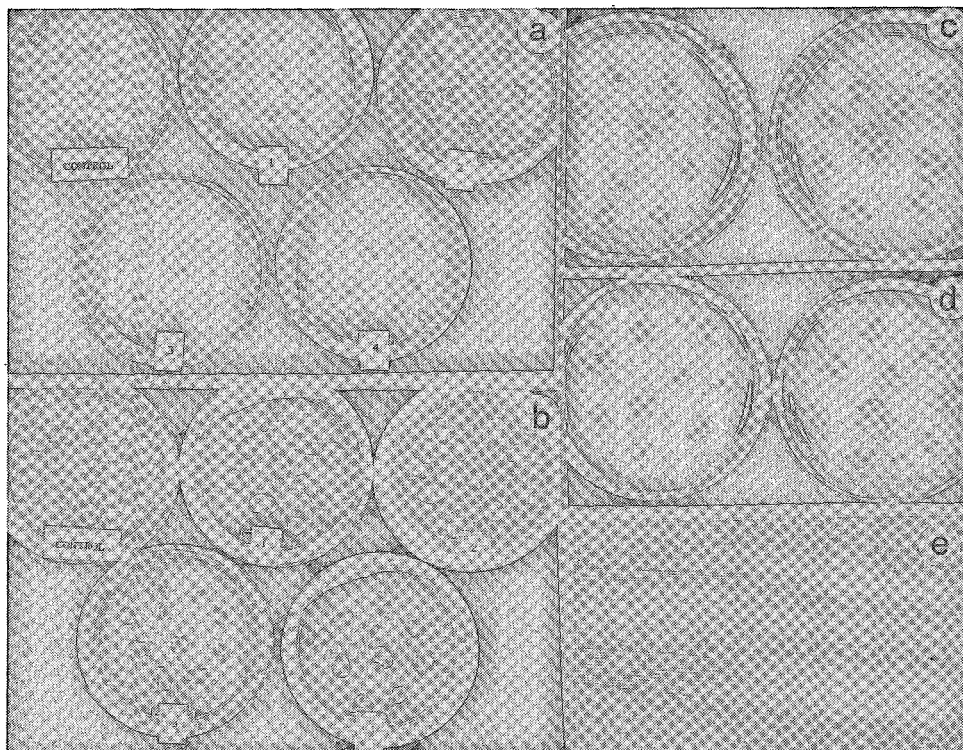


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New hybrids between wheat and *Agropyron*. IV. Transmission of telo and whole chromosomes, and fertility of alien addition lines of *Agropyron trachycaulum* and *A. ciliare* in wheat¹

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Introduction

Earlier attempts to hybridize *Agropyron trachycaulum* (Link) Malte ($2n=28$, SSHH), and *A. ciliare* (Trin.) Franch ($2n=28$, SSYY) with wheat were unsuccessful (Smith 1942). Intergeneric hybrids of *Triticum aestivum* L. with these species were reported and described for the first time by Sharma and Gill (1983a). In these crosses, wheat was used as the male parent, and embryo rescue was required. *A. trachycaulum* (female) \times *T. aestivum* cv. Chinese Spring (CS), and *A. ciliare* (female) \times CS hybrids were advanced to BC₃ stage using CS pollen (Sharma and Gill 1983b). Transmission of the alien chromosomes and telosomes through pollen, and fertility of the alien addition lines are reported in this communication.

Materials and Methods

Two monosomic addition plants, t₂₋₁₋₃ and t₂₋₁₋₄, of *A. trachycaulum* \times CS cross, both having $2n=43$, and two plants, c₃₋₁₋₄ and c₃₋₁₋₂, of *A. ciliare* \times CS cross, having $2n=44$ and 46 , respectively, were used in the present study. From the selfed progenies (BC₃F₂) of the monosomic additions, t₂₋₁₋₃ and t₂₋₁₋₄, plants having a chromosome number $42+t'$ or 43 were used to pollinate CS to isolate euplasmic alien addition lines. Similarly, from the selfed progenies of c₃₋₁₋₂ and c₃₋₁₋₄, plants having a chromosome number $42+t'$ or 43 were used to pollinate CS. The transmission rate of both telo and whole chromosomes through the male gametes was estimated by examining the chromosome composition of the hybrid seedlings. The fertility of the hybrids was measured by the total number of seeds set on each plant.

Results and Discussion

The chromosome composition of the BC₃F₂ plants used as pollen source to cross with CS, and the chromosome number and frequency of hybrid progenies are given in Table 1. A total of 4 plants from each of the two intergeneric derivatives were used as pollen source, and 35 and 36 hybrid plants, respectively, were analyzed cytologically (Table 1). The *Agropyron* chromosome involved

1: Contribution No. 12242, Purdue Univ. Agric. Exp. Stn., W. Lafayette, Indiana 47901. I thank Dr. B.S. Gill for sharing the seed and for comments, and Drs. R. Shukle and C. Johns for review.

Table 1. Transmission of telo and whole chromosome of *Agropyron trachycaulum* (t), and *A. ciliare* (c) through the male gamete in crosses with Chinese Spring

Female parent	Male parent plant number	Chromosome No. of male parent plant	No. of seeds analyzed	Chromosome number and frequency (%) of seeds		
				42	42+t'	43
Chinese Spring	t ₂₋₁₋₃ ⊗ 3, 8	42+t'	20	55.0	45.0	—
"	t ₂₋₁₋₄ ⊗ 4	42+t'	5	80.0	20.0	—
"	t ₂₋₁₋₄ ⊗ 12	43	10	90.0	—	10.0
"	c ₃₋₁₋₄ ⊗ 10	42+t'	10	90.0	10.0	—
"	c ₃₋₁₋₄ ⊗ 2, 3	43	18	88.9	—	11.1
"	c ₃₋₁₋₂ ⊗ 9	43	8	100.0	—(-)	—(-)

in t₂₋₁₋₃ was median. The *Agropyron* chromosomes involved in t₂₋₁₋₄ and c₃₋₁₋₄ were sub-median (Sharma and Gill 1983b). The *Agropyron* chromosome in t₂₋₁₋₄ has now been identified as 1H^t (B.S. Gill, pers. commun.).

The rate of transmission of the sub-median *A. trachycaulum* chromosome through pollen was 10% compared to 20% transmission of telo of the same chromosome (Table 1). The transmission of telosome from the median *A. trachycaulum* chromosome was 45%. There was no transmission of

Table 2. Chromosome number, and number of seeds produced on selfing by plants raised from the reverse cross of Table 1 with Chinese Spring (CS) as the female parent

Reverse cross	Chromosome number	No. of plants	Seed number	
			Mean	Range
Cs × t ₂₋₁₋₃ ⊗ 3, 8	42	11	1011.4	800-1360
	42+t'	9	869.7	653-1015
Cs × t ₂₋₁₋₄ ⊗ 4, 12	42	13	1008.3	719-1323
	42+t'	1	1087.0	1087
	43	1	742.0	742
Cs × t ₃₋₁₋₄ ⊗ 10, 2, 3	42	25	522.8	188- 768
	42+t'	1	584.0	584
	43	2	473.0	379- 567

the whole chromosome derived from c_{3-1-2} . The transmission of the sub-median chromosome from c_{3-1-4} was 11.1% (Table 1). In the sample studied, the telo transmission from c_{3-1-4} was 10%. Thus, whereas the rate of transmission of sub-median chromosomes of *A. trachycaulum* and *A. ciliare* was alike, the rate of transmission of telosome of *A. trachycaulum* appeared to be higher than that of *A. ciliare*. Also, the rate of telosome is higher than that of the whole chromosome of *A. trachycaulum*. When a decaploid *A. elongatum* chromosome 6 was added to the hexaploid wheat complement, it was transmitted through the pollen with a reduced frequency (13.9%). However, a telocentric for the same chromosome was transmitted with the expected frequency (Knott 1964). Pollen carrying the added chromosome was at a competitive disadvantage, but the telocentric chromosome was not deleterious to the pollen.

Theoretically an extra chromosome should be transmitted by 50% of the gametes on the female as well as on the male side. This assumption is seldom realized (Simeone et al 1985). Transmission rate of extra chromosome through male gamete varied from 0% in barley (Tsuchiya 1960) to 52% in maize (Einset 1943). Sears (1956) obtained only 1.3% disomics from selfing monosomic addition of an *Aegilops umbellulata* chromosome to hexaploid wheat. In wheat-barley crosses, Islam et al (1978) found that the transmission of barley chromosomes was very low. Mochizuki (1963) recovered no disomic addition line from an *A. elongatum* monosomic addition line to tetraploid wheat. The *A. intermedium* chromosome conditioning stripe rust resistance in wheat was transmitted through 10% of the male gametes, whereas the chromosome imparting stem rust resistance had a selective advantage through the pollen with transmission of 85-96% (Cauderon and Rhind 1976). Transmission rates of *A. trachycaulum* and *A. ciliare* chromosomes studied here lie within these limits.

In the euplasmic condition the vigor and fertility of both euploids and those having alien telo were better than those having whole chromosome (Table 2, Fig. 1). Apparently the alien whole

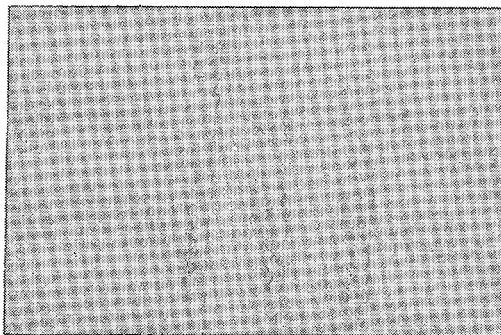


Fig. 1. Spikes of euplasmic plants Chinese Spring \times (t_{2-2-p_3}): left-plant No. 1 ($2n=42$); middle-plant No. 3 ($2n=42+t$); right-plant No. 10 ($2n=43$). Plants having $2n=42$ were the most and those having $2n=43$ were the least vigorous, but all were normal and fertile.

chromosomes of *A. trachycaulum* and *A. ciliare* had some negative effect. The seed number per plant was lower in plants involving *A. ciliare* than in plants involving *A. trachycaulum*. Even though all the plants appeared normal, comparisons showed that plants having $2n=42$ were the most vigorous and those having $2n=43$ were the least vigorous. Yield of most addition lines of *A. intermedium* in wheat was lower than the wheat parent (Cauderon and Rhind 1976). All ditelosomic additions except 3S, 4S, 4L of barley in wheat were less fertile than wheat parent (Islam 1983).

References

- Cauderon Y and Rhind J (1976) The effect on wheat of an *Agropyron* chromosome carrying stripe rust resistance. *Ann Amélior Plantes* 26: 745-749
- Einset J (1943) Chromosome length in relation to transmission frequency of maize trisomics. *Genetics* 28: 349-364
- Islam AKMR (1983) Ditelosomic additions of barley chromosomes to wheat. *Proc 6th Int. Wheat Genet Symp, Kyoto, Japan* pp. 233-238
- Islam AKMR, Shepherd KW and Sparrow DHB (1978) Production and characterization of wheatbarley addition lines. *Proc 5th Int Wheat Genet Symp, New Delhi, India* pp. 365-371
- Knott DR (1964) The effect on wheat of an *Agropyron* chromosome carrying rust resistance. *Can J Genet Cytol* 6: 500-507
- Mochizuki A (1963) *Agropyron* addition lines of durum wheat. *Wheat Inf Serv* 15-16: 50-53
- Sears ER (1956) The transfer of leaf rust resistance from *Aegilops umbellulata* to wheat. *Brookhaven Symp in Biol* 9: 1-22
- Sharma HC and Gill BS (1983a) New hybrids between *Agropyron* and wheat. 2. Production, morphology and cytogenetic analysis of F_1 hybrids and backcross derivatives. *Theor Appl Genet* 66: 111-121
- Sharma HC and Gill BS (1983b) New hybrids between *Agropyron* and wheat. 3. Backcross derivatives, effect of *Agropyron* cytoplasm, and production of addition lines. *Proc 6th Int Wheat Genet Symp, Kyoto, Japan* pp. 213-221
- Simeone R, Blaco A and Giorgi B (1985) Chromosome transmission in primary trisomics of durum wheat. *Cereal Res Commun* 13: 27-31
- Smith DC (1942) Intergeneric hybridization of certain cereals and other grasses. *J. Agric. Res* 64: 33-37
- Tsuchiya T (1960) Cytogenetic studies of trisomics in barley. *Jap J Bot* 17: 177-213



Utilization of durum wheat (*Triticum turgidum* L. var. *durum*) landraces to improve yield and yield stability in dry areas

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Introduction

Cereals landraces are important sources of genetic variation (Frankel 1977; Chang 1985) for varietal improvement in dry areas. In the case of durum wheat, this is particularly evident in low rainfall areas of North Africa and West Asia where 70 to 80% of the areas reserved for this cereal are annually covered by landraces, such as Haurani in Jordan, Syria and Lebanon, or selections from landraces such as Kyperounda in Morocco and Cyprus, Zenati Bouteille in Algeria, and Mahmoudi in Tunisia. The use of durum wheat landraces in hybridization programs shows that progress can be made in improving cultivars for dry areas. Omrabi, a line derived from a cross involving Haurani, and Jori C69, a high yielding cultivar when grown in relatively more favorable environments, has been found to be superior to its parents in both favorable and dry environments (Nachit and Ketata 1986). In Jordan, Haurani, the local durum landrace, is mainly grown by farmers in rainfed areas because of its drought tolerance and stability, while Stork, a high yielding variety introduced from CIMMYT, had limited acceptance and is grown mainly in more favorable rainfed and irrigated areas. The current project was undertaken to combine the stress tolerance of Haurani with the yield potential and earliness of Stork since yield stability is a prerequisite to Jordanian wheat cultivation.

Material and Methods

Twenty-two advanced lines (F_8 and F_9) from Haurani/Stork crosses along with their parents were evaluated in two locations in Jordan (Ramtha; 277 mm and Jubeiha; 490 mm) and one location in Syria (Tel Hadya; 330 mm) over three years. Two replications in each location were used. Data were recorded on grain yield, biological yield, and plant height. Stability analysis was performed for all recorded parameters according to the model of Eberhart and Russell (1966). Analysis of variance was used to estimate $G \times E$ interactions.

Results and Discussion

Variety \times location, variety \times year and variety \times location \times year interactions were all important sources of variation. The estimate of the variance components are given in Table 1. For grain yield, biological yield and plant height the locations, years, locations \times years components were all

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Table 1. Analysis of variance for grain yield, biological yield, and plant height

Source of Variation	D.F.	Mean square ($\times 10^3$)		
		Grain yield (kg/ha)	Biological yield (kg/ha)	Plant height (cm)
Location (L)	2	186179.36***	527827.13***	76.60***
Year (Y)	2	24811.15***	167308.50***	18.08***
L \times Y	4	8926.74***	166793.16***	3.05***
Reps in L and Y	9	204.61	702.20	0.04
Genotypes (G)	23	804.48***	3413.57*	0.79***
G \times L	46	565.42***	3329.92**	0.18***
G \times Y	46	368.72***	1354.85ns	0.56***
G \times L \times Y	92	237.19***	1872.39ns	0.142***
Error	207	123.85	2287.48	0.04

ns: not significant, *, **, and *** significant at 5, 1 and 0.1% level, respectively.

significant at 0.1% level. The variance components of genotypes, genotypes \times locations, genotypes \times years and genotypes \times locations \times years were significant at 0.1% level for grain yield and plant height. The variance components of biological yield for genotypes and genotypes \times locations were significant at 5 and 1% level, respectively, however, for genotypes \times years and genotypes \times locations \times years no significance was found. In dryland crop production is frequently affected by environmental stresses such as limited water availability, high or low temperatures and mineral stresses (Christiansen 1982). Occasional periods of stress may occur during the growing season, even when the crop is grown in an "optimum moisture environment", resulting in yield decreases. Genetic resistance to stresses is therefore required to attain acceptable yield levels. The genetic manipulation of plants to produce satisfactory economic yields in the presence of abiotic stresses is the most economical solution. Identification of crop varieties carrying genetic stress resistance and yield stability is the most easily transferable part of a technology package to rainfed areas where environmental stresses are the major limiting production factors. Crosses of Haurani with Stork show that it is possible to develop cultivars that combine yield potential, yield stability and stress tolerance (Table 2). Cross 4 and 20 were tested in rainfed areas of Jordan and Syria in a range of environments with various amounts of rainfall. These crosses also showed better tolerance to environmental variation. The high stability values of these crosses are reflected in their high mean yields, coefficients of regression similar to one and low residual variances. These results corroborate earlier studies on incorporating landrace moisture stress tolerance to improve varieties yielding ability under dryland conditions (Nachit and Ketata 1986).

