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INTENSIFICATION OF PLANT BREEDING

AND

SEED PRODUCTION

P O L A N D

Triticale Breeding

by

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FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS

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UNDP-FAO Project "Triticale Breeding" Consultant mission June 29-July 13, 1980
(POL/77/007)

Program

Sunday	29.06	Arrived in Warzawa and transferred to hotel.
Monday	30.06	Visit to the Ministry of Agriculture and the UNDP-FAO office (Dr. B. Kramski, Director). In the afternoon visited the breeding stations Laski and Dańków of PHR (Poznańska Hodowli Róslin) with doc. Dr. T. Wolski and E. Tymieniecka where their triticale breeding program and nutritional laboratories were seen, and various problems were discussed.
Tuesday	01.07	Visit to IHAR (Inst. Hodowli i Aklim. Roslin) breeding station at Radzikow with Dr. W. Sowa, where his triticale breeding program was seen and breeding methodology problems were discussed.
Wednesday	02.07	Departure to Poznań by air. Trip from Poznań by car to visit the ZD HAR breeding station at Malyszyn. Met with Dr. W. Maćkowiak and saw his triticale breeding program and nutritional laboratories in detail. Stayed overnight at Gorzów Wlkp.
Thursday	03.07	Visited the ZD HAR breeding station at Malyszyn and discussed breeding methodology with Drs. Sowa and Maćkowiak.
Friday	04.07	Trip by car to Poznań with Dr. Sowa. Visit to the Plant Genetics Institute of the Polish Academy of Sciences, and met with the director Prof. Dr. Wiatroscak.

Friday	04.07	Met and discussed triticale cytology and mutation breeding with Drs. Patyna, Lukaszewski, and Lapinski. Met and discussed triticale cytology with Dr. St. Rogalska from the Institute of Genetics and Plant Breeding, Academy of Agriculture, Poznan.
Saturday	05.07	Trip by car to visit the Research Centre for Testing Cultivars (COBORU) at Slupia Wielka to see the national field trials and to discuss the methods of introduction of new varieties. In the afternoon, visited the (PHR) breeding station Rogaczewo-Choryn where the triticale breeding program was viewed and breeding problems were discussed with doc. Dr. T. Wolski and E. Tymieniecka.
Sunday	06.07	Trip to Krakow by train with Dr. Sowa. Dr. Dwurzna from the IHAR met the train.
Monday	07.07	Visit to the Department of Cereals - IHAR, Krakow. Discussed triticale breeding and genetic problems with Dr. St. Nalepa.
Tuesday	08.07	Visit to the IHAR breeding station Grodkowice (near Krakow) where the triticale experimental fields of Dr. St. Nalepa were located.
Wednesday	09.07	Trip to Lublin by train with Dr. St. Nalepa. Visit to the Plant Breeding and Seed Production Department at the Agricultural Academy in Lublin where triticale cytology problems were discussed with Prof. Dr. Cz. Tarkowski and Dr. G. Stefanowska. Visit to the triticale field experiments at Czeslawice near Lublin with Prof. Dr. Cz. Tarkowski.

Thursday	10.07	Trip to Warzawa by train.
Friday	11.07	Visit to the IHAR station at Radzikow where a meeting was held with the director Prof. Dr. St. Starzycki and the Polish triticale breeding programs were discussed. A lecture was presented on the University of Manitoba triticale program and impressions of the various Polish breeding programs. Visit with the biological testing program at Radzikow under the direction of Prof. Maria Rakowska.
Saturday	12.07	Visited Warzawa.
Sunday	13.07	Departed Warzawa at 7:00 and arrived in Winnipeg at 15:30.

Comments on the Project

The four breeding programs, located in Radzikow, Laski, Malyszyn, and Krakow are very impressive in the quality of their organization and their germplasm. The breeders have defined very well the various problems present in Poland and have designed their individual breeding programs in order to give them the best possible results with the available germplasm. All of the programs were involved not only in the production of varieties, but were also conducting agronomic studies on sowing applications, growth performances on poor soils, and coordinating work with biological evaluation on advanced lines.

