND CYTOPLASMIC INTROGRESSION FROM HEXAPLOID WHEAT</u>	 111
 <u>APPENDIX I</u> Embryo Culture Media and Procedures	 113
<u>APPENDIX II</u> Expansion of the Binomial $C_I^{14}P^IQ(14-I)$	118
<u>APPENDIX III</u> Correlation Between BC_1 Characters	119
<u>APPENDIX IV</u> Observations of BC_1F_2 Single Rows (2 metres long)	121
 <u>BIBLIOGRAPHY</u>	 125

LIST OF TABLES

TABLE 1	Crossability of 4x Wheat Species with Cultivated Rye (After Sanchez-Monge, 1958)	10
TABLE 2	Crossability of 4x Wheat Species with Cultivated Rye (After Pienaar, 1973)	11
TABLE 3	Crossability of <u>Secale triticum</u> with <u>Triticum aestivum vulgare</u> (From Pienaar, 1973)	13
TABLE 4	Results of a study carried out by Fedorova and Polenova (1975) using the method of crossing Triticales and Hexaploid Wheat to introduce Germplasm.	18

TABLE 5	Results of Experiment I (September, 1973) The fate of embryos cultured on Schenk and Hildebrandt media incorporating different levels of hormone.	41
TABLE 6	Results of Experiment II (May, 1974) The fate of embryos cultured on Schenk and Hildebrandt media at constant hormone level with two methods of embryo removal.	42
TABLE 7	Results of Experiment III (September, 1974) The fate of embryos cultured on Schenk and Hildebrandt media incorporating two hormone levels with activated carbon added at the higher hormone level.	42
TABLE 8	Efficiency of embryo culturing in terms of labour and expenses. Minimum labour required to embryo culture 100 embryos.	45
TABLE 9	Details of generation, treatment, planting and harvesting dates - Group 1.	50
TABLE 10	Table of Primary and Secondary Crosses.	51
TABLE 11	Details of generation, treatment, planting and harvesting dates - Group 2.	54
TABLE 12	Results of Primary Crosses (F_1 hybrids) - Groups 1 and 2.	59
TABLE 13	Results of Secondary Crosses (BC_1) - Groups 1 and 2.	60
TABLE 14	Results of reciprocal crosses between Triticale and Wheat.	61
TABLE 15	Cytology of F_1 : Hexaploid Triticale X Hexaploid Wheat.	64
TABLE 16	Genotype Effect on F_1 Fertility.	64
TABLE 17	BC_1 seed set and viability data.	66
TABLE 18	Summary of BC_1 Data.	68
TABLE 19	Meiosis - BC_1 Plants.	71
TABLE 20	Observed mean frequency of transmission of chromosome 5R, having <u>Hp</u> gene, to the BC_1 plants.	72

	<u>Page no.</u>
TABLE 21 Observed and expected frequencies of eggs having specific numbers of transmitted univalent chromosomes.	76
TABLE 22 Results collected on BC_1F_2 .	83
TABLE 23 Yield trial - BC_1F_3 .	86
TABLE 24 Notes on yield trial - BC_1F_3 .	87
TABLE 25 Comparison of Triticale vs. Wheat for suitability as Female Parent.	97
TABLE 26 F_1 Results	100
TABLE 27 Results of BC_1	101

LIST OF FIGURES

FIG. 1 From Larter and Hsam (1973)	6
FIG. 2 From Lange and Wojciechowska (1976)	13
FIG. 3 Somatic chromosome distribution of BC_1 plants.	70
FIG. 4 Diagram of plotted expected frequencies and observed frequencies of eggs.	78
FIG. 5 Diagram showing probabilities of a minimum of 5 or 6 homoeologous groups being represented.	80
FIG. 6 Histogram of observations of BC_1F_2 single rows.	89
FIG. 7 Summary of results of Group 1 plants.	90
FIG. 8 Summary of results of Group 2 plants.	91
FIG. 9 Results - Chapter 3.	102

LIST OF PHOTOGRAPHS

PHOTOGRAPH 1 Embryo Culture.	42B
PHOTOGRAPH 2 F_1 hybrid grain type as compared to the triticale parent.	62B
PHOTOGRAPH 3 Grain type of the parents, F_1 and BC_1 .	62B