Several morphological, phenological and physiological traits (Duwayri and Shelalkeh 1986; Nachit and Jarrah 1986) are related to yield under moisture stressed conditions. Grain yield was associated with dry matter production ($r=+0.71***$), straw yield ($r=+0.53**$)

Table 2. Stability parameters and grain yield of high performing, stable durum wheat lines selected from Haurani/Stork crosses

Genotypes	Mean yield (kg/ha)	Percentage increase over Haurani	Regression coefficient	Residual variance
4	2313.4	123.3	1.14	66241.4
6	2389.8	127.4	1.28	374433.2
16	2307.0	123.0	1.08	580927.4
20	2311.0	123.2	1.15	266756.7
Stork	1730.5	92.2	1.10	513546.6
Haurani	1876.0	100.0	0.68	312001.8

and also with 1000 kernel weight ($r=+0.57^{**}$). The ability to grow at low temperature leads to the improved growth of canopy and ground cover, increased amount of water available for transpiration and reduced evaporative water loss. Plant height was also positively associated with grain yield ($r=+0.63^{***}$) under moisture stressed conditions.

These results indicate that gene pools from landraces can be used to further increase durum wheat yields under rainfed conditions. The results also suggest that stress tolerance increases yield stability. Further studies are needed to quantify the genetic diversity available in durum wheat landraces, to study the genetic variation of various morphophysiological characters under dry conditions, and establish their association with drought tolerance.

Acknowledgement

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Literature Cited

- Christiansen MM (1982) World environmental limitations to food and fiber culture. In (Christiansen MM and Lewis CF, eds) "Breeding plants for less favorable environments", Wilded Interscience, New York
- Eberhardt SA and Russell WA (1966) Stability parameters for comparing varieties, *Crop Science* 6: 36-40
- Fischer RA (1980) Influence of water stress on crop yield in semiarid regions. In (NC Turner and PJ Kramer, eds) "Adaptation of plants to water and high temperature stress", Wilded Interscience, New York 323-329
- Frankel OH (1977) Natural variation and its conservation. In "Genetic diversity in plants" (A Muhammed, R Askel and RV Von Borstel eds), Plenum New York 21-44
- Nachit MM and Ketata H (1986) Breeding strategy for improving durum wheat in Mediterranean rainfed conditions. International Wheat Conference, May 2-9, Rabat, Morocco
- Nachit MM and Jarrah M (1986) Association of some morphological characters to grain yield in durum wheat under Mediterranean dryland conditions. *Rachis* 5: 33-34
- Shaladeh M and Duwayri M (1986) Inheritance of morphophysiological characters and grain yield in durum wheat crosses. *Rachis* 5: 37-42



Resistant wheat plants against *Helminthosporium sativum* from embryo derived callus cultures

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Introduction

Wheeler and Luke (1955) first used phytotoxins in resistance breeding. Correlation of resistance to a parasite and to its toxins is a necessary prerequisite for such a use of phytotoxin. Another important factor is high regeneration ability of the genotype. Large scale screening of cell populations using *in vitro* procedures successfully produced resistant plants against phytotoxins (reviewed by Wenzel 1985).

In this experiment toxins from *Helminthosporium sativum* P.K. & B. were used for selection. This fungus produces two toxins namely, helminthosporal (Ludwig 1957) and victoxinine (Pringle 1976) which causes seedling blight, root rot, head blight and leaf spot. This paper describes the selection of immature embryo derived calli of wheat against phytotoxins of *H. sativum* and the testing of regenerated plants.

Materials and Methods

The callus cultures were established from immature embryos of the wheat varieties 'Atys' and 'Pitic 62' as described by Chawla and Wenzel (1987a).

Isolation of toxin

Helminthosporium sativum P.K. & B. collection strain no. 62606 (supplied by Dr. H. Nirenberg, Berlin) was used in the study. For the preparation of toxic culture filtrate, the seeding production flasks were prepared by the method of Pringle and Scheffer (1963) in modified Fries medium and then 1 ml of this medium was put in 20 ml of modified Fries medium and kept at 25°C for 28 days. The culture filtrate containing the phytotoxic compounds was filtered through several layers of cheese cloth and then through Sartorius filter (0.2 μ) to discard the mycelium and spores. The culture filtrate was concentrated *in vacuo* at 40°C to 10% of the original volume. An equal volume of cold methanol was added and the solution was stored overnight at 5°C. Precipitates were removed by filtration, washed with cold methanol and this methanol was removed in a vacuum freeze drier. The oily substance formed was dissolved in methanol and further dilutions were made with water. Toxicity of purified culture filtrate was tested by root bioassay method (Pringle and Braun 1957).

Testing of lethality of toxins on callus

Small pieces of callus (approx. 30 mg) were placed in a petri dish containing different units of toxin concentration and fresh weight increase of each inoculum was determined after 3 weeks.

Callus selection and regeneration procedures

After 3-4 months of callus initiation, small calli pieces of about 30 mg fresh weight were put in a petridish with 10 ml of toxic medium. Continuous method of selection was performed in which 4 cycles of selections were done on toxic medium by transferring healthy calli after 3 weeks interval. After selection the resistant calli were grown on maintenance medium and parts of calli were put on regeneration medium containing 0.35 mg/l naphthalene acetic acid and 1 mg/l 6-benzyl-amino-purine. Regenerated plants were transferred to soil and grown under semicontrolled green house conditions.

In vivo testing of plants against pathogen

Leaves of regenerated plants from callus after selection and non-toxin treated control plants were inoculated with 5 μ l spore suspension (10^6 /ml), and kept in humid chambers. In susceptible plants, dark brown spots appeared on the leaves within 3-4 days after inoculation. The plants from *in vitro* cultures were categorised as resistant (R), intermediate (I) and susceptible (S).

Results

Lethality of toxin was tested on five levels of toxin conc. and increase in fresh weight was determined after 3 weeks of growth (Fig. 1a). At the highest toxin conc. in B4T medium, almost

Table 1. Response of immature embryo derived calli to the toxin preparation of *Helminthosporium sativum*, regeneration frequency of resistant callus lines and in vivo reaction of regenerated plants to the pathogen

Genotype	No. of calli	No. of surviving calli after selection cycles			
		1st	2nd	3rd	4th
Atys	1360	331 (24%)	243 (18%)	189 (24%)	158 (12%)
Pitic 62	500	118 (24%)	85 (17%)	66 (13%)	54 (11%)

	Regeneration frequency			Disease reaction			
	Calli	Reg.	Green shoots	Plants	R	I	S
Atys	92	45 (38.9%)	4 (4.4%)	4	3	1	—
Pitic 62	44	11 (25%)	7 (15.9%)	5	4	1	—

R: Resistant, I: Intermediate, S: Susceptible

all the calli died. At the next lower level of toxin conc. in B3T medium, 10-30% of the calli survived and a growth rate of around 30% was observed. At the lower levels in B1T and B2T no significant effect on growth of calli compared to control B0T level was observed. A control experiment with uninoculated fungal growth medium, processed in the same way as the toxin preparation was tested with different levels of conc. in callus growing medium. There was no effect on growth of callus (Fig. 1b).

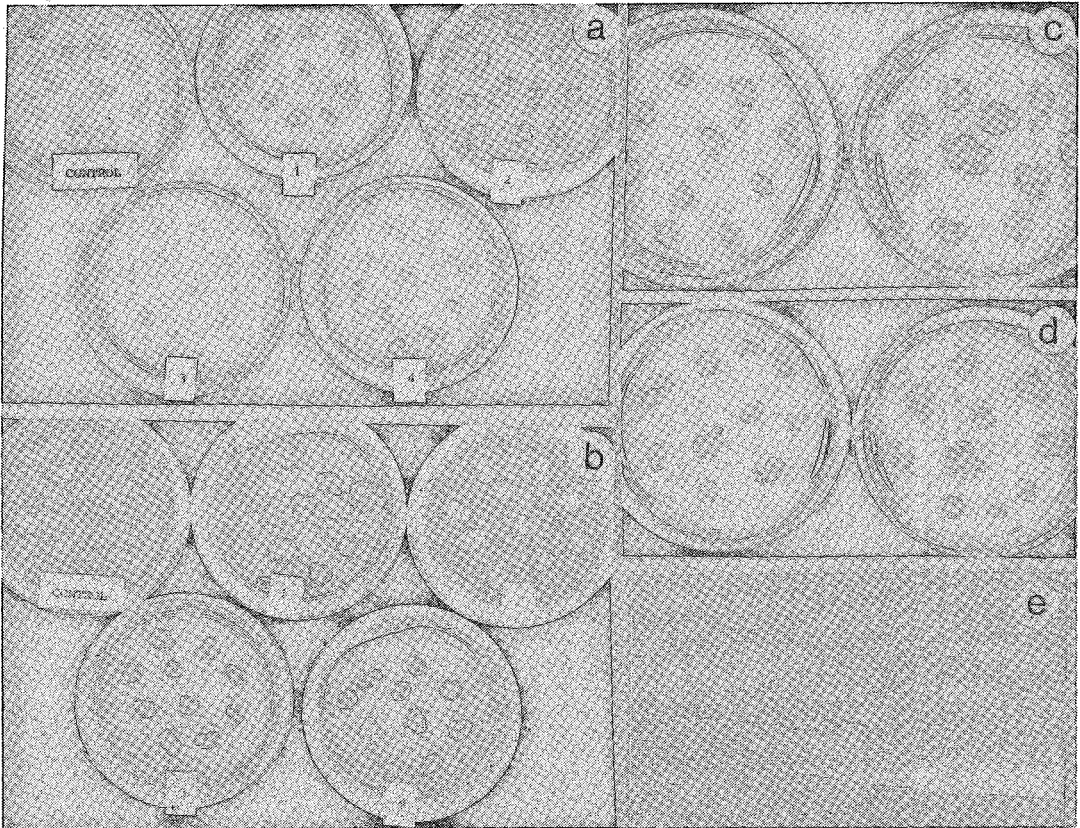


Fig. 1. a: Effect of phytotoxins at concentrations of 0, 5, 20 (upper row), 100 and 200 units on callus growth of wheat.
 b: The same experiment with extract units from uninoculated fungal culture medium added.
 c: Wheat calli after the first and second cycles of selection on toxic media.
 d: Wheat calli after third and fourth cycles of selection.
 e: In vivo reaction of leaves of in vitro selected (top) and an unselected control plant to $5\mu\text{l}$ spore suspension with 10^6 *Helminthosporium sativum* spores per ml.

Selection of resistant calli was done by putting small pieces of calli (10/petri dish) containing toxic medium and transferring the insensitive calli after 3 weeks of growth to the next cycle (Fig. 1c & d). After the 1st cycle on toxic medium the two genotypes showed 24% survival of calli (Table 1). In the 2nd cycle 6-7% of the calli still died but there after only 2-4 % of the calli died indicating insensitivity to toxic medium was exhibited after 2nd cycle of selection.

After 4 selection cycles the resistant calli were grown on maintenance medium without hormones and parts of good looking calli were placed on regeneration medium. The frequency of regeneration varied from 25 to 48%. As compared to this maximum of 16% green shoot formation frequency was observed. The observation on *in vivo* reaction of leaves of regenerated plants showed 7 plants were insensitive to the pathogen and 2 showed intermediate reaction (Fig. 1e).

Discussion

In addition to the efforts made in resistance breeding through conventional approaches, attempts have been made in the last few years to produce novel resistant plants through cell culture techniques. In the present work helminthosporal and victoxinine toxins produced by the fungus in the liquid culture filtrate were used for selection of resistant calli. The earlier experiments on selections conducted by Gengenbach and Green (1975) and Hartman et al (1984) increased the conc. of toxin in the media slowly and others have used low to moderate conc. of toxin (Behnke 1979; Thanutong et al, 1983). But here a high toxin conc. from the beginning was employed to kill in the first cycle of selection itself 70-80% of the calli.

The previous report on these genotypes has revealed that 14 to 25% regeneration potentiality is retained by old callus cultures (Chawla and Wenzel 1987a). It revealed 16% green shoot formation after selection in one of the genotypes. Also, the plants regenerated from calli resistant to the toxins expressed the resistance character. However, the character was not expressed as a qualitative trait in all cases. The regenerated plants also showed intermediate reaction i.e. few symptoms and at a later stage. This variability of the regenerated plants in the trait selected for has been found in rapeseed for *Phoma lingam* resistance (Sacristan 1982) and in barley for fusaric acid toxin (Chawla and Wenzel 1987b). The variation for resistance obtained in the plants indicates that probably the nuclear genome has an influence on the resistance rather than mitochondrial DNA as reported for *Helminthosporium maydis* resistance in maize (Brettell et al 1980).

Aknowledgments

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References

- Behnke M (1979) Selection of potato callus for resistance to culture filtrates of *Phytophthora infestans* and regeneration of plants. Theor Appl Genet 55: 69-71
- Brettell RIS, Thomas E and Ingram DS (1980) Reversion of Texas male sterile cytoplasm maize in culture to give fertile, T-toxin resistant plants. Theor Appl Genet 58: 55-58
- Chawla HS and Wenzel G (1987a) Regeneration potential of callus from wheat and barley. Arch Zuchtungs-forsch 17: 337-343
- Chawla HS and Wenzel G (1987b) In vitro selection for fusaric acid resistant barley plants. Plant Breeding Crop Sci 15: 645-649
- Gengenbach, BG and Green CE (1975) Selection of T-cytoplasm maize callus cultures resistant to *Helminthosporium maydis* race T pathotoxin. Cro Sci 15: 645-649
- Hartman CL, McCoy TJ and Knous TR (1984) Selection of alfalfa (*Medicago sativa*) cell lines and regeneration of plants resistant to the toxin(s) produced by *Fusarium oxysporum* f.sp. *medicagnis*. Plant Sci Lett 34: 183-194
- Ludwig RA (1957) Toxin production by *Helminthosporium sativum* and its significance in disease development. Can J Bot 35: 291-304
- Pringle RB (1976) Comparative biochemistry of the phytopathogenic fungus *Helminthosporium*. XVI. The production of victoxinine by *H. sativum* and *H. victoriae*. Can J Biochem 54: 783-787
- Pringle RB and Braun CA (1957) The isolation of the toxin of *Helminthosporium victoriae*. Phytopathol 47: 369-371
- Pringle RB and Scheffer RP (1963) Purification of the selective toxin of *Periconia circinata*. Phytopathol 53: 785-787
- Sacristan MD (1982) Resistance responses to *Phoma lingam* of plants regenerated from selected cell and embryogenic cultures of haploid *Brassica napus*. Theor Appl Genet 61: 193-200
- Thanutong P, Furusawa I and Yamamota M (1983) Resistant tobacco plants from protoplast derived calluses selected for their resistance to *Pseudomonas* and *Alternaria* toxins. Theor Appl Genet 66: 209-215
- Wenzel G (1985) Strategies in unconventional breeding for disease resistance. Ann Rev Phytopath 23: 149-172
- Wheeler HE and Luke HH (1955) Mass screening for disease resistant mutants in oats. Science 122: 1229-1234



Wheat breeding for resistance to *Fusarium* diseases, especially to *Fusarium graminearum* Schw.

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Summary

Over 130 wheat sources of resistance were tested under conditions of artificial infection and chosen 7 genotypes were mutually crossed by using the scheme of partial diallel. Sources of resistance markedly differed in level of resistance, which ranged from 0, 65 to 3, 89 (rating scale 0 – 5). Improving the level of resistance was obtained in F_1 generation as well as in $F_1 \times F_1$ crosses, in the several combinations, in comparison with their parental components. Additive gene effect (i.e. minor gene effects) and partial dominance were noticed. The highest level of resistance was found in F_1 generation and in $F_1 \times F_1$ crosses of combinations involving genotypes Bizel and Poncheau.