The programs could not be faulted on the testing and design of agronomic experiments, nor on the priorities they established for selecting the various agronomic traits of interest. One of the limiting factors in any breeding program is the ability the staff has of getting large amounts of material planted and harvested, and the various programs were efficiently utilizing their staff and space. However, it was felt that in general several of the breeding programs should try and increase the number of lines in yield trial for the first time in order to be able to evaluate a larger variety of germplasm (this was discussed with the individual programs). All of the programs were making a wider variety of crosses, involving triticale, wheat, and rye, than most other programs around the world and were making good progress with these crosses in improving several agronomic characters.

I. Equipment

A) In general the breeding programs were very well equiped except in some minor areas.

The IHAR programs at Radzikow and Malyszyn need two plant threshers at each location, and the PHR program at Laski and Choryn need two plant threshers at each location. These plant threshers can be obtained from the Allen Machine Co., 272 Des Moines Street, P. O. Box 1803, Ames, Iowa 50010, U.S.A.

B) All of the programs need more seed cleaning equipment. (The IHAR program at Krakow has not received the equipment ordered in 1978.) Several small seed cleaners are available from the Allen Machine Co. as well as other manufacturers, which would meet the requirements of all of the programs.

C) Because of the importance of testing for sprouting resistance every program should have the Hagberg equipment in order to test for falling number. The IHAR station at Malyszyn does not have this equipment.

D) Because of the necessity for all the breeding programs in Poland to share their data it is advisable for each program to have access to a copying machine in order that valuable time is not spent writing out duplicate field books and data sheets. The PHR stations do not have access to this type of equipment.

E) The IHAR station at Malyszyn indicated the need for a second heating unit for use in protein analysis. All other programs indicated that their laboratory testing equipment for grain quality was adaquate.

F) Because all of the programs are increasing the number of spike selections they make each year it is essential that each program acquire 2 head threshers from the Precission Machine Co., Lincoln, Nebraska, U.S.A. (a total of 10 head threshers are required: two at each of the following locations, Radzikow, Laski, Malyszyn, Choryn, and Krakow).

II. Recommendations

A) Primary triticale production

Every program visited was involved in making large numbers of crosses between hexaploid wheat and rye. However, none of the programs was treating the wheat/rye hybrids with colchicine in order to double the chromosomes and make them fertile. Instead all of the untreated hybrids were used directly in the program that created them by making crosses with existing triticales. This is an extremely important and valuable way of utilizing new germplasm immediately without having to resort to the colchicine techniques for doubling chromosomes. However, this method for utilization of primary triticales does not allow for the conservation of the newly created hybrid for use in the future by other programs. In fact the breeder who made the original cross will have to remake the cross each time he wants to use a particular hybrid, and because of the variability present in rye the original hybrid could never be recreated.

It is recommended that each wheat/rye cross in every program be made so that enough hybrid seed is available for immediate use in the field (as in the past), and is available for treating with colchicine in order to create a fertile amphiploid. This can be accomplished by having the necessary chemicals and supplies available to each program so that they can treat their own amphiploids. (The technique used at the University of Manitoba will be supplied to each program: see Appendix I.)

Once the fertile amphiploids have been created there should be an immediate seed increase. The increased seed supply should be utilized in several ways: 1) an adequate amount should be permanently stored

at Radzikow, thus creating a Polish winter triticale amphiploid collection for future use in Poland and around the world; 2) the program creating the amphiploid should keep some seed for its own use; and 3) the remainder of the seed should be placed in a primary triticale crossing block, which should be distributed every year to every triticale program in Poland for their use. This will make amphiploids (from the best Polish wheats and ryes) available to everyone as well as being preserved for future generations.

If durum/rye crosses are to be made then an embryo culture laboratory needs to be set up at one location where durum/rye amphiploids can be made for all the programs (The University of Manitoba techniques will be supplied: see Appendix I). The IHAR station at Radzikow would be the best location for this laboratory.