	<u>Page no.</u>
PHOTOGRAPH 4 Meiosis of BC_1 plants.	70B
PHOTOGRAPH 5 Univalent and mitotic chromosome counts of BC_1 plants.	70D
PHOTOGRAPH 6 Vigour of BC_1F_2 plants.	83B
PHOTOGRAPH 7 Seed set of BC_1F_2 .	83B
PHOTOGRAPH 8 F_1 hybrid seed set.	97B
PHOTOGRAPH 9 Parents and the resulting F_1 hybrid.	97B

SUMMARY

The objective of the research conducted for this thesis was to develop methods which plant breeders could use to introduce genetic material from the genetically-improved bread wheat (Triticum aestivum L.) into triticales (x Triticosecale Wittmack). The transfer of this improved germplasm has been recommended by triticales workers (Sanchez-Monge, 1958; Kiss, 1966b; Larter, 1973 and Merker, 1975) as one of the ways of improving triticales.

Initial experiments with wheat ♀ X triticales ♂ resulted in severe problems due to endosperm incompatibility in the initial cross. Experiments conducted on the culturing of embryos from the above cross produced two changes in technique which significantly improved efficiency.

Firstly, only removal of the endosperm is necessary for successful culturing of the embryo. The additional removal of the enveloping pericarp and scutellum is harmful, due to the increased sensitivity of the embryo to the micro-environment.

Secondly, the problem of post-germination callusing can be overcome by the addition of activated carbon which acts as a buffering agent by stabilizing fluctuating hormone levels.

Embryo culturing, using even the most efficient techniques is very time and labour consuming. Alternatively, the reciprocal cross (triticales ♀ X wheat ♂) produces the same number of F₁ plants per head (emasculated and pollinated) without the use of embryo culturing. This is due to the presence of more endosperm in the F₁ seed.

The conclusion of the author is that the introduction of Triticum aestivum germplasm into triticales, whether for a specific

purpose (disease resistance genes) or simply to increase the genetic variance, does not require embryo culturing. Embryo culturing is not efficient in terms of time or materials compared to methods described later in this summary.

Hybrid plants from the triticale-wheat cross were found to be self-sterile under glasshouse conditions and required backcrossing with hexaploid triticale pollen to set seed. Plants resulting from the backcrossing of the hybrid were studied and the following observations were noted: a) mitotic chromosome count; b) meiosis (univalent count); c) seed set; d) germination percentage of selfed seed; e) dosage of hairy peduncle; f) height; g) tillering and spikelet number. Correlations were attempted between certain of the observations:

mitotic chromosome count/total seed per plant

mitotic chromosome count/average seed per tiller

mitotic chromosome count/height

height/seed per plant

height/tiller number

tiller number/seed per plant

All were found to be non-significant.

Observations of the transmission frequency of the Hp gene (.461) to the BC₁ generation was not significantly different from the expected transmission rate calculated for univalents (.465) using the BC₁ mitotic chromosome counts of the plants with Triticum timopheevi cytoplasm.

Selfing of the BC₁ plants was carried out to the BC₁F₄ generation and observations noted at each generation of the seed set and genetic segregation. Results indicate that stable fertile lines are produced

in the BC_1F_3 generation. Maintenance of all lines was possible from the BC_1F_2 generation onwards, although fertility did vary from 5 seed per head to complete seed set in all primary, secondary and tertiary florets. Recovery of some self-fertility (seed set when selfed) was unexpectedly rapid, increasing from only 55% of the BC_1 plants to 100% of BC_1F_2 plants. Phenotypic segregation was as great within a line derived from a single BC_1 plant when observed in the BC_1F_3 generation as between lines derived from differing BC_1 plants.

Inferences about the female gametes produced by the hybrid plants (triticale X wheat) were made from observations recorded on the BC_1 plants. The findings thus inferred about the female gametes suggested that very high selection occurred for eggs with five or more univalent chromosomes present. It was found that Triticum timopheevi cytoplasm produced greater numbers of viable eggs per thousand florets compared to Triticum aestivum cytoplasm. This is attributed to the lowering of selection pressures for viability in eggs having 4 to 7 transmitted univalents. Selection coefficients were calculated on the basis of assumptions of a univalent transmission frequency of .25 and transmission of any univalent is independent of the transmission of any other univalent. This, together with a simple binomial expansion, $C_I^{14} (P^I Q^{14-I})$, gives an expected frequency of any class I (number of transmitted univalents).