Introduction

The genus *Fusarium* belongs to the order Moniliales and class Fungi imperfecti (Deuteromyceteae). The principal pathogen that causes wheat scab is *Fusarium graminearum* Schw. (the perfect stage is *Gibberella zeae* /Schw./Petch.). The pathogen that causes head blight and shrivelling of kernels, causes also rot on secondary roots, develops in the soil as facultative parasite, and usually lives saprophytically on debris. It is permanently present in the soil and thus greatly makes the study of inheritance of resistance difficult if the work is carried out under field conditions.

Attack of *Fusarium graminearum* Schw. in wheat has been known in Yugoslavia, long time ago (Milatović 1960; Perišić 1963; Kostić and Smiljaković 1966; Jovičević 1969) During recent years this disease has been frequently occurred in Yugoslav agricultural practice, especially affecting heads of susceptible varieties. Disease attack is more expressed when high temperatures are accompanied with high air humidity during heading and flowering time. Since 1975 attack of *Fusarium graminearum* Schw. is increasing in our country.

Severe outbreaks of this disease is the consequence of narrow crop rotation of the two main crops (corn-wheat), which are the most common hosts for *Fusarium graminearum* Schw. Some times also happens that wheat comes after wheat. Increase of the mineral fertilisation, and especially discordance of nitrogen to other components favour the disease occurrence. The quantity of N fertilizer usually applied nowadays is as high as 200 kg per hectar of pure nitrogen. Yield reductions of wheat, caused by *Fusarium graminearum* Schw. forced us to improve investigations on this disease. So, in the year 1978 we started with wheat breeding program of resistance

to *Fusarium graminearum* Schw. in Institute for Breeding and Production of Field Crops in Zagreb.

The program of incorporating wheat resistance to *Fusarium* spp., especially to *Fusarium graminearum* Schw. is very complex, because the pathogen itself is a facultative organism to which mechanism of breeding and model of inheritance is also complex. Influence of the environmental conditions in expression of the genetic resistance is rather high.

The objective of our investigations is to determine the reaction of the wheat sources of resistance to fusarium head scab under conditions in Yugoslavia, with the purpose to use the best sources in our breeding program.

Materials and Methods

Investigations of wheat resistance to scab caused by *Fusarium graminearum* Schw. were conducted in field experiments in the experimental nursery of the Institute for Breeding and Production of Field Crops in Zagreb. Here were included 130 wheat genotypes obtained from the world various centers. The obtained sources of resistance are mainly extensive genotypes, taller in habit and with less expression of the resistance to other diseases. They often possess some undesirable traits, too.

In our investigations we used both natural and artificial infections. Infected materials were collected from the large wheat growing area in Yugoslavia to be used for artificial infection. *Fusarium* from collected samples were developed on PDA and used for productions of pure culture by using infected wheat kernels, namely its juice for inoculation and for the identification of *Fusarium* species as well.

Artificial infection of spikes in field, took place at flowering stage using "spray-method". The second infection is falling in a week later. Attack severity was estimated in two intervals – first at the milk or milk-dough stage and the second seven days later (at the growth stage 11,1, and 11,3 of the Feekes scale). The rating scale 0 – 5 was used for estimating fusarium attack (0 = no infection, 5 = more than 75% diseased plants/spikes).

Using partial diallel crossings with seven sources of resistance were performed in 1981. The following sources were used: Toropi, Roazon, Encruzilhada, Bizel, Mironovskaya 808, Balayacerkov and Poncheau. In the year 1982 were made crosses $F_1 \times F_1$. Next year we put offsprings in the experiment parallel with corresponding F_1 generation and their parents (sources of resistance). The obtained F_1 crosses and the crosses of $F_1 \times F_1$ were set in trials using randomized block design in 5 replications. Each combination was planted in a 1,20m row, 10 kernels/row. The parents (sources of resistance) were planted parallel in two rows with 0,30m row spacing. The trial was artificially inoculated by using "spray-method". Two ratings of disease attack in field were taken.

Results and Discussion

Over 130 sources of resistance were collected and tested, seven were chosen and crossed (using the scheme of partial diallel) in order to determine the level of wheat resistance to fusarium head scab. Apart from considering the level of resistance, efforts were made to select as divergent material as possible with good agronomic traits.

According to the results from Table 1 the selected materials differ markedly among themselves in the level of resistance. The level ranged from 0,65 (Encruzilhada) to 3,89 (Roazon) and the selected materials expressed types of reaction from resistant to moderately susceptible (R, MS). This level of resistance was determined using the rating scale 0 – 5. By this scale types rated 0 – 2 were marked R, MR, while types estimated 3 – 5 were marked MS, S, AS (VS).

Improvement of the resistance was observed in F_1 generation in comparison with relative parental components (i.e. sources of resistance). This was particularly evident in the following combinations: (Toropi \times Encruzilhada), (Bizel \times Mironovskaya 808), (Bizel \times Poncheau) and (Balaya-

Table 1. Mean disease severity and reaction of the sources of resistance to *Fusarium graminearum* Schw. in wheat compared with their single and double crosses

Entry	Mean disease severity*	Reaction
Sources of resistance		
Roazon	3.89	MS
Mironovskaya 808	1.72	R
Balayacerkov	2.89	MR
Poncheau	1.33	R
Bizel	1.14	R
Toropi	2.85	MR
Encruzilhada	0.65	R
F_1 (single cross)		
Roazon \times Mironovskaya 808	2.80	MR
Balayacerkov \times Poncheau	1.04	R
Roazon \times Bizel	3.33	MS
Bizel \times Poncheau	0.95	R
Roazon \times Poncheau	2.59	MR
Bizel \times Mironovskaya 808	0.77	R
Toropi \times Encruzilhada	0.75	R
$F_1 \times F_1$ (double cross)		
(Roazon \times Mironovskaya 808) \times (Balayacerkov \times Poncheau)	0.50	R
(Roazon \times Bizel) \times (Bizel \times Poncheau)	1.00	R
(Roazon \times Poncheau) \times (Bizel \times Poncheau)	1.33	R
(Bizel \times Poncheau) \times (Toropi \times Encruzilhada)	1.66	R
(Bizel \times Mironovskaya 808) \times (Balayacerkov \times Poncheau)	1.33	R

* scale 0 – 5;

0 – 2 = R, MR

3 – 5 = MS, S, AS (VS)

0 = no infection

5 = more than 75% diseased plants/spikes

cerkov x Poncheau), the resistance level of which ranged from 0,75 (Toropi x Encruzilhada) to 1,04 (Balayacerkov x Poncheau). In the presented combinations in F_1 generation of single crosses there is obviously additive gene effect present. Additive gene effect and the more expressed level of resistance particularly appeared in crosses with Bizel, Poncheau and Encruzilhada, where as somewhat less in combinations involving genotypes Mironovskaya 808, Toropi and Balayacerkov. Reaction type of crosses was resistant (R).

Furthermore, partial dominance was also noticed in combinations of crosses such as: (Roazon x Poncheau), (Roazon x Mironovskaya 808) and (Roazon x Bizel), which expressed the level of resistance from 2,59 (Roazon x Poncheau) to 3,33 (Roazon x Bizel). Partial dominance is present in those combinations involving genotypes with less expressed level of resistance, in this case crosses with Roazon. Reaction type of crosses was moderately resistant to moderately susceptible (MR - MS).

Even more expressed improvement of the level of resistance in comparison to parental components was found in F_1 generation of double crosses ($F_1 \times F_1$), in which the level of resistance ranged from 0,50 / (Roazon x Mironovskaya 808) x (Balayacerkov x Poncheau) / to 1,66 / (Bizel x Poncheau) x (Toropi x Encruzilhada) /. Increased level of resistance was also expressed in the following combinations of double crosses ($F_1 \times F_1$); / (Roazon x Bizel) x (Bizel x Poncheau) /, / (Bizel x Mironovskaya 808) x (Balayacerkov x Poncheau) /, as well as in / (Roazon x Poncheau) x (Bizel x Poncheau) /. These crosses expressed the resistant (R) type of reaction.

From all said above probably the polygenic mode of inheritance with its minor gene effects is present here.

The results given in Table 1 prove that among all wheat sources of resistance to fusarium head scab, the highest level of resistance was found in genotypes Encruzilhada, Bizel, Poncheau and Mironovskaya 808. In connection with the investigations of the level of resistance to fusarium head scab similar findings were reported also by other workers (Schroder and Christensen 1963; Feekes and Wieten 1967; Wildermuth and Purss 1971; Luzzardi et al 1974, 1975, 1984; Mesterhazy 1977, 1978, 1983; Saur and Trotter 1981; Saur, 1984; Bekele 1984; Liu 1984; Milatovic, Vlahovic and Tomasovic 1982; Tomasovic 1981, 1984).

Conclusions

Based on several investigations of wheat sources of resistance to fusarium head scab under artificial field infection, we might have the following conclusions:

1. A considerable difference in the level of resistance among the selected genotypes was found, ranging from 0,65 to 3,89 (rating scale 0 - 5).
2. Testing of resistance in adult stage has revealed the improvement of the level of resistance in F_1 progenies as well as in $F_1 \times F_1$ crosses in comparison with their parental components.
3. Additive gene effect is noticed in F_1 including increased level of resistance as well as partial dominance.

4. Those combinations involving genotypes Bizez and Poncheau, shows the highest level of resistance whether it was in F_1 generation or in $F_1 \times F_1$ crosses.

References

- Bekele GT (1984) Head scab screening methods used at CIMMYT. Wheats for more tropical environments. A Proceedings of the International Symposium. September 24-28, 1984 Mexico, D.F. The United Nations Development Programme and CIMMYT 169-173.
- Feekes W and Wieten DT (1967) Breeding wheat for resistance to *Septoria Fusarium*. Techn. Ber. Sticht. Nederl. Graan-Centrum.
- Jovicevic B (1969) Prilog proučavanju *Fusarium* spp. na semenu pšenice. Savremena poljoprivreda 11-12: 523-530, Novi Sad.
- Kostic B and Smiljakovic H (1966) Bolesti pšenice u uslovima intenzivne proizvodnje i mere za njihovo suzbijanje. Agrohemija; 7-8, 331-342, Beograd.
- Liu ZZ (1984) Recent advances in research on wheat scab in China. Wheats for more tropical environments. A Proceedings of the International Symposium. September 24-28, 1984 Mexico, D.F. The United Nations Development Programme and CIMMYT 174-181.
- Luzzardi GC, Pierobom CR, Osorio EA, Moreira JCS, Wetzel MVS and Dias eJCA (1974) Melhoramento de trigo para resistencia a "Gibberella". Reunia-Latinoamericana de trigo conferencia latinoamericana del trigo, Porto Alegre, Brazil
- Luzzardi GC, Pierobom CR, Osorio EA, Moreira JCS, Wetzel MVS and Dias eJCA (1975) Wheat Breeding for Resistance to *Gibberella zeae* (Schw.) Petch. Annual Wheat Newsletter, Kansas State University and Canada Department of Agriculture, 21 (1): 53.
- Luzzardi GC (1984) Wheat Breeding for scab resistance. Wheats for more tropical environments. A Proceedings of the International Symposium. September 24-28, 1984 Mexico, D.F. The United Nations Development Programme and CIMMYT, 158-168
- Mesterhazy A (1977) Reaction of Winter Wheat Varieties to Four *Fusarium* Species. Phytopathology Z. 90. 104-112.
- Mesterhazy A (1978) Comparative analysis of artificial inoculation methods with *Fusarium* spp. on winter wheat varieties. Phytopathologische Zeitschrift 93: 12-25
- Mesterhazy A (1983) Breeding winter wheat varieties to four *Fusarium* species. *Fusarium culmorum* Z. Pflanzung, 91: 295-311
- Milatovic I (1960) Pregled najrasirenijih bolesti pšenice na području Slavonije i Podravine. Agronomski glasnik, 11-12: 600-607, Zagreb
- Milatovic I, Vlahovic V and Tomasovic S (1982) Otpornost klasova pšenice prema *Fusarium graminearum*. Schw. Zastita bilja, Vol. 33 (4), 162, 389-396, Beograd
- Perisic M (1963) *Fusarium graminearum* Schwabe - parazit pšenice u Slavoniji, Zastita bilja, 14, 527-532, Beograd
- Saur L and Trotter M (1981) Problemes poses par le jugement du comportement de lignees de ble tendre et especes voisines vis-a-vis de deux parasites de l'epi *Septoria nodorum* Berk. et *Fusarium roseum* (Link.) Sn. et H. Mise en evidence de quelques sources de resistance. C.R. Acad. Fr., 67: 139-145
- Schroeder HW and Cristensen JJ (1963) Factors effecting resistance of wheat to scab by *Gibberella zeae*. Phytopathology 53, 831-838
- Tomasovic S (1981) Breeding of wheat for resistance to *Fusarium* diseases, especially to *Fusarium graminearum* and creating a model for inheritance of resistance in new wheat cultivars. Genetika 13(12): 177-187, Beograd
- Tomasovic S (1984) Improving resistance of Wheat (*Triticum aestivum* ssp. *vulgare*) to *Fusarium* spp. by applying recurrent selection. Annu. Wheat Newsletter, Vol. 30, Colorado State University and Canada Dept. of Agriculture
- applying recurrent selection. Annu. Wheat Newsletter, Vol. 30, Colorado State University and Canada Dept. of Agriculture.
- Wildermuth GB and Purss GS (1971) Further source of field resistance to crown rot (*Gibberella zeae*) of cereals in Queensland. Aust Exp Agr and Hubs 11: 455-459



Polyphenol oxidase enzyme in isogenic lines of wheat in relation to leaf rust resistance

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Introduction

It has been observed that fungal infection results in changes of multiple forms and activity of enzymes in the host parasite complex. Enzymic studies have been conducted on various plant species after infection (Desai and Pillai 1974; De Witt and Bakker 1980; Kanugo and Chawla 1988) but limited studies have been made to characterise the healthy wheat genotypes (Narasimham and Chawla 1984). In view of this role of polyphenoloxidase which is one of the phenol oxidising enzymes involved in disease resistance (Batra and Kuhn 1975) was studied for its activity and isozymes in near isogenic lines of wheat with different leaf rust resistance, *Lr* genes in different genetic backgrounds.

Materials and Methods

Material consisted of nine near isogenic lines of wheat with different leaf rust resistance - *Lr* genes in different genetic back grounds (Thatcher - Tc; Prelude - Pr, Chinese spring - CS) and two susceptible cultivars which have been numbered as a to k.

The lines with their accession numbers were:

(a) RL 6028 - *Lr1* (Pr), (b) RL 6002 - *Lr3* (Tc), (c) RL 6029 - *Lr3* (Pr), (d) RL 6007 - *Lr3ka* (Tc), (e) RK 6030 - *Lr3ka* (Pr), (f) RL 6024 - *Lr3bg* (Tc), (g) Acc 1003 - *Lr9* (CS), (h) Acc 1407 - *Lr9* (Tc), (i) Acc 1417 - *Lr19* (Tc) and two susceptible Cvs (j) Sonalika and (k) WL 711.

Seed material was grown in petri dishes in an incubator for different germination stages of 0, 24, 48, 72, 96 hrs and one week at a temp of 25°C. 2g sample material of each was homogenised in 3 ml of cold 0.9% NaCl solution and centrifuged at 12,000g for 20 min at 0°C. O-diphenolase (PPO) activity was determined by the method of Haskins (1974). One unit of enzyme activity was the change in O.D. by 0.1 unit per minute. Benjamin and Montgomery (1973) procedure for staining the polyphenol oxidase bands was followed after polyacrylamide gel electrophoresis.