B) Coordination of the data from various international nurseries.

Several international nurseries from the International Maize and Wheat Improvement Center (CIMMYT), Mexico, and the University of Manitoba, Canada are grown every year in several locations in Poland. One or two persons, either in IHAR or PHR, should be assigned the task of obtaining copies of all the results from every foreign nursery (from every location) and putting this data into a single report for distribution to all Polish breeders. This means that each breeder will have data from several locations on all the nurseries one year before he could obtain that data by himself. This would be extremely valuable in deciding on the lines to retain in as short a time span as possible.

C) Winter triticale performance nursery.

The winter wheat performance nursery from the U.S.A. has been extremely

valuable to breeders around the world in giving them performance information on their own material, and in allowing them to access material from other breeding programs. At the present time there is no winter triticale performance nursery and one should definitely be organized. Because of the impact of Polish breeding programs it would be logical for Poland to initiate a winter triticale performance nursery. The possibility should be explored of coordinating this nursery with the wheat nursery from the U.S.A. If such a nursery could be organized and sent around the world it would supply Polish breeders with an excellent accessment of the winterhardiness and disease patterns existing in their germplasm. The University of Manitoba will gladly grow such a nursery.

D) Spring variety evaluation.

The winter triticale varieties are adaquately tested in preliminary and national yield trials, but the spring varieties have no such testing program. Either separate preliminary and national tests should be organized for spring triticale varieties, or the spring varieties should be included in the spring wheat tests.

E) Cooperative research projects within Poland.

There were several centers in Poland conducting basic research on winter triticale: 1) the Institute of Plant Genetics, Polish Academy of Science, Poznan; 2) the Institute of Genetics and Plant Breeding, Academy of Agriculture, Poznan; 3) the Plant Breeding and Aklimatization Institute, Krakow; and 4) the Institute of Plant Breeding and Seed Production, Lublin. IHAR, Krakow has one of the largest basic research programs that is closely coordinated with an applied research breeding program. It is recommended that much closer ties be established between

The other basic and practical research programs. This close relationship would supply the basic research programs with many good projects which arise out of the breeding programs. On the other hand, the breeding programs obtain a great deal of information from the basic research projects that could not have been obtained from the field. These projects would result in some excellent publications, and could be organized by having the basic research scientists tour the various breeding programs with the breeders at least once a year.

F) An Intra-Poland screening nursery.

In addition to an international winter triticale performance nursery it would be extremely valuable to have an earlier generation intra-Poland screening nursery. This nursery could function as a preliminary screening nursery of Polish material (at several locations) before it is placed in the international winter triticale performance nursery. This nursery could be used to evaluate material from the various Polish breeding programs for winterhardiness, disease resistance, adaptation as early as the F_4 generation.

G) Joint research projects on the international level.

The possibility of joint research projects between Polish Institutes, the University of Manitoba, and the International Maize and Wheat Improvement Center (CIMMYT) are excellent and should be explored in depth.

1. Present projects:
   a) A project studying the effects of rye chromosomes on various agronomic characteristics is currently underway. Dr. W. Sowa, IHAR, Radzikow, and Dr. J.P. Gustafson, University of Manitoba, Winnipeg.

b) The University of Manitoba is evaluating lines from the PHR station at Laski for winterhardiness.

2. Possible projects that could be organized:*

* None of these possible collaborative projects involving CIMMYT can be initiated until the scientists at CIMMYT are consulted and agree to the project. The CIMMYT scientists should be consulted as soon as possible.

a) Polish scientists could be involved in helping to plan and organize various winter triticale nurseries with CIMMYT and the University of Manitoba.

b) Polish scientists (Drs. A. Aniol and W. Sowa, IHAR, Radzikow) could organize an international nursery for the study of Aluminum toxicity in wheat and triticale in collaboration with CIMMYT, scientists in the U.S.A., and other countries that have soil problems similar to those present in Poland.