The derived frequencies of viable eggs was considered to conform with the observed frequencies if a further assumption was made requiring that the transmitted univalents fit into the maximum number of homoeologous groups. For example, an egg having the chromosomal constitution of 14 A and B genome chromosomes, plus the following

five transmitted univalents - 1R, 1D, 2R, 2D, 3R, chromosomes - would not be viable because only three homoeologous groups are represented by transmitted univalents. A possibly viable example is where the five transmitted univalents are the following - 1R, 2D, 3R, 5D, 7R chromosomes. Here five homoeologous groups are present and only two are missing.

A test of the above assumption was conducted where a triticales, having two or three D genome chromosomes, was crossed with wheat. The D chromosomes pair at meiosis in the F_1 , producing eggs with a high frequency of two or three homoeologous groups present in the third genome, which, plus the .25 transmission frequency of the univalents, gives an average of about 5.0 homoeologous groups being represented in the female gametes. F_1 plants of the above chromosome constitution, when backcrossed with any triticales, would then have 50% viable eggs, based on the above hypothesis.

The triticales Arabian (ITYN-5-13), having two or three D genome chromosomes in its third genome, was crossed to two wheat varieties and backcrossed with a triticales. The egg viability obtained from the hybrid was .46, which agrees with that expected on the basis that at least five homoeologous groups must be present in the third genome for an egg resulting from a triticales-wheat cross to be viable.

The integrated triticales ITYN-5-13 was found to cause only partial endosperm incompatibility when crossed as male to wheat. A brief study was conducted of this line in reciprocal crosses with wheat, and in backcrosses of the hybrid plants with integrated third genome and full rye genome triticales.

Germination of the hybrid seed was 57% using wheat as the female in the cross, compared with 100% using ITYN-5-13 as the female, and

In spite of the superiority of triticales as the female parent in the introgression of hexaploid wheat germplasm into triticales, the large-scale use of triticales as the female parent is limited due to the lack of an available male-sterility system. Therefore large-scale field crossing of triticales and wheat will require the use of wheat as the female because of available genetic male-sterility and cytoplasmic male-sterility systems.

seed set, when backcrossed with full rye genome triticales, was 44% with a BC₁ seed germination of 85%.

→ With these latter findings, it is suggested by the author that large scale introgression of bread wheat germplasm is possible, using a bread wheat population segregating for genetic male sterility maintained at a frequency of 1 fertile : 1 sterile. Wind pollination of the male sterile plants with triticales pollen from varieties found to cause only partial endosperm incompatibility and pollination of the resulting F₁ plants using any triticales, would result in massive amounts of germplasm being introduced into triticales with little effort by the breeder.

Alternatively, hand emasculation of the integrated triticales and hand pollination with wheat pollen is more labour intensive, but the resulting germplasm transfer into triticales will be no more difficult than standard intraspecific transfer of germplasm carried out by crop breeders.

AUTHOR'S DECLARATION

This thesis contains no material which has been accepted for the award of any other Degree or Diploma in any University and that, to the best of my knowledge and belief, contains no material previously published or written by any other person, except when due reference is made in the text of the thesis.

RAYMOND LEE BRENGMAN //

ACKNOWLEDGEMENTS

I acknowledge with gratitude the advice and criticism of my supervisor, Professor C.J. Driscoll, and the assistance of Mr. Allen Lisle of the Queensland Department of Primary Industries, Biometry Branch, in carrying out a computer simulation to test the validity of the theoretical probabilities presented in Figure 5.

Appreciation is expressed to Dr. N.L. Darvey and to my fellow graduate students for their suggestions and assistance. A special thanks is given to the University of New South Wales, the Waite Institute and Roseworthy Agricultural College for the use of equipment and facilities during this study.

Special thanks are extended to Mr. G.J. Hollamby and fellow colleagues of the Roseworthy Wheat Breeding Team for the time and encouragement given while I was employed at Roseworthy Agricultural College.

In conclusion my sincere thanks to my wife for her understanding, encouragement and for the painstaking typing of this thesis.