Results and Discussion

Polyphenol oxidase showed an increase in its activity from 0 hr to 48 hr stage of germination followed by a decline at 72 hr stage (Table 1). The activity was almost same at 96 hr stage but then again an increase in the activity was recorded in one week old seedlings but lower than 48 hr

Table 1. Polyphenoloxidase activity at different stages of germination in wheat

Genotype	Stages of germination					
	0hr	24 hr	48 hr	72 hr	96 hr	One week (whole plant)
<i>Lr</i> 1 (Pr)	107.5	162.5	562.5	112.5	105.5	275.0
<i>Lr</i> 3 (Tc)	120.0	150.0	575.0	67.5	69.0	150.0
<i>Lr</i> 3 (Pr)	131.2	331.2	750.0	67.5	68.5	150.0
<i>Lr</i> 3 ka (Tc)	153.7	200.0	718.5	63.0	65.0	140.0
<i>Lr</i> 3 ka (Pr)	105.5	343.7	700.0	102.0	100.0	225.0
<i>Lr</i> 3 bg (Tc)	97.5	160.0	750.0	112.5	105.0	187.5
<i>Lr</i> 9 (Tc)	112.5	218.7	850.0	112.5	108.0	237.5
<i>Lr</i> 9 (CS)	101.2	212.5	750.0	137.5	121.5	212.5
<i>Lr</i> 19 (Tc)	78.7	343.7	662.5	112.5	108.0	200.0
Sonalika	108.7	150.0	718.7	112.5	108.0	125.0
WL 711	146.2	250.0	725.0	67.5	78.5	112.5

stage of germination. Maximum activity was observed at 48 hr stage which was 4 to 9 fold more over the 0 hr stage. Kruger (1976) reported similar changes in activity of polyphenol oxidase in germinating wheat seeds. All the isolines with *Lr* genes showed greater activities than the susceptible entries at one week old stage, but significant differences between these two susceptible entries and resistant isolines could not be established in the early stages of germination.

A total of 7 isozyme forms of PPO were observed with maximum of four bands could be seen at appeared. Similar changes were observed in the case of peroxidase (Narasimham and Chawla 1984) and were reported in case of O-diphenolase during various stages of germination (Taneja and Sachar 1974). These changes are the basis of possible differential gene activities during the development of a sporophyte.

Two slow moving isozymes viz. band nos 5 and 6 whenever observed were always present together and with almost similar intensity which made us to speculate that perhaps these are coded by a single locus.

Isolines with *Lr* genes could not be differentiated from susceptible cultivars with a characteristic isozymic pattern. All the lines showed the same isozymic pattern especially at one week stage.

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References

- Batra GK and Kuhn CW (1975) Polyphenol oxidase and peroxidase activities with acquired resistance and its inhibition by 2-thiourea in virus infected soybean. *Physiol Plant Pathol* 5: 239-248

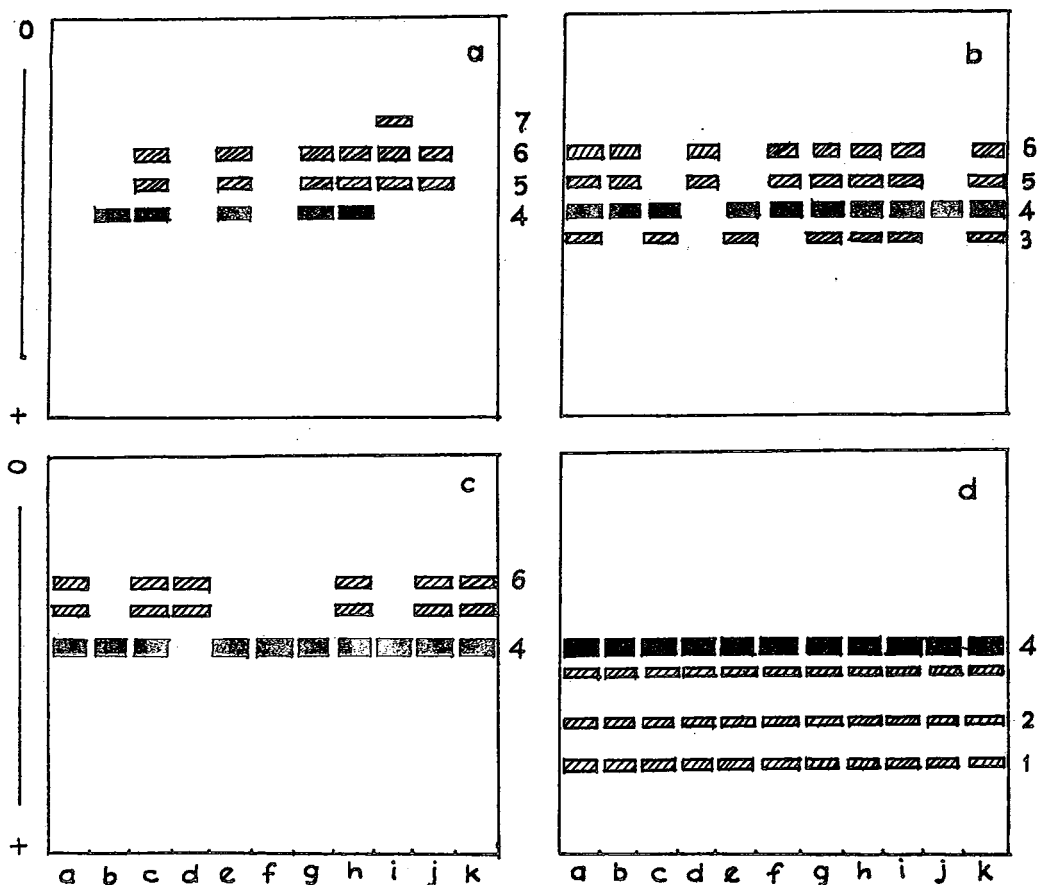


Fig. 1. Zymogram showing polyphenol oxidase isozymes at different stages of seed germination. a - 0 hr; b - 24 hr; c - 48 hr; d - one week. In vertical columns: a - *Lr1* (Pr); b - *Lr3* (Tc); c - *Lr3* (Pr); d - *Lr3ka* (Tc); e - *Lr3ka* (Pr), f - *Lr3bg* (Tc), g - *Lr9* (CS), h - *Lr9* (Tc), i - *Lr19* (Tc), j - Sonalika, k - WL 711.

- Benjamin, ND and Montgomery MW (1973) PPO of Royal ann cherries: purification and characterisation. *J Food Sci* 38: 799
- Desai MV and Pillai SN (1974) Phenolic compounds in relation to black shank (*Phytophthora parasitica*) resistance in tobacco. *Ind J Mycol Plant Pathol* 4: 17-20
- De Witt PTG and Bakker J (1980) Differential changes in insoluble tomato leaf proteins after inoculation
- Haskins PA (1974) Changes in the activities of several enzymes during germination and seedling development in corn. *Plant Physiol* 30: 74-78
- Kanugo US and Chawla HS (1978) Changes in ribonucleases and protein content after powdery mildew inoculation on different genotypes of wheat. *Wheat Inf Serv* 65: 25-27
- Kruger JE (1976) Changes in the PPO of wheat during kernel growth and maturation. *Cereal Chem* 53: 201-213
- Narasimham JV and Chawla HS (1984) A study of peroxidase enzyme in isogenic lines of wheat in relation to leaf rust resistance. *Ind J Plant Physiol* 27: 340-344
- Taneja SR and Sachar RC (1974) Induction of PPO in germinating wheat seeds. *Phytochem* 13: 2695-2702



Hybrid weakness in *Triticum dicoccum* Schubl.

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Introduction

Genetic traits such as hybrid necrosis and hybrid chlorosis are controlled by two independent gene systems. In both kinds of hybrid weakness the F₁ hybrid is lethal or semi-lethal resulting in gradual death or debility. Hybrid necrosis and hybrid chlorosis are frequently met with in inter- and intraspecific wheat crosses and are serious barriers to the transference of genes in a planned hybridization programme. Hybrid necrosis is governed by two dominant complementary genes *Ne1* and *Ne2*, located on chromosomes 5B and 2B respectively (Tsunewaki 1966) while hybrid chlorosis is controlled by two dominant complementary genes *Ch1* located on 2A (Hermesen and Waninge 1972) and *Ch2* on 3D (Tsunewaki and Kihara 1961). The present investigations were carried out to identify the genes for necrosis and chlorosis in nine Indian varieties of *T. dicoccum*.

Materials and Methods

Nine varieties of *Triticum dicoccum* Schubl. were crossed to two *T. aestivum* testers, C306 (*Ne1ne2ch1Ch2*) and Sonalika (*ne1Ne2ch1Ch2*). The F₁ hybrids and parents were raised in the greenhouse under optimum conditions of growth. The F₁ hybrids were critically observed for the occurrence of hybrid necrosis and hybrid chlorosis and genotype of the parents with respect to the genes for necrosis and chlorosis were determined from the phenotype of the hybrids.

Results and Discussion

With the exception of HW 178-A and HW 489, all the varieties of *T. dicoccum* when crossed to C 306 produced strong chlorotic F₁ hybrids indicating that the varieties carry the *Ch1* gene (Table 1). The F₁ hybrid plants between Sonalika and four *T. dicoccum* varieties, namely, HW 43, HW 1018, HW 1046 and Khapli-53 Yellow expressed the symptoms of both necrosis and chlorosis simultaneously indicating that these varieties also carry the gene *Ne1*. The variety HW 178-A produced normal and necrotic F₁ off-spring when crossed to C 306 and Sonalika respectively. Results indicate that the variety is non-carrier for chlorosis gene while it carries the gene *Ne1*. Normal hybrids were obtained in the crosses of HW 489 with both the testers indicating that this variety is non-carrier for both necrosis and chlorosis genes. The F₁ hybrids between Sonalika,

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a *Ne2 Ch2^m* - carrier (Kochumadhavan et al 1984), and three *T. dicoccum* varieties, viz., HW 1016, HW 1017 and Sangli 2-2, exhibited only chlorosis and it is likely that chlorosis may obscure the expression of necrosis. Consequently, this type of epistatic gene action caused difficulty in identifying the genotype of these varieties of *T. dicoccum* as to have *Ne1* or *ne1*. When

Table 1. Genotype of *T. dicoccum* varieties with respect to the genes for hybrid necrosis and hybrid chlorosis

Varieties	Testers		Genotype of the variety tested
	C306 (<i>Ne1ne2ch1Ch2</i>)	Sonalika (<i>ne1Ne2ch1Ch2</i>)	
HW43	c	nc	<i>Ne1ne2Ch1</i>
HW178-A	—	n	<i>Ne1ne2ch1</i>
HW1016	c	c	? <i>ne2Ch1</i>
HW1017	c	c	? <i>ne2Ch1</i>
HW1018	c	nc	<i>Ne1ne2Ch1</i>
HW1046	c	nc	<i>Ne1ne2Ch1</i>
Khapli-53 Yellow	c	nc	<i>Ne1ne2Ch1</i>
Sangli 2-2	c	c	? <i>ne2Ch1</i>
HW489	—	—	<i>ne1ne2ch1</i>

— = Normal; n = necrotic; c = chlorotic; ? = gene could not be determined.

both the complementary gene systems for hybrid necrosis and hybrid chlorosis operate in F_1 hybrids, the phenotypic expression of one hybrid weakness over the other or the simultaneous occurrence of both kinds of hybrid weakness depends on the relative strength of alleles existing at *Ne*- and *Ch*- loci. Therefore, the genes with respect to necrosis in the varieties: HW 1016, HW 1017 and Sangli 2-2 could not be determined for want of single-gene tester (*ne1Ne2ch1ch2*) for necrosis or a two-gene tester (*ne1Ne1Ch1ch2*) for necrosis and chlorosis with the authors. While the single-gene tester is extremely rare, the two-gene tester has not been established in polyploid wheats.

Varieties of *T. dicoccum*, like other tetraploid species of wheat, are either *Ne1* - carriers or noncarriers (Nishikawa 1967; Tsunewaki 1969b; Tomar et al 1987). *Ne2* - carriers have not yet been reported in 4x wheats despite the location of this gene in B genome. Therefore, the *Ne2* gene, found restricted to the western 6x wheats is presumed to have originated by mutatio at the hexaploid level in Europe (Tsunewaki and Kihara 1962).

Hermesen (1966) reported that the Indian emmer wheat Khapli carried the *Ch1* gene. The present study reveals a high prevalence of *Ch1*-carriers in *T. dicoccum* varieties and this further corroborates the observation of Kochumadhavan et al (1984) that the gene *Ch1* widely occur among the Indian varieties of *T. dicoccum*.

References

- Hermesen JGTh (1966) Hybrid necrosis and red hybrid chlorosis. Proc. 2nd Int Wheat Genet Symp Lund, Hereditas Suppl 2: 439-452
- Hermesen JGTh and Waninge J (1972) Attempts to localize the gene *C_h1* for hybrid chlorosis in wheat. Euphytica 21: 204-208
- Kochumadhavan M, Tomar SMS and Nambisam PNN (1984) Hybrid necrosis and hybrid chlorosis in Indian varieties of *Triticum dicoccum* Schubl. Euphytica 33: 1-6
- Nishikawa K (1967) Identification and distribution of necrosis and chlorosis genes in tetraploid wheat. Seiken Ziho 19: 37-42
- Tsunewaki K (1960) Monosomic and conventional analysis in common wheat. III. Lethality. Jap J Genet 35: 71-75
- Tsunewaki K (1969) Origin and phylogenetic differentiation of common wheat revealed by comparative gene analysis. Proc 3rd Int Wheat Genet Symp pp 71-85
- Tsunewaki K and Kihara H (1961) F₁ monosomic analysis of *Triticum macha*. Wheat Inf Serv 12: 1-3
- Tsunewaki K and Kihara H (1962) Comparative gene analysis of common wheat and its ancestral species. I. Necrosis. Jap J Genet 37: 474-484

II. Proceedings of the 15th Wheat Genetics Symposium of Japan

The 15th Wheat Genetics Symposium of Japan was held on April 5, 1989 at Yokohama, Japan, chaired by Dr. M. Tanaka under sponsorship of Kihara Institute for Biological Research. Seventy-eight wheat researchers attended the symposium with hot discussions. The followings were the abstracts of the presentations. Addition to the research reports, Drs. M. Tanaka and S. Sakamoto introduced a slide presentation on the Botanical Expedition to Tibet in summer, 1988.

Phylogeny of wheat and its relatives as viewed from nuclear, chromosomal and chromosome arm DNA content

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Bhaskaran and Swaminathan (1960), Pai et al (1961), Upadhy and Swaminathan (1965) emphasized the diminution of chromosome length and nuclear DNA content in connection with polyploidy in wheat. However, Rees (1963), and Rees and Walters (1965) could not detect such diminution. In relation to the functional diploidization in polyploid and phylogeny of wheat, DNA content per nucleus of single-layer smeared pollen tetrad in wheat and its relatives, DNA content of individual chromosome at the first meiotic metaphase in hexaploid wheat and individual telocentric chromosome in mitotic root tip cell of common wheat were measured Feulgen-cytophotometrically. The results are summarized as follows:

1. Nuclear DNA content in di-, tetra- and hexaploid wheat is not simple ratio of 1:2:3, but 1.0:1.6:2.4 (Nishikawa and Furuta 1969).
2. There is no intraspecific variation in *Triticum monococcum* (Furuta et al 1978), *T. urartu* (Furuta et al 1986) and *T. aestivum* (Nishikawa and Furuta 1967).
3. *T. aestivum* has the sum of DNA of *T. turgidum* and *Aegilops squarrosa* (Nishikawa and Furuta 1969, Furuta et al 1974), indicating no significant change in DNA content of *T. aestivum* since its origin.
4. There are variation in tetraploid wheat,
 - A: *T. timopheevi* has 95% DNA of *T. turgidum* (Rees and Walters 1965).
 - B: Small differences between strains of *T. turgidum* (Nishikawa et al 1978).
5. Interspecific variation occurs among putative genome donors to polyploid wheat (Furuta et al 1986): *Ae. squarrosa* (relative ratio; 0.86) < *Ae. speltoides* (0.92) < *T. urartu* (0.98)

= *T. monococcum* (1.00) < *Ae. bicornis* (1.07) = *Ae. searsii* (1.08) < *Ae. longissima* (1.21) < *Ae. sharonensis* (1.27).