c) A winter/spring rye crossing program could be organized with CIMMYT, which would help increase the variability present in spring rye. Dr. St. Nalepa (IHAR, Krakow) has indicated his willingness to make the winter/spring crosses using the spring rye 'Snoopy' as a base for the spring germplasm.

d) Further projects will be explored with the CIMMYT staff during the consultants next visit to Mexico.

e) The University of Manitoba is always willing to help organize research projects and have Polish scientists visit Winnipeg. Polish students could even be sent to the University of Manitoba or elsewhere to work on an advanced degree while conducting a research project beneficial to Poland.

g) The final recommendation would be that the Food and Agricultural Organization of the United Nations continue supporting this project for at least 3 to 5 years.

The project "Intensification of Plant Breeding and Seed Production" is rapidly drawing to an end with impressive results. The original project was setup to purchase equipment and train scientists, with the chief goal in mind being the production of high-yielding triticale varieties without any decrease in protein or quality. A variety is presently in the National testing program and should be released for commercial production in 1981. Better material is currently in the breeding programs and should be available in about 3 years.

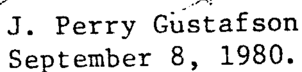
Once a variety is produced then additional problems will arise in the areas of seed production, general agronomy, seed cleaning and handling, storage, and end product usage. In addition to these problems the breeding programs will find increased pressure for the production of new and better varieties which will have specific characteristics for end product usage, at a faster rate than before. These areas are the ones that need further support for the next 3 to 5 years after that the entire system all the way from the breeding program to the end product use should be firmly established.

III. Nutritional, and biochemical analysis.

The laboratories for testing of nutritionally important characteristics of the grain were inspected at every station visited. All of the laboratories were very well equipped except for the one at Malyszyn which needed an additional heater for protein analysis (see equipment needed). The Polish

programs are doing a very good job in testing the material in their programs for quality. The consultant's report (2) on the Biological Evaluation of Nutritional Value of Feed Grains by Bjorn O. Eggum made the same comments that could be made in this report. No additional comments are necessary, because the nutritional testing programs were found to be very well organized and run by capable people.

In summary I would like to point out that it was a great pleasure for me to be able to collaborate with the various Polish scientists on this project. The various breeders (especially Dr. W. Sowa) had worked out a very good program for my visit. They were very generous with their time in showing me around their programs and answering my questions. I enjoyed very much the many fruitful discussions and look forward to further exchanges of ideas and germplasm in the future between the various Polish breeding programs, the University of Manitoba, and the International Maize and Wheat Improvement Center (CIMMYT).



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Appendix I

Production of new amphiploids begins with the creation of the intergeneric hybrid, which involves crossing various forms of *T. aestivum* and *T. turgidum* with various species of *Secale*. Crosses between the hexaploid wheat and diploid rye generally do not require embryo culturing as do the tetraploid wheat-diploid rye crosses. If the embryos need to be cultured, culturing usually takes place between 14 and 20 days after fertilization, depending on the particular cross involved.

Culturing embryos requires an area where there are no air currents that could carry bacterial and fungal spores. The culturing area should be sterilzed by exposure to UV light for half an hour prior to use; or the entire area can be wiped clean with a cloth dampened with 95 per cent alcohol. If the alcohol cloth is used, overhanging lamps, walls immediately surrounding the work area, and the dissecting microscope should be wiped clean also.

Once the work area has been sterilized, seeds containing the embryos to be cultured can be brought to the area. Because the seeds have bacterial and fungal spores on their pericarp, they need to be surface-sterilized in a 5 per cent solution of sodium hypoclorite for one minute. The seed is then rinsed in two changes of distilled water. The scalpel and two pairs of find pointed forceps used in dissecting the embryo are sterilized by dipping in 95 per cent alcohol and flamed over an alcohol lamp. It is recommended that two sets of instruments be available so that one set can cool while the other is being used.