6. There is about twice variation in nuclear DNA content among diploid species of wheat and *Aegilops*. Three diploid species which are remotely related and whose species are diagnostic to one another, *Ae. squarrosa*, *Ae. caudata* and *Ae. umbellulata* belonging to the lower DNA group and are known to be donors of the unaltered or pivotal genomes to polyploid relatives as pointed out by Zohary and Feldman (1962). On the other hand, the genomes (S and M genome groups) of higher DNA group are modified in polyploid species (Furuta 1975).
7. Considerable intraspecific variation occurs in three diploid species; *Ae. squarrosa* (Furuta et al 1975), *Ae. longissima* and *Ae. sharonensis* (Furuta et al. 1977, 1986).
8. DNA content of AB genome component of common wheat is the same to that of *T. turgidum* (Furuta et al 1974) and is similar to the sum of diploid wheat and *Ae. bicornis*, *Ae. searsii*, *Ae. longissima* or *Ae. sharonensis* (Furuta et al 1986).
9. The result of individual chromosomal (univalent chromosomal) DNA content of seven strains belonging to six subspecies of Dinkel wheat reveals variations in DNA content between three genomes and homologous chromosomes. The ratio of their genomic DNA content is 1.00:1.20:0.83 in A:B:D (Furuta et al 1984).
10. Individual telocentric chromosomal DNA content in monotelodisomic 1BS and monotelodisomic or double telotrisomic for another chromosomes and double monotelodisomic 1BS-1BL of al 1988).
1.00:1.12:0.82 for A:B:D, and high positive correlations ($r=0.90$ and 0.99 , respectively) between length and DNA content of telosomes and DNA content of individual chromosome and the sum of DNA of two arms for the 21 individual chromosomes (Furuta et al 1988).

All evidence mentioned above indicates no significant reduction of genetic materials or DNA in polyploid wheat, but the additive relationship in DNA content between polyploid and its genome donors and suggests that relative DNA content of the B genome in common wheat is more than nuclear DNA content of *Ae. speltoides*, but comparable to the nuclear DNA content of *Ae. longissima*, *Ae. searsii*, *Ae. bicornis* or probably *Ae. sharonensis* belonging to section Sitopsis of the genus *Aegilops*.

References

- Bhaskaran S and Swaminathan MS (1960) Metaphase chromosome length and DNA content in relation to polyploidy in *Triticum* species. *Exp Cell Res* 20: 298-299
- Pai RA, Upadhyaya MD, Bhaskaran S and Swaminathan MS (1961) Chromosome diminution and evolution of polyploid species in *Triticum*. *Chromosoma* 12: 398-409.
- Furuta Y, Nishikawa K and Haji H (1978) Uniformity of nuclear DNA content in *Triticum monococcum* L. *Japan J Genet* 58: 361-366
- Furuta Y, Nishikawa K and Kimizuka T (1977) Quantitative comparison of the nuclear DNA in section Sitopsis of the genus *Aegilops*. *Japan J Genet* 52: 107-115

- Furuta Y, Nishikawa K and Makino T (1975) Intraspecific variation of nuclear DNA content in *Aegilops squarrosa* L. Japan J Genet 50: 257-263
- Furuta Y, Nishikawa K, Makino T and Sawai (1984) Variation in DNA content of 21 individual chromosomes among six subspecies in common wheat. Japan J Genet 59: 83-90
- Furuta Y, Nishikawa K, and Simokawa K (1988) Relative DNA content of the individual telocentric chromosomes in Chinese Spring wheat. 7th Intern Wheat Genet Symp, Cambridge, 1: 281-186
- Furuta Y, Nishikawa K, and Tanino T (1974) Stability in DNA content of AB genome component of common wheat. Japan J Genet 49: 179-187
- Furuta Y, Nishikawa K, and Yamaguchi S (1986) Nuclear DNA content in diploid wheat and its relatives in relation to phylogeny of tetraploid wheat. Jpn J Genet 61: 97-105
- Rees H (1963) Deoxyribonucleic acid and the ancestry of wheat. Nature 198: 108-109
- Rees H and Walters MR (1965) Nuclear DNA and the evolution in wheat. Heredity 20: 73-82
- Upadhyia MD and Swaminathan MS (1963) Deoxyribonucleic acid and the ancestry of wheat. Nature 200: 713-714
- Zohary D and Feldman M (1962) Hybridization between amphidiploids and the evolution of polyploids in the weat (*Aegilops-Triticum*) group. Evolution 16: 44-61

Genome analysis of *Aegilops mutica* Boiss. and its phylogenetic position in the genera *Aegilops* and *Triticum*

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Introduction

To estimate the genome relationship between *Ae. mutica* and the other diploid species of the genera *Aegilops* and *Triticum* by the chromosome pairing at MI of meiosis in their F₁ hybrids, ten diploid *Aegilops* and a diploid *Triticum* species were crossed with *Ae. mutica*. Among the crosses the F₁ hybrids were obtained from the crosses of nine *Aegilops* and a *Triticum* species × *Ae. mutica*. One of the difficulties in estimating the genome relationships among diploid species by the meiotic chromosome pairing in their F₁ hybrids is that non-homologous but partially homologous or homoeologous chromosomes from the both parents pair with their counterparts. In the present work, B-chromosomes of *Ae. mutica* were used as a useful tool for suppressing such a homoeologous chromosome pairing. It is well known that they effectively suppress the frequency of the pairing between homoeologous chromosomes though they do not affect that between fully homologous ones. The simplified principle of the present genome analysis using B-chromosomes is shown in Fig. 1. Based on the chromosome pairing at MI in the F₁ hybrids both with and without B-chromosomes between the diploid species and *Ae. mutica*, the genomes of the diploid species are classified into the following three classes according to the degree of their homology with the genome of *Ae. mutica* designated as Mt in Fig. 1: (a) the genome designated as X has no homology with the genome of *Ae. mutica* when chromosomes derived from the two parents do not pair at all and only 14 univalents of A-chromosomes are observed in the F₁ hybrids both with two B-chromosomes (2B hybrids) and without B-chromosomes (OB hybrids); (b) the genome Y is partially homologous or homoeologous with that of *Ae. mutica* when the OB hybrids show a very high frequency and an almost regular configuration of chromosome pairing as seven bivalents while the 2B hybrids show a drastically low frequency of chromosome pairing and form only 14 univalents of A-chromosomes; and (c) the genome Z is homologous with that of *Ae. mutica* when both the OB and 2B hybrids show a very high frequency and a regular configuration of A-chromosome pairing.

Chromosome pairing in the present F₁ hybrids was analyzed based on this principle of the genome analysis (Ohta and Tanaka 1983; Ohta 1988). *Ae. uniaristata*, *Ae. caudata* and *Ae. umbellulata* were concluded to be distantly related to *Ae. mutica*, because even their OB hybrids with *Ae. mutica* showed a low frequency and a complicated configuration of A-chromosome pairing and because their 2B hybrids showed a drastically low frequency of A-chromosome pairing. *Ae.*

	(a)	(b)	(c)
Genome constitution of parental species and cross combination	: XX × MtMt	YY × MtMt	ZZ × MtMt
Genome constitution of the F ₁ hybrids	: XMt	YMt	ZMt
No. of B-chromosomes in the F ₁ hybrids	: <hr/> 0B 2Bs	<hr/> 0B 2Bs	<hr/> 0B 2Bs
Configuration of A-chromosome pairing at MI in the F ₁ hybrids	: 14 _I 14 _I	7 _{II} 14 _I	7 _{II} 7 _{II}
Relationship between the parental genomes	: not homologous at all	partially homologous or homoeologous	homologous

Fig. 1. The principle of the present genome analysis based on the chromosome pairing at MI of meiosis in the F₁ hybrids between the diploid *Aegilops-Triticum* species and *Ae. mutica* with and without B-chromosomes.

bicornis, *Ae. longissima*, *Ae. sharonensis*, *Ae. comosa* and *T. boeoticum* showed a good genomic affinity with *Ae. mutica* and their OB hybrids with *Ae. mutica* showed a high frequency and an almost regular configuration of chromosome pairing. However, their genomes were concluded to be only homoeologous with that of *Ae. mutica* because their 2B hybrids with *Ae. mutica* formed 14 univalents of A-chromosomes. In contrast with those species, both the OB and 2B hybrids from the crosses of *Ae. speltoides* or *Ae. squarrosa* × *Ae. mutica* showed a high frequency of A-chromosome pairing. In addition, most of the OB hybrids between *Ae. speltoides* and *Ae. mutica* formed some degree of normal pollen grains and egg cells with seven chromosomes while those from the crosses of the other species including *Ae. squarrosa* × *Ae. mutica* were almost completely sterile. These results indicate very close genetic relationship between *Ae. mutica* and *Ae. speltoides* and I propose that the genome symbol S^m to *Ae. mutica* as a closely related genome to the genome S of *Ae. speltoides*.

Sakamoto (1973) classified the genera of the tribe Triticeae into two major groups from their geographical distribution: the Arctic-temperate group and the Mediterranean group. He concluded that the establishment of the former group in the Late-Tertiary is earlier than the establishment in the Quaternary of the latter including the genera *Aegilops* and *Triticum*. *Ae. mutica* is the only species in the genera *Aegilops* and *Triticum* that has a lot of morphological characteristics common to the genera of the Arctic-temperate group. Moreover, the karyotype of *Ae. mutica* consists of only the chromosomes with median and submedian centromeres and *Ae. mutica* is almost self incompatible while the other species in the genera *Aegilops* and *Triticum* are more or less self compatible. These characteristics of *Ae. mutica* are common to the genera of the Arctic-temperate group, and according to Stebbins (1957) self compatible plant species are most probably always derived from self incompatible species. From these evidences *Ae. mutica* is

thought to be the least specialized from the other genera of the tribe Triticeae among the species of the genera *Aegilops* and *Triticum*. And from these facts, I conclude that, in the genera *Aegilops* and *Triticum*, *Ae. mutica* is the most similar to the putative common ancestor of this plant group.

References

- Ohta S (1988) Further evidence for the close genetic relationship between *Aegilops mutica* Boiss, and *Ae. speltoides* Tausch. Proc. 7th Int Wheat Genet Symp, Cambridge, pp 133-138
- Ohta S and Tanaka M (1983) Genome relationships between *Ae. mutica* and the other diploid *Aegilops* and *Triticum* species, based on the chromosome pairing in the hybrids with or without B-chromosomes. Proc. 6th Int Wheat Genet Symp, Kyoto, pp 983-991
- Sakamoto S (1973) Patterns of phylogenetic differentiation in the tribe Triticeae. Seiken Sihō 24: 11-31
- Stebbins GL (1957) Self fertilization and population variability in the higher plants. Amer Nat 91: 337-354

Application of fluorescence *in situ* hybridization to molecular cytogenetics of wheat

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In recent years it has been possible to recognize specific repeated sequences and even single-copy sequences on metaphase chromosomes using biotin-labeled probes. In our wheat molecular cytogenetic program some efforts have been made towards exploring the possibilities of the useful application of fluorescence *in situ* hybridization with biotin-labeled probes. The results of such studies are presented here.

Wheat ribosomal RNA gene (rDNA) and the 120-bp repeated DNA family of rye (pSC119) were used as probes. Probe DNA was labeled with biotin-16-dUTP by random primer method. Hybridization reactions were incubated at 37°C for 6 hr. Fluorescent detection of hybridized biotinylated DNA was carried out using the rabbit anti-biotin antibody and the FITC-goat-rabbit antibody.

The fluorescence hybridization of rDNA to a metaphase spread of monosomic 1A line of *Triticum aestivum* cv. Chinese Spring is shown in Figure 1. A rDNA locus on chromosome 1A is clearly visible in addition to rDNA loci on chromosome 1B, 6B, and 5D. *In situ* hybridization using the rDNA probe was also conducted in the 1R(1B) substitution line of wheat, Burgas 2, which was regenerated from immature embryo culture. This line was found lacking in one of the two rDNA loci on the chromosome 1R, although it had a pair of chromosome 1R. In metaphase spread, the NOR present on the wheat chromosome 6B was stretched whereas the rye NOR remained contracted. Interphase nuclei of Burgas showed that the NOR loci of 6B were dispersed and thus were being actively transcribed. On the contrary, the rye NOR locus was condensed, indicating no activity. The activity of the rye rDNA genes always remained suppressed in the wheat background. The degree of rDNA gene expression was visible by fluorescence detection after hybridization. In F₁ hybrid between Chinese Spring wheat and Prolific rye, the chromosomes were readily distinguished from wheat chromosomes on the basis of *in situ* hybridization patterns to pSC119 probe (Fig. 3). The highly repeated sequences on both wheat and rye chromosomes were seen as yellow segments. The rye chromosomes were entirely orange in color, whereas the wheat chromosomes appeared red. The individual chromosomes of rye were also easily identified. As shown in Figure 4a, the increased probe concentration resulted in brighter detection signals relative to Figure 3. Rye chromosomes can be strikingly visualized. In Burgas wheat, the two rye chromosomes are clearly seen in the metaphase spread, as are their domains in the interphase nucleus (Fig. 4b). We can call this approach "chromosome or genome painting." Thus, fluorescence *in situ* hybridization with biotin-labeled probe seems to be a powerful tool for identification of specific chromosomes in wheat and its relatives and for detection of alien chromosomes in their hybrids.

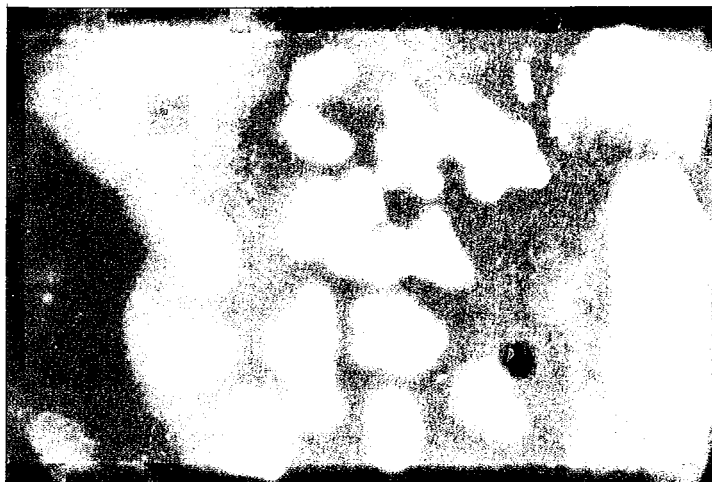


Fig. 1. Fluorescence hybridization of biotin-labeled rDNA probe to a metaphase spread of *Triticum aestivum* cv. Chinese Spring (monosomic 1A). Chromosomes are counterstained with propidium iodide, which fluoresces red. Biotin-labeled rDNA probe is stained with fluorescein which fluoresces yellow. Arrowhead indicates the rDNA on chromosome 1A.

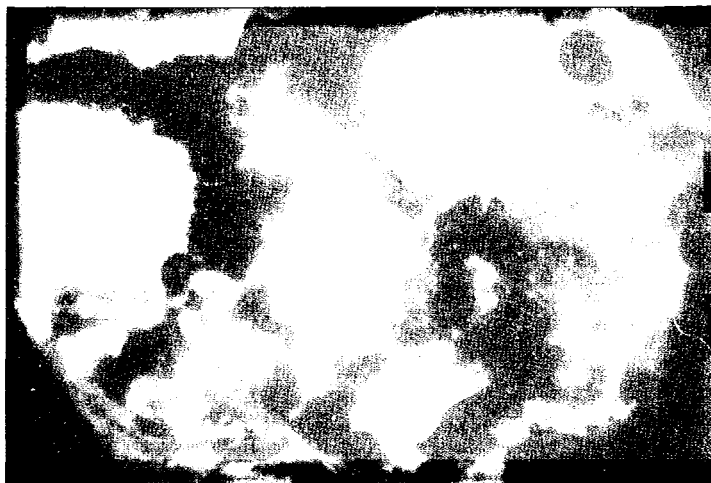


Fig. 2. Fluorescence *in situ* hybridization of biotin-labeled rDNA probe to metaphase chromosomes and nucleus of a 1R(1B) substitution line, *T. aestivum* cv. Burgas 2. One of the pair in rye rDNA is missing.