One pair of forceps is used to hold the seed while an incision is made through the pericarp and seed coat close to the scutellum. The second pair

of forceps then is used to peel away the pericarp and seed coat exposing the embryo. By inserting the scalpel between the scutellum and endosperm, the embryo is removed and placed on the culture medium with the plumule towards the top of the vial. Gently heat the lip of the culture vial before the embryo is placed in it and afterwards before the vial is capped to force out any contaminated air present. A summary of this technique as well as the technique for excising embryos from mature seed is summarized by Kaltsikes (1974). The contents of the embryo culture medium and its preparation appears in Tables 1 and 2.

The vials are placed then in the dark at about 21°C until root growth had been initiated. Then the vials are placed under continuous light at 21°C until plant growth reaches the top of the vial, at which time the weak-appearing plantlets are transplanted into small pots containing a mixuture of 3 parts vermiculite, 1 part soil, and 1 part peat moss. The soil and peat moss provide good retention capacity for the nutrient solution (Tables 3 and 4) that is used to water the plantlets until they are established well enough to survive without an artificial supply of nutrients. At that time, they are transferred to a larger pot with ordinary soil. The large, vigorous plantlets are transplanted directly from culture vials to pots containing soil and are watered. If the vials are deep enough to allow the plantlet to reach the third or fourth leaf stage, then the vermiculite and usage of nutrient solution can be eliminated.

The second stage in production of new amphiploids is the doubling of the F_1 hybrid plants chromosome number. The usual method of doubling chromosomes involves treating the hybrid with the alkaloid colchicine.

Several procedures have been used throughout the years and are summarized in Table 5 and described in detail by Kaltsikes (1974).

A new method used at the University of Manitoba, utilizing a surfactant, dimethyl sulfoxide (DMSO), works very well. Soil is washed away from the roots of seedlings at the three-leaf stage and the crown and roots are immersed in a solution of 0.05% colchicine and 1.5% DMSO for five hours with air being bubbled through the solution. The seedlings were then rinsed in water and replanted.

Nitrous oxide also has been used successfully to double chromosomes of tetraploid wheat (Kihara and Tsunewaki, 1960) and barley and what-*Aegilops* hybrids (Dvorak *et al.*, 1973). This method is currently under study for possible adaptation to wheat-rye hybrids, but at the present time, the results are still inconclusive.

Another method proposed by Tsuchiya and Larter (1968) involves crossing parents previously doubled. They obtained a higher seed set using this technique compared to the conventional method. Their main problem was that they had a very low rate of doubling the parents as compared to doubling the hybrids. A similar method to Tsuchiya's was proposed by Darvey (personal communication), and involves crossing either autopolyploids or allopolyploids of wheat and ryes, resulting in direct synthesis of triticale. The major difference in the methods is that in Darvey's method, both the wheats and the ryes will be already in a heterozygous state when they are crossed so that an infinite number of genetically different lines are potentially possible.

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Table 1. *Embryo culture medium currently being used at the University of Manitoba (Murashige and Skoogs, 1962).*

Stock solution	Ingredients	Final conc. mg./l	Concentration in stock solution
			gm./l deionized water
A	NH_4NO_3	1650.0	8.250
	KNO_3	1900.0	9.500
	$CaCl_2 \cdot 2H_2O$	440.0	2.200
	$MgSO_4$	170.0	0.850
	KH_2PO_4	170.0	0.850
	$MnSO_4 \cdot H_2O$	16.9	0.0845
	$ZnSO_4 \cdot 7H_2$	8.6	0.043
	H_3BO_3	6.2	0.031
			mg./250 ml. deionized water
B	KI	.83	207.50
	$CuSO_4 \cdot 5H_2O$	.025	6.25
	$Na_2MoO_4 \cdot 2H_2O$	.25	62.50
	$CoCl_2 \cdot 6H_2O$	.025	6.25
			mg./50 ml. deionized water
C	$FeSO_4 \cdot 7H_2O$*	24.9	1245.00
			mg./100 ml. deionized water
D	Indolacetic acid	1.0	20.00
			mg./100 ml. deionized water
E	Kinetin	1.0	10.00
			mg./250 ml. deionized water
F	Glycine	2.0	25.00
	Nicotinic acid	0.5	6.25
	Pyridoxine HCl	0.5	6.25
	Thiamine HCl	0.1	1.25

* Take 1.305g. EDTA + 10 ml H_2O add 13.4 mil. 1M KOH then shake and dissolve all the EDTA. pH should be 7.0 then add 1.245 g. $FeSO_4$; $7H_2O$ and bubble air overnight. After adjusting resulting solution to a pH 5.2 add H_2O to a volume of 50 ml.