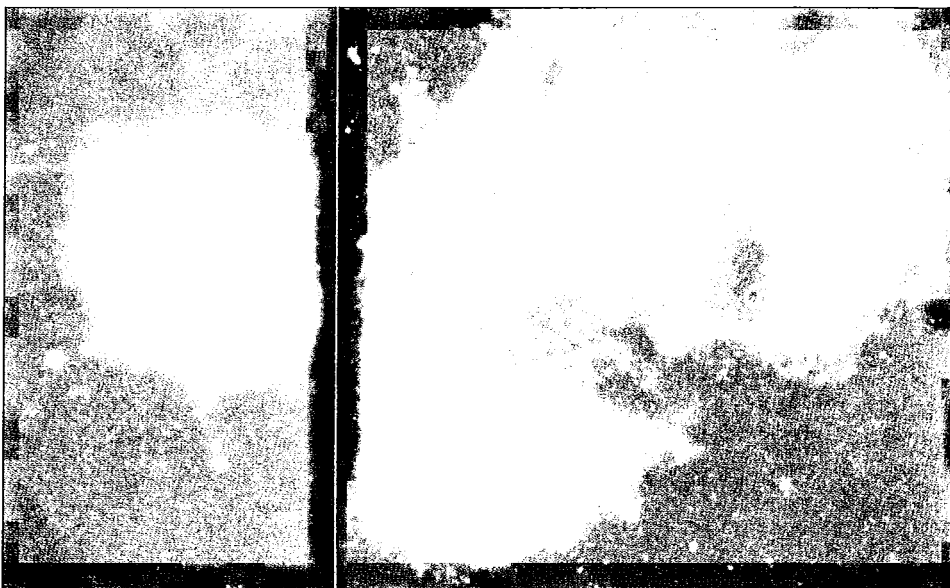


Fig. 3. Fluorescence hybridization of biotin-labeled pSC119 probe to nucleus (left) and metaphase chromosomes (right) of the F₁ hybrid between Chinese Spring wheat and Prolific rye.

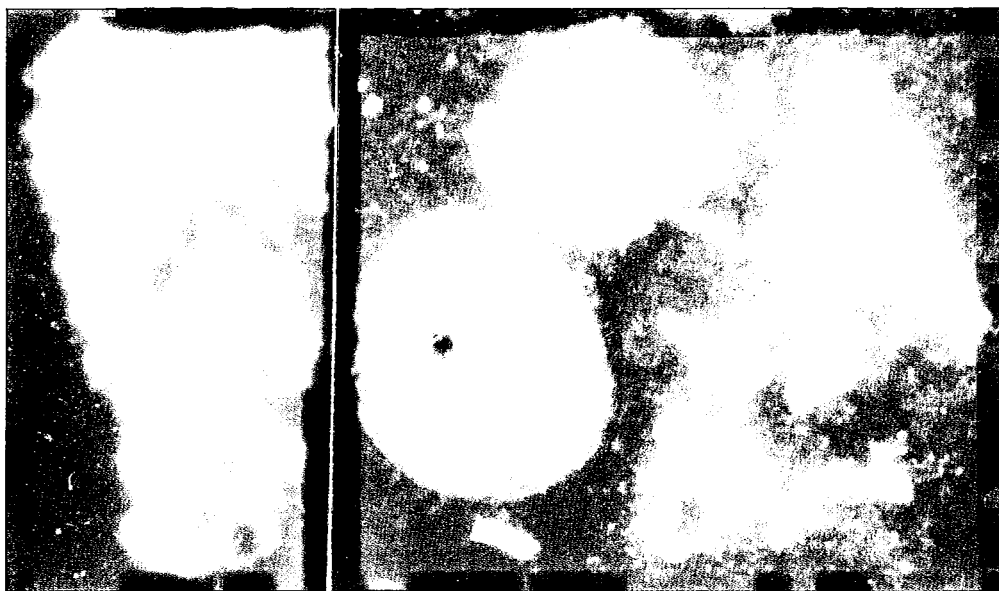


Fig. 4. Detection of rye chromosomes in a wheat-rye hybrid (Chinese Spring wheat x Prolific rye (left) and a 1R(1B) substitution line (Burgas 2) (right) by fluorescence *in situ* hybridization using high concentration probe of biotin-labeled pSC119.

Identification of wheat and rye chromosomes by *in situ* hybridization with repeated DNA sequences

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The proportion of repeated DNA sequences has been estimated at more than 80% of common wheat DNA. Using technique of *in situ* hybridization with biotin-labeled probe, repeated DNA sequences proved useful as cytological markers for chromosome identification. *In situ* hybridization using pSC119 probe containing repeated sequences (120-bp repeated family) from rye heterochromatin was conducted on rye, triticale, and wheat-rye substitutions to identify rye and/or wheat chromosomes.

Using high labeling DNA by random primer method and biotin-16-dUTP, high-resolutive *in situ* plates were obtained. Individual chromosomes of Prolific rye were distinguished from each other by *in situ* hybridization patterns to pSC119 (Fig. 1). The 120-bp family detected by pSC119 were clustered at 28 sites distributed over the seven chromosomes.

In a hexaploid triticale, Rosner six pairs of rye chromosomes were identified (Fig. 2). Rye chromosomes were readily distinguishable from the wheat chromosomes. In addition to dark brown labeling of heterochromatic regions, all chromosomes of rye gave light brown color indicating the dispersed nature of the repeated sequences, whereas those of wheat appeared blue.

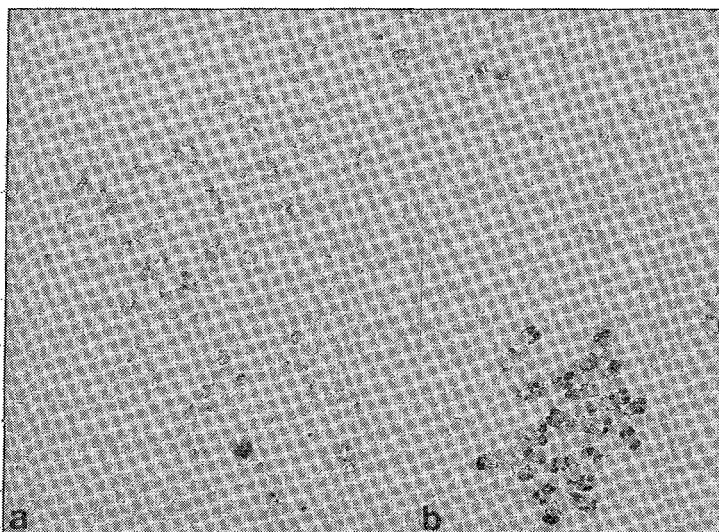


Fig. 1 *In situ* hybridization of biotin-labeled pSC119 probe to metaphase chromosomes of *Secale cereale* cv. Prolific: the concentration of probe in (a) is lower than that in (b).

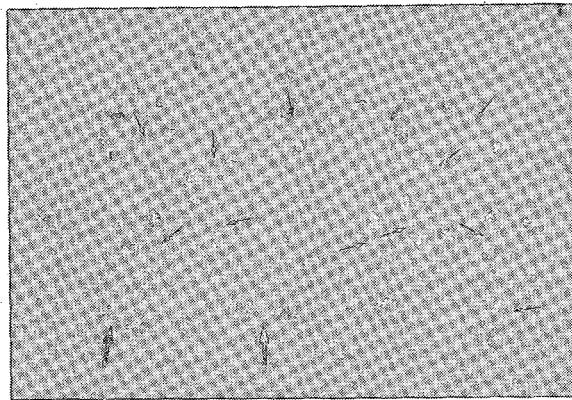


Fig. 2. *In situ* hybridization of biotin-labeled pSC119 probe to a metaphase cell of *Triticale* cv. Rosner. Small arrows indicate the rye chromosomes. Large arrows indicate chromosome 4B pair.

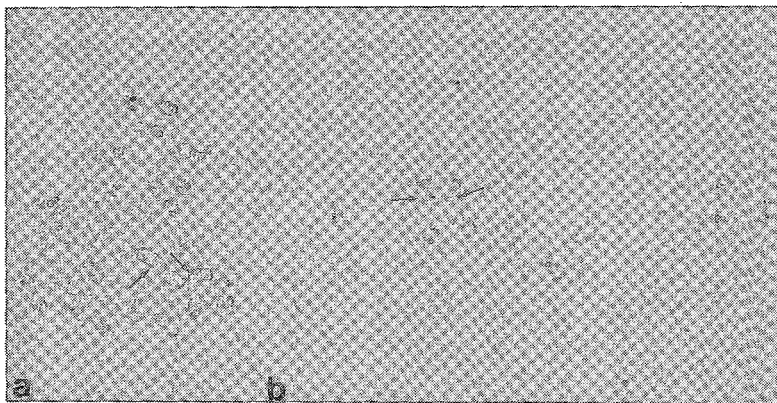


Fig. 3. *In situ* hybridization of biotin-labeled pSC119 probe to chromosomes of *Triticum aestivum* cv. Burgas 2 (1R(1B) substitution line): low (a) and high (b) stringency in hybridization. Arrows indicate chromosome 1R pair.

Rayburn and Gill (1985) reported that chromosome 4B (formerly 4A) of wheat had four major hybridization sites, one terminal and two intercalary on the long arm and one terminal on the short arm. They also suggested that chromosome 4B has been stable during the evolution of the polyploid wheats. In chromosome 4B of Rosner, however, an additional interstitial site on the short arm was observed.

In situ hybridization to biotin-labeled pSC119 in a 1R(1B) substitution line, Burgas 2 was shown in Fig. 3, in which the two rye chromosomes were easily identified. By modifying the amount of biotinylated probe or stringency in hybridization, the tone of color of rye chromosome could be controlled (Fig. 1, Fig. 3).

Visualization of the genetic activity of ribosomal RNA genes in wheat by *in situ* hybridization using biotinylated probe

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Ribosomal RNA genes (rDNA) are fundamental components of the protein synthetic machinery and are tandemly repeated in higher plants. They are mostly present in the secondary constrictions of satellited chromosomes. *In situ* hybridization studies established the location of the rDNA on chromosomes 1B, 6B and 5D of *Triticum aestivum* cv. Chinese Spring. In the present study, behavior of the rDNA during the wheat cell cycle was visualized using *in situ* hybridization with a biotinylated rDNA probe of wheat.

The *in situ* hybridization experiment showed that the rDNA sites were seen as brown color on the blue chromosomes at early prophase to metaphase stages (Fig. 1). Nucleoli were stained purple. In prophase, rDNA of chromosome 6B separated into two condensed parts, while that of 1B localized near its short arm only. Further, rDNA on chromosomes 1B and 6B was always accompanied with nucleolus. Ribosomal RNA genes being active were recognized as dispersed part within the nucleolus. We considered the condensed parts to be genetically inactive. These parts were probably methylated. The rDNA on chromosome 5D was seldom accompanied with nucleolus. Stretched chromosomes in early metaphase showed that most rDNA on chromosomes 1B and 6B were condensed on both the ends of secondary constriction.

In both interphase and early prophase nuclei of the F₁ hybrid between Chinese Spring wheat and Prolific rye, wheat rDNA was always accompanied with nucleolus, whereas rye rDNA was not, showing no activity of rye ribosomal gene (Fig. 2). Wheat rDNA on chromosomes 1B and 6B at metaphase was partially stretched. None of the rye rDNA was found stretched but remained condensed. Thus, in the presence of wheat NOR chromosomes 1B and 6B, the obvious suppression of rDNA on the chromosome 1R is evident, as the rye rDNA is no longer dispersed.

In all cases of other derivatives of wheat-rye hybrids, such as triticale, addition lines, substitution and translocation lines (Fig. 3), we found that rDNA on the chromosome 1R of rye was suppressed by the presence of wheat chromosomes. In conclusion, as also reported by Gustafson et al (1988), *in situ* hybridization using biotin-labeled probe can be effectively used to visualize genetic activity or gene expression at the rDNA sites with the help of light microscope.

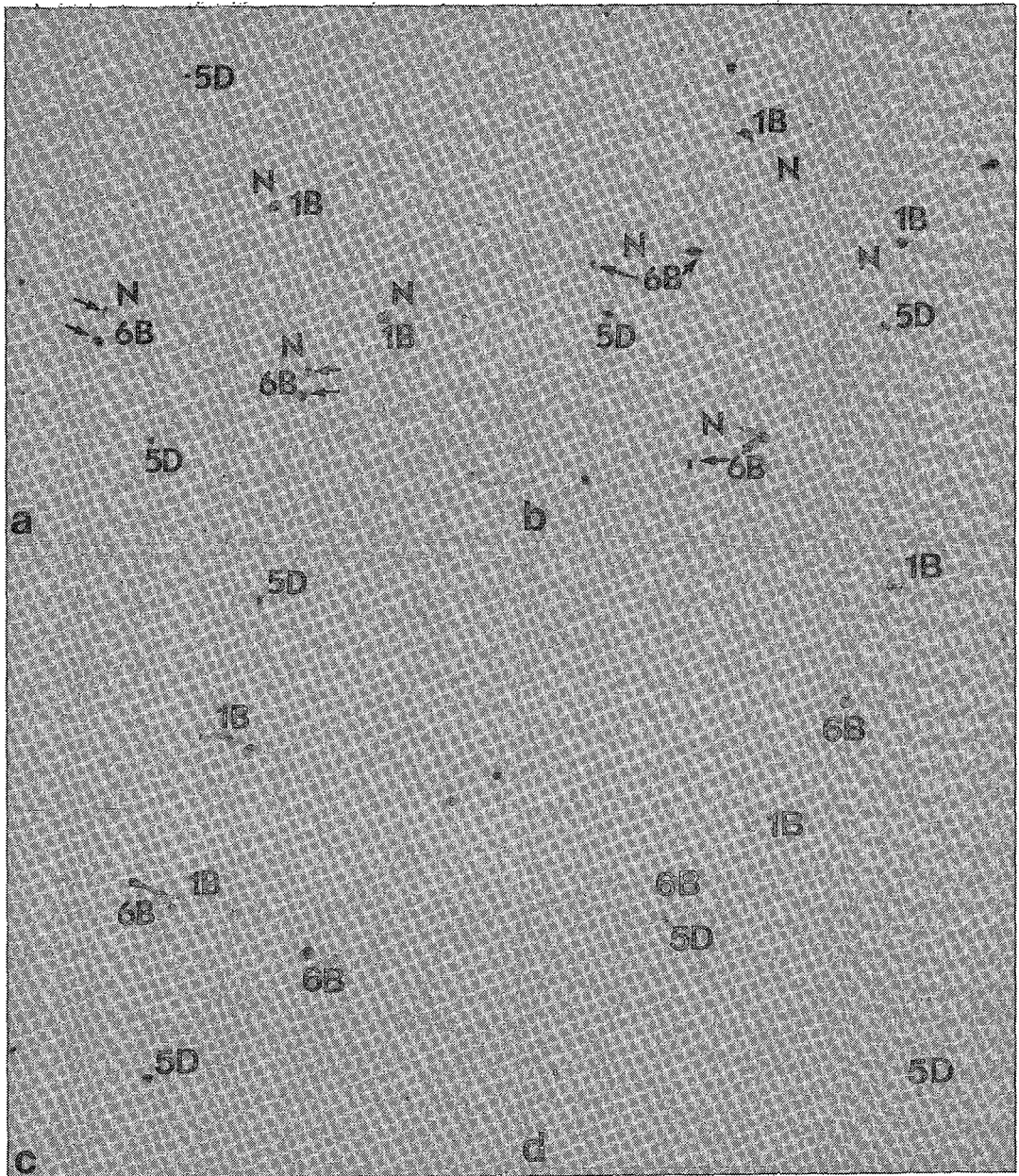
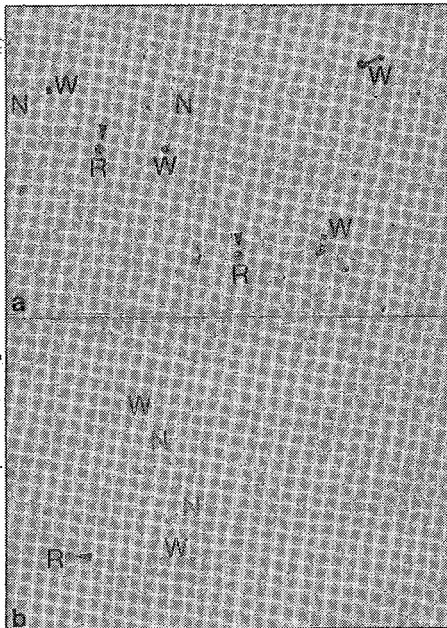


Fig. 1. The behavior of rDNA on prophase to metaphase chromosomes revealed by *in situ* hybridization of *Triticum aestivum* cv. Chinese Spring with biotin-labeled rDNA probe: (a) early prophase; (b) late prophase; (c) early metaphase; (d) mid-metaphase. N: nucleolus.

200-2 100 2000 1000 200



interphase nucleus and metaphase chromosomes (a) and prophase chromosomes (b) of the F₁ hybrid between Chinese Spring wheat and Prolific rye. W: wheat rDNA (1B or 6B). R: rye rDNA. N: nucleolus.

Fig. 2. *In situ* hybridization of biotin-labeled rDNA probe to interphase nucleus and metaphase chromosomes (a) and prophase chromosomes (b) of the F₁ hybrid between Chinese Spring wheat and Prolific rye. W: wheat rDNA (1B or 6B). R: rye rDNA. N: nucleolus.

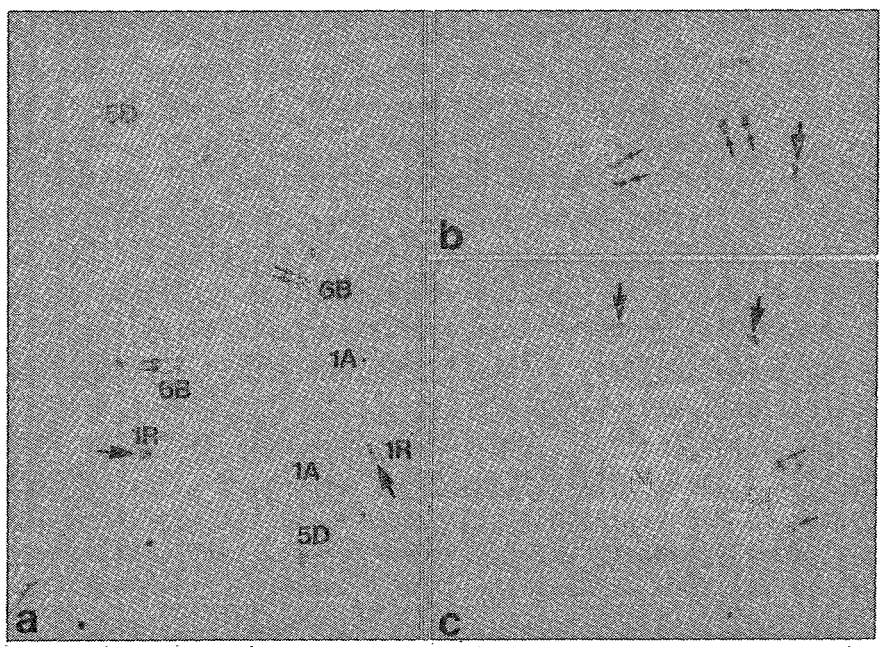


Fig. 3. *In situ* hybridization of a 1BL-1RS translocation line, *T. aestivum* strain Salmon using biotin-labeled rDNA probe: (a and b) metaphase; (c) prophase. N: nucleolus. The chromosome 6B is always marked by the condensed region of rDNA which is composed of two parts one each on both the ends of secondary constriction.

Development of reciprocal translocation homokaryotypic lines by X-rays irradiation of polyhaploid in wide cross hybrid

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X-rays irradiation is well known to be useful in inducing chromosomal translocation. In wheat breeding Sears (1956) and Knott (1968) used X-rays irradiation to translocate alien genes into wheat chromosomes. However in the case of X-rays irradiation of disomic plant the induced translocations are mostly heterozygous. Therefore a translocation homozygote must be selected from the selfed progeny of a heterokaryotype.

We propose the following procedures using polyhaploids for the development of reciprocal translocation homozygotes; (1) irradiating polyhaploid material seeds or calli with X-rays to induce translocations, (2) treating the irradiated polyhaploid materials with colchicine solution to obtain fertile plants. As a result, any chromosomal changes induced in the polyhaploid cells should be present in a state of homokaryotype due to chromosome doubling. As an example, the scheme of the development of reciprocal translocation homozygotes from wheat x rye polyhaploids is shown in Fig. 1.

An experiment was made on wheat-rye polyhaploid hybrids. The polyhaploid seeds between the common wheat variety Chinese Spring and inbred rye lines were irradiated with X-rays (15-40 kR) and then the plants from the irradiated seeds were treated with 0.05% colchicine solution at the 2-3 tiller stage in order to recover seed fertility. In the progenies (X2C2) of the fertile plants obtained chromosomal structural changes were cytologically identified by the modified method of acetocarmine-Giemsa staining (Nakata et al 1977) where the Wright solution was used instead of the Giemsa solution.

As the result, 44 translocated chromosomes were identified in 19 out of 65 X2C2 lines examined and were present as homokaryotype. 77.3% (34 chromosomes of 17 pairs) of 44 translocated chromosomes were the products of reciprocal translocations. Most of translocations were non-centric translocations, that is, most of translocated positions were located on interstitial regions of chromosome arms. 10 out of 17 pairs of reciprocal translocations occurred between chromosomes of rye and of wheat. However only two pairs (5B-5R and 6B-6R) were between homologous chromosomes. The result indicates that in the procedures using polyhaploid the reciprocal translocations occur independently from homoeologous group, and translocated chromosomes become homozygous in the fertile progeny obtained.

If in remote crossing a hybrid is scarcely obtained, reciprocal translocations can be induced in the calli initiated from particular organs (young spike segments, embryo and so on) of the polyhaploid hybrids. In our Laboratory the polyhaploid calli between three *Aegilops* species and rye were

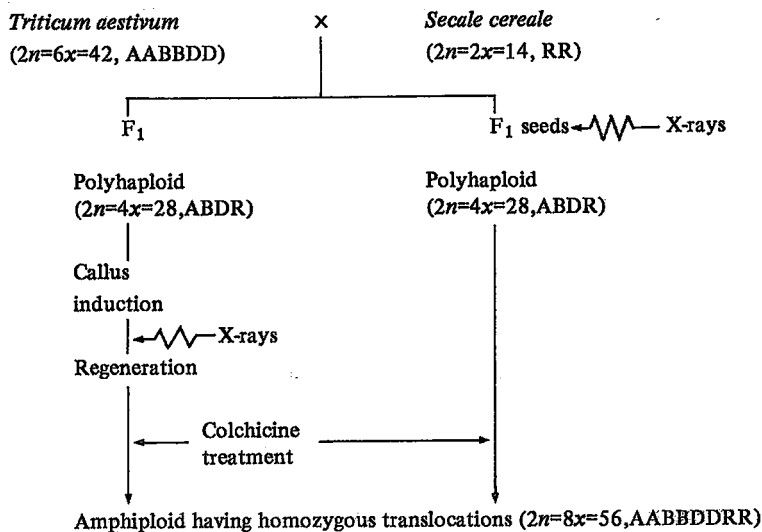


Fig. 1. Procedures for development of reciprocal translocation homozygote by X-rays irradiation of polyhaploid.

irradiated with X-rays of 625, 1250, 2500 and 5000 R. These calli were induced from young spikes of the respective hybrid. X-rays of 5000R inhibited the growth and regeneration of calli. However X-rays of other doses did not noticeably affect the growth and regeneration of the calli and induced chromosomal structural changes in the callus cells. And, a plant was obtained having reciprocal translocation between chromosomes of *Ae. umbellulata* and rye among regenerated plants from the irradiated calli.

Thus the procedures that combine polyhaploid as material, X-rays irradiation for induction of translocation and colchicine treatment for chromosome doubling are useful in developing a reciprocal translocation homozygote in wide cross hybrid.

Reference

- Knott, DR (1968) Translocations involving *Triticum* chromosomes and *Agropyron* chromosomes carrying rust resistance. *Can J Genet Cytol* 10: 695-696
- Nakata N, Yasumuro Y and Sasaki M (1977) An acetocarmine-Giemsa staining of rye chromosomes. *Jpn J Genet* 52: 315-318
- Sears ER (1956) The transfer of leaf-rust resistance from *Aegilops umbellulata* to wheat. in "Genetics in Plant Breeding", Brookhaven Symp Biol 2: 1-22

Production of wheat x barley hybrids and preferential elimination of barley chromosomes

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In order to introduce early heading and/or early maturing traits of barley, *Hordeum vulgare* L., into common wheat, *Triticum aestivum* L., we produced barley x wheat hybrids (Shimada et al 1987). However, pistillody and low female fertility were found in the backcrossed generations by wheat, probably due to interaction between nucleus and barley cytoplasm. Thus, we decided to produce wheat x barley hybrids, though the cross is reported to be more difficult than the barley x wheat cross (Fedak 1980; Islam et al 1981).

In the winter of 1987-1988, intergeneric crosses were carried out using four wheat cultivars and four barley cultivars (strains) (Table 1) in a greenhouse under 15-25°C and long day photoperiod conditions. Two days prior to pollination, emasculation and injection of 100mg/l 2,4-D to wheat stems were carried out.

Crossabilities (no. of embryos obtained/no. of florets pollinated) were different among the cross combinations between wheat and barley cultivars (Table 1). Among wheat cultivars, Norin 12, Norin 61 and Shinchunaga showed extremely higher crossabilities than that of Chinese Spring. Since Chinese Spring and Shinchunaga are known to have *kr1kr2* genes (Sasaki 1987), this result suggests that the crossability between wheat and barley is controlled by the gene(s) other than *kr*. Clear difference was also found among barley cultivars in the male side, i.e., Betzes barley showed the highest crossability to wheat. Thus, the cross Norin 12 x Betzes showed the highest crossability in this experiment and it was higher than those reported previously (Fedak 1980; Islam et al 1981).

The embryos were rescued by culture on N₆ medium supplemented with 0.5mg/l GA₃, 0.5mg/l IBA and 400mg/l Casein Hydrolysate at 14-20 days after pollination, and then 74 plants were obtained.

Chromosome number variation was observed in the root tip cells of the 60 seedlings of the hybrids. Among them, 28 seedlings had 28 chromosomes, as expected, comprising of 21 wheat and seven barley chromosomes, and eight seedlings had 21 chromosomes which were assumed to be haploids of wheat. The other 23 seedlings were aneuploid hybrids which had 22-27 chromosomes and one had 29 chromosomes. About half of the aneuploid hybrids showed mosaic chromosome number.

Meiosis in pollen mother cells of the hybrids showed lower chromosome pairing frequencies as compared with the case of barley x wheat hybrids reported before (Shimada et al 1987).

Table 1. Results of the cross, wheat × barley (no. embryos produced/no. florets pollinated and the percentages in parentheses).

Wheat cv. (female parent)	Barley cv. (male parent)				Total
	Betzes	Nyugoruden	Harunanijou	Kinai 5	
Chinese Spring	1/156 (0.64%)	2/225 (0.89%)	0/170	0/304	3/855 (0.35%)
Norin 12	17/206 (8.25%)	1/212 (0.47%)	1/204 (0.49%)	0/136	19/758 (2.51%)
Norin 61	18/298 (6.04%)	9/238 (3.78%)	3/378 (0.79%)	1/180 (0.56%)	31/1094 (2.83%)
Shinchunaga	10/200 (5.00%)	4/182 (2.20%)	10/656 (1.52%)	5/100 (5.00%)	29/1138 (2.55%)
Total	46/860 (5.35%)	16/857 (1.87%)	14/1408 (0.99%)	6/720 (0.83%)	82/3845 (2.13%)

Table 2. Patterns of presence of the isozyme bands which mark barley chromosomes in the hybrids of wheat × barley (+: present, -: absent).

Cross combination	No. of chromosomes	Barley chromosome (isozyme marker)				
		1(CP)	2(G6P)	4(PGM)	5(6PG)	6(GOT)
WB-36 Scn × Haruna	28	+	+	+	+	+
WB-63 Scn × Betzes	28	+	+	+	+	+
WB-25 CS × Betzes	27	-	+	+	+	+
WB-61 Scn × Betzes	27	+	+	+	+	+
WB-67 N61 × Betzes	27	+	+	+	+	-
WB-45 Scn × NG	26, 27	+	+	+	-	+
WB-78 Scn × Betzes	25, 27	-	+	+	-	+
WB-64 Scn × Betzes	26	-	-	+	+	+
WB-59 Scn × Betzes	24, 26	-	+	+	-	+
WB-74 N61 × Betzes	24, 26	-	+	+	-	+
WB-54 N12 × Betzes	24, 25	+	+	+	-	-
WB-77 N12 × Betzes	24	+	-	+	-	+
WB-66 N61 × Betzes	23	-	-	+	-	-
WB-72 N61 × Betzes	23	-	+	-	+	-
WB-50 N61 × Betzes	21, 22	-	-	-	-	-
WB-26 N12 × Betzes	21	-	-	-	-	-
WB-60 Scn × Betzes	21	-	-	-	-	-
WB-68 N61 × Betzes	21	-	-	-	-	-
WB-69 N61 × Betzes	21	-	-	-	-	-

Abbreviation: Scn; Shinchunaga, CS; Chinese Spring, N61; Norin 61, N12; Norin 12, Haruna; Harunanijou, NG; Nyugoruden, EP; Endopeptidase, G6P; Glucose-6-phosphate dehydrogenase, PGM; Phosphoglucosmutase, 6PG; 6-phosphogluconate dehydrogenase, GOT; Glutamate oxaloacetate transaminase.

In order to examine the chromosome constitution of the hybrid plants, isozyme analyses were carried out by using five isozyme markers of barley chromosomes which showed different isozyme bands from those of wheat (Table 2). Among 19 plants examined, two plants having 28 chromosomes were shown to be the hybrids having 21 wheat and seven barley chromosomes, and four plants having 21 chromosomes were the haploid plants of wheat. Preferential elimination of barley chromosomes were examined in the thirteen aneuploid hybrids. Barley chromosomes 1, 5 and 6 were eliminated in eight, eight and five plants, respectively, and chromosome 4 was eliminated in only two plants. From the report by Linde-Laursen and Bothmer (1988) and this experiment, it is assumed that chromosome 1, 5, 6 and 7 of cultivated barley are preferentially eliminated in the early stage of embryogenesis in the interspecific and intergeneric hybrids.

We are now producing wheat-barley chromosome addition lines by back crossing wheat to these hybrids.

Details of this experiment will be described elsewhere.

References

- Fedak G (1980) Production, morphology and meiosis of reciprocal barley-wheat hybrids. *Can J Genet Cytol* 22: 117-123
- Islam AKMR, Shepherd KW and Sparrow DHB (1981) Isolation and characterization of euplasmic wheat-barley chromosome addition lines. *Heredity* 46: 161-174
- Linde-Laursen I and Von Bothmer R (1988) Elimination and duplication of particular *Hordeum vulgare* chromosomes in aneuploid interspecific *Hordeum* hybrids. *Theor Appl Genet* 76: 897-908
- Sasaki M (1987) Triticale cytogenetics and breeding. *Japan J Breed* 37 (Suppl 1): 2-5
- Shimada T, Koba T, Otani M and Niizeki H (1987) Morphology, meiosis and *in vitro* propagation of barley-wheat hybrids. *Barley Genetics V (Proc 5th Int Barley Genet Symp)*: 343-350

A triticale breeding strategy drawn from genetic analysis of nucleo-cytoplasmic interaction

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The wheat cytoplasm is euplasmic for wheat chromosomes but alloplasmic for rye chromosomes of triticale (TC), the converse being the case in TC with rye cytoplasm (Sasaki et al 1983). Thus both the wheat and rye cytoplasm suppress the expression of nuclear genes of TC in the way of various kinds of interaction. We have accumulated different cases of the genetic interactions between nuclear genes and cytoplasm using eu- and alloplasmic lines of Chinese Spring (CS) ditelosomics, rye and TC. Those interactions can be classified as shown in Table 1.

Examples of the Genetic Interaction Types

I. NN-type interaction

- (1) Hybrid chlorosis between wheat and rye was controlled by complementary genes, *Hch1* and/or *Hch2*, on the 1BS and 1DS of CS, and *Hch3* on the 6R of inbred rye line IR14 (Sasaki and Yasumuro 1974, Nakata et al 1989).
- (2) Nucleolar organizer activity of the 6B of wheat was epistatic effect over the 1R of rye even in rye nuclei (Nakata et al 1986b).