Table 2. *Preparation of culture medium from the stock solutions.*

To be used on large, well-formed embryos

1. Add 600 ml. denionized water to a 1500 ml. flask
2. Add 200 ml. stock solution A
3. Add 1 ml. stock solution B
4. Add 1 ml. stock solution C
5. Add 30 gm. sucrose
6. Add denionized water until a 1000 ml. volume has been reached
7. Adjust pH to 5.9 using 0.1N HCl or 0.1N NaOH
8. Add 10 gm. agar and boil until dissolved
9. Pour into small vials and stopper
10. Autoclave for 20 minutes at 250°F at 1 atm. and allow to cool

To be used if embryos are small

1. Steps 1, 2, 3, 4, and 5 are the same as above
2. Add deionized water until a volume of 965 ml. has been reached
3. Adjust pH to 5.9 as above
4. Add 10 gm. of agar as above
5. Autoclave
6. Place in a 2000 ml. sterlized separatory funnel
7. Add 5 ml. sterile filtered stock solution D
8. Add 10 ml. sterile filtered stock solution E
9. Add 20 ml. sterile filtered stock solution F
10. Pour into small sterilized vials and allow to cool

Table 3. *The nutrient solution, used to water the plantlets after transplantation from the culture vial, is made up as follows (Machlis and Torrey, 1956).*

Stock solution	Ingredients	Concentration in stock solution
1	1M $Ca(NO_3)_2 \cdot 4H_2O$	236.150 g./1. deionized water
2	1M KNO_3	101.108 g./1. deionized water
3	1M $MgSO_4 \cdot 7H_2O$	246.498 g./1. deionized water
4	1M KH_2PO_4	136.091 g./1. deionized water
5	$FeSO_4 \cdot 7H_2O$*	1.245 g./50ml. deionized water
6	H_3BO_3	2.86 g./1. deionized water
	$MnCl_2 \cdot 4H_2O$	1.81 g./1. deionized water
	$ZnCl_2$	0.11 g./1. deionized water
	$CuCl_2 \cdot 2H_2O$	0.05 g./1. deionized water
	$Na_2MoO_4 \cdot 2H_2O$	0.025 g./1. deionized water

* Take 1.305 g. EDTA + 10 ml deionized H_2O add 13.4 ml. 1 M KOH then shake and disolve all the EDTA. pH should be 7.0 then add 1.245g. $FeSO_4 \cdot 7H_2O$ and bubble air overnight. After adjusting resulting solution to pH 5.2 add deionized H_2O to a volume of 50 ml.

Table 4 *Preparation of the nutrient solution from the stock solutions is as shown below.*

1.	Add 10 ml. stock solution 1
2.	Add 10 ml. stock solution 2
3.	Add 4 ml. stock solution 3
4.	Add 2 ml. stock solution 4
5.	Add 1 ml. stock solution 5
6.	Add 2 ml. stock solution 6
7.	Add 1971 ml. deionized water until total solution volume is 21.

Table 5 *Procedures used to treat hybrids with colchicine for the purpose of doubling chromosomes.*

Proceedure	Reference
Seed treatment	Sears, 1939
Crown method	Sears, 1941
Capping method	Bell, 1950
Injection method	Bell, 1950
Schumann's method	Schumann, 1960
Tiller method	Cauderon and Saigne, 1961
Modified inversion or dropper method	Siddigui, 1971
Root immersion	Welleniek, 1947
Rutherford's method	Rutherford, 1969