Table 1. Classification of various nucleo-cytoplasmic interactions revealed in our experiments

Interaction type	Factor combination	Description
NN-type	$N_i \times N_j$	Interaction between wheat and rye nuclear genes.
NC-type	$N_i \times C_i$	Interaction between nucleus and cytoplasm of the same species.
	$N_i \times C_j$	Interaction between nucleus and cytoplasm of different species.
CNN-type	$C_i (N_i \times N_j)$	Double interaction between cytoplasm and NN-type interaction.
	$C_k (N_i \times N_j)$	C_k is the third cytoplasm.
NCN-type	$(N_i \times C_j) \times N^i$	Double interaction between NC-interaction and nuclear gene.

Where, N stands for nuclear gene, C for cytoplasmic genome, i, j, k stand for different species of N and C. N^i stands for non-allelic gene within the same nuclear genome.

II. NC-type interaction

(1) Ni × Ci: This type of interaction showed a strong affinity between nuclear and cytoplasmic genes of the same species.

(i) Midget chromosome originated from rye chromosome 1R was found in the CS nucleus with rye cytoplasm. The midget chromosome was necessary for the endosperm development of the wheat line with rye cytoplasm, i.e., a strong interaction was found between the gene on the 1R and rye cytoplasmic genome (Nakata et al 1986c).

(ii) The CS line with *Agropyron intermedium* cytoplasm had a chromosome of *intermedium* origin which had a recovering effect of the plant vigour (Tokiwa et al 1986).

(iii) Chromosome 2D in TC lines with *aestivum* cytoplasm and chromosome 2R in TC lines with *cereale* cytoplasm were responsible for the high seed fertility (Yasumuro et al 1987).

(2) Ni × Cj: This type of interaction showed deleterious effect contrary to Ni × Ci.

(i) The chiasma frequency of an inbred rye line with *aestivum* cytoplasm ((*aes*)-IR115) was lower than that of the line with rye cytoplasm ((*cer*)-IR115), though the cytoplasmic differences were not found in chiasma frequency in the other IR lines (Nakata et al 1986a).

III. CNN-type interaction

(1) Hybrid weakness between wheat and rye was caused by complementary gene action and this effect was inhibited by *speltoides* cytoplasm. Complementary genes *De1* on the 7BL of CS and *De2* of the inbred rye line, IR 129 (chromosome not identified) caused weak plant growth in the wheat-rye hybrid lines with wheat, rye or some *Aegilops* cytoplasm (NN-type interaction), however, the line with *speltoides* cytoplasm showed normal plant growth, that is, this cytoplasm had the inhibitor (*Ide*) to the nuclear genotype *De1-De2*-, recovering normal plant vigour (Yasumuro et al 1983; Sasaki et al 1986).

(2) Abnormal pollen tetrad formation was found at high frequency in the TC lines with *timophevi* cytoplasm in the cross-combinations of CS with IR46 or IR129 (Yasumuro et al 1985).

IV. NCN-type interaction

(1) Wheat endosperm glutenin genes on the 1BL and 1DS of CS were suppressed by a complementary effect between the 1AS of CS and cytoplasmic genomes of *aestivum* or *ovata* (Yasumuro et al 1985).

Discussion and Conclusion

An independent major effect of cytoplasm was not detected in our experiments. The NC-type interaction was the most frequent cases in which the Ni × Ci interactions showed a strong complementation between nuclear genes and cytoplasmic genome in the same species, on the other hand, Ni × Cj interactions showed a deleterious effect owing to inharmonious combination of nuclear gene with cytoplasm being different species origin.

These results indicate that the most suitable nuclear genes had been selected intensively under

the background of cytoplasmic genome of the species. Thus the interaction effect is so strong as inducing chromosomal structural changes in the 1R (the case II-(1)-(i)) and as establishing a new composite genome from D and R genomes through 2D-2R substitution (the case II-(1)-(iii)).

We can draw a conclusion for triticale breeding strategy that the elimination of deleterious interactions mainly caused by the Ni x Cj is essential to select the most suitable nuclear genotype under a given cytoplasm of TC, which lead to build up a harmonious combination of nuclear and cytoplasmic genomes. As for the choice of cytoplasm for TC, the wheat cytoplasm might be the best and the *caudata* could be next candidate in addition to rye (Sasaki et al 1986).

References

- Nakata, N, Muraoka S, Yasumuro Y and Sasaki M (1986a) Japan J Breed 36 (Supple 1): 122-123 (Japanese)
Nakata N, Yasumuro Y, Hamaoka H and Sasaki M (1986b) Japan J Breed 36 (Supple 2): 154-155 (Japanese)
Nakata N, Yasumuro Y and Sasaki M (1986c) WIS 63: 39-41
Nakata N, Haba Y, Yasumuro Y and Sasaki M (1989) Japan J Breed 38: (Supple 2): 328-329 (Japanese)
Sasaki M and Yasumuro Y (1974) Japan J Breed 24 (Supple 2): 104-105 (Japanese)
Sasaki M, Yasumuro Y and Nakata N (1983), 6th Int Wheat Genet Symp 1179-1184
Sasaki M, Muraoka S, Nakata N and Yasumuro Y (1986) Proc Int Triticale Symp 436-441
Sasaki M, Nakata N and Yasumuro Y (1986) Japan J Breed 36 (Supple 2): 152-153 (Japanese)
Tokiwa K, Yasumuro Y, Nakata N and Sasaki M (1986) Japan J Breed 36 (Supple 1): 328-329 (Japanese)
Yasumuro Y, Sasaki M and Nakata N (1983) Proc 6th Int Wheat Genet Symp 1145-1148
Yasumuro Y, Nakata N, Hirao A and Sasaki M (1985) J Fac Agr, Tottori Univ 20: 1-7
Yasumuro Y, Nakata N, Kawahito S and Sasaki M (1987) Japan J Breed 37: 397-404
Yasumuro Y, Nakata Y, Fukumoto A and Sasaki M (1988) Proc 7th Int Wheat Genet Symp 681-686

Microspore development during *in vitro* anther culture of wheat

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Haploid plants of wheat have been obtained by anther culture since 1973 (Ouyang et al 1973, Picard and Debuysse 1973). We have cultured the anthers of some Japanese wheat cultivars and found that the frequencies of anthers producing pollen embryoids varied with genotypes (Shimada and Otani 1988) and the yield of green plantlets from pollen embryoids were very low (Shimada and Otani 1989). In this experiment, the development of microspores during *in vitro* anther culture was observed.

Materials and Methods

Spikes of *Triticum aestivum* cv. Norin 61 were collected from greenhouse-grown plants and sterilized in 70% ethanol for 1 - 2 minutes. Anthers containing microspores in the late-uninucleate stage were inoculated on Potato-2 (Chung et al 1978) medium and cultured at 28°C in the dark. To observe the division occurring *in vitro* in the microspores, anthers were taken randomly from the cultures after inoculation. The anthers were placed on a glass slide in a few drops of acetocarmine and tapped softly through the coverglass.

Results and Discussion

Almost all microspores cultured at the stage of late-uninucleate started nuclear division within the first few days (Fig. 1a, 2). The first mitosis produced either identical nuclei by equal division of the microspore nucleus or a vegetative like and a generative like nuclei which were recognized by the different intensities of acetocarmine staining (Fig. 1b). Fig. 2 shows the development of microspores which indicates the relationship between culture period and the number of nuclei. After five to seven days of culture the microspores with four to five nuclei whose cell membrane was obscure were observed (Fig. 1c). Although the surviving microspores decreased drastically after a week, some of them with multinuclei developed into multicellular microspores (Fig. 1d). At 14 days, microspores with over 20 cells were observed, where the cell division occurred inside the exine of microspores (Fig. 1e). Some multicellular microspores broke the exine and grew into proembryoids after three weeks in culture (Fig. 1f).

About 30% of the anthers had more than 30 viable multicellular microspores (Fig. 1e). However, the frequencies of anthers with embryoids in Norin 61 was 10.7% and average number of embryoids in one anther was around two (Shimada and Otani 1988). Only a few percents of

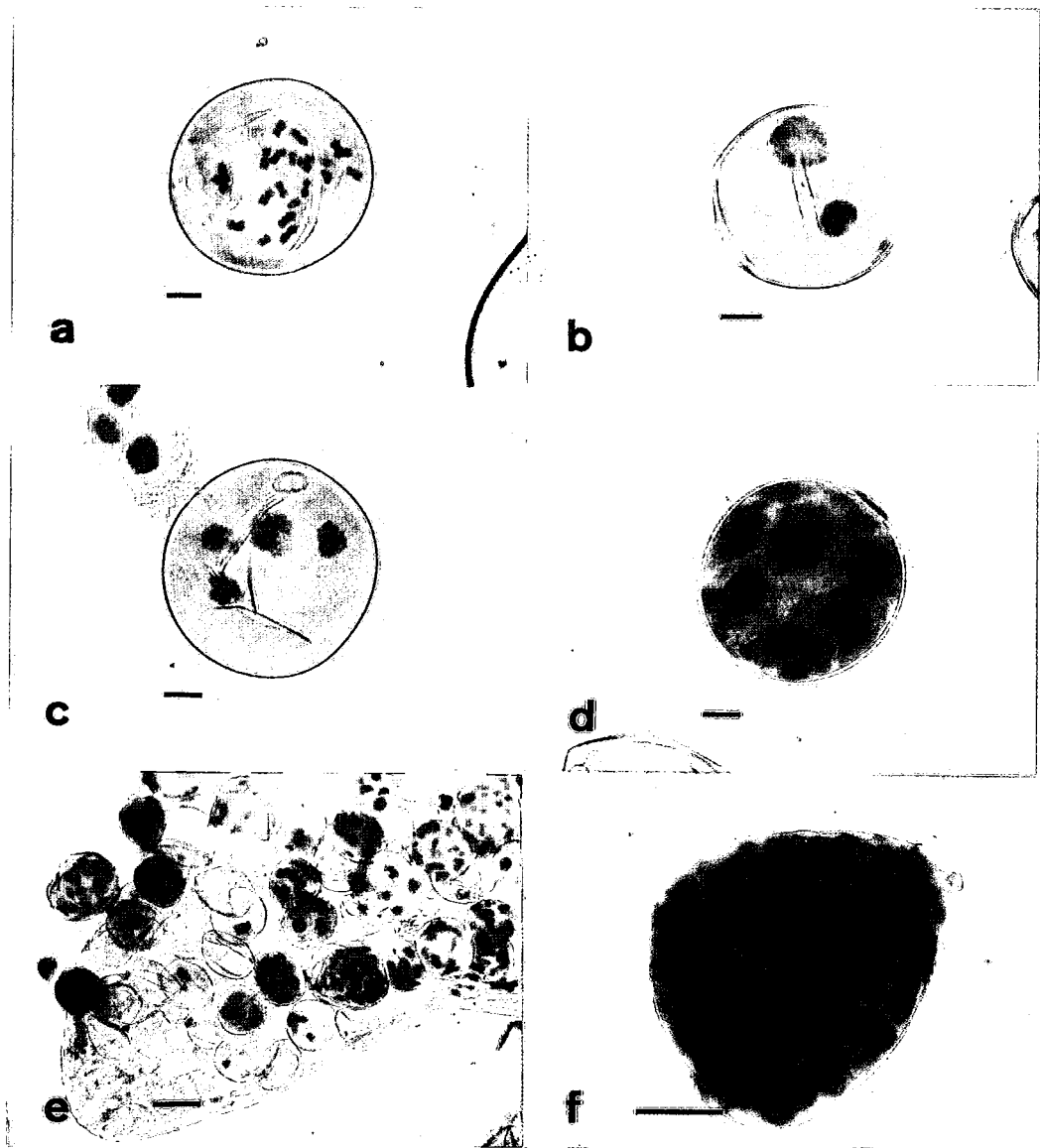


Fig. 1. Developing microspores during anther culture of Norin 61 on Potato-2 medium. Bar = 10μ (a, b, c and e), 50μ (f and g)

- a) First division in microspore on the first day.
- b) Microspore with two nuclei, a vegetative like and a generative like nucleus.
- c) Equal four nuclei are free in microspore.
- d) Multicellular microspore at a week of culture.
- e) Many multicellular microspores are observed in an anther at 2 weeks of culture.
- f) Proembryoid.

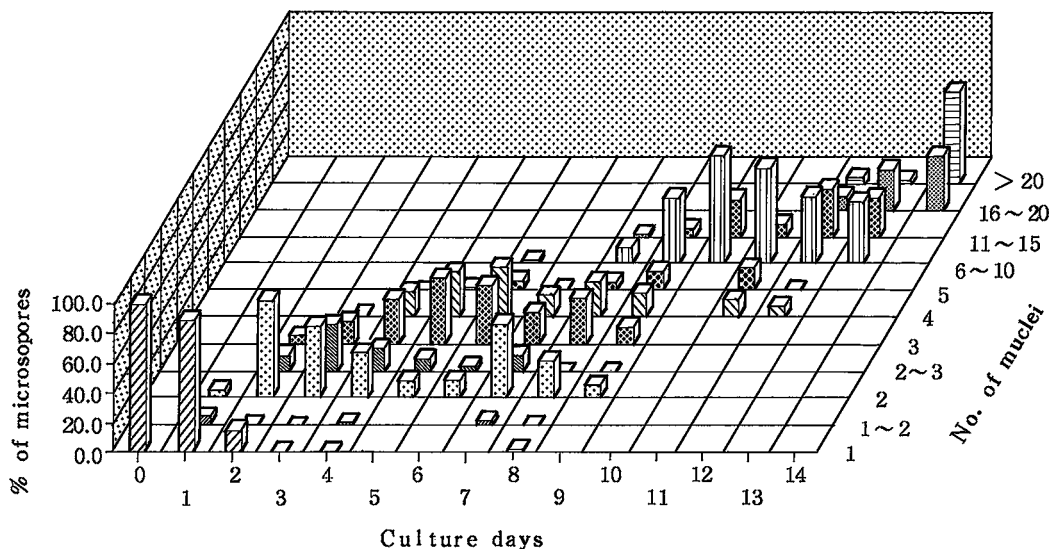


Fig. 2. Microspore development at day 0 and subsequent to culture initiation

multicellular microspores could develop into embryoids.

There are two steps in the development of microspores which are from the multinuclear to the multicellular stage, and from the multicellular stage to proembryoids. At both steps the frequencies of surviving microspores decreased drastically. More than 100 multicellular microspores which would be potential embryoids were observed in some anthers. The induction frequency of pollen plants could be increased to satisfy the requirements for breeding by further improvement of the satisfactory conditions in anther culture.

References

- Chuang CC, Ouyang JW, Chia A, Chou SM and Ching CK (1978) A set of potato media for wheat anther culture. In: Proc Symp Plant Tissue Culture, Science Press, Peking pp. 51-56
- Ouyang JW, Hu H, Chung CC and Tseng CC (1973) Induction of pollen plants from anthers of *Triticum aestivum* L. cultured in vitro, Sci Sinica 16: 79-95
- Picard E and Debuyser J (1973) Obtention de plntules haploides de *Triticum aestivum* L. a partir de cultures d'antheres in vitro. CR Acad Ser D 281: 127-130
- Pan JL, Gao GH and Dan H (1983) Initial types of pollen cells and their development in anther culture. In "Cell and tissue culture techniques for cereal improvement" Proc. Workshop cosponsored by the Institute of Genetics, Academia Sinica and the International Rice Research Institute, Science Press, Beijing pp. 117-129
- Shimada T and Otani M (1988) Efficiency of potato medium on induction of pollen embryoids in anther culture of Japanese wheat cultivars. Japan J Breed 38: 212-222
- Shimada T and Otani M (1989) Varietal differences in green plant regeneration ability of wheat pollen embryoids. Japan J Breed 39: 187-194

III. Gene Symbol

Catalogue of gene symbols for wheat. (1989 supplement)

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A complete update of the Catalogue was included in the Proceedings of the 7th International Wheat Genetics Symposium held at Cambridge, England, in July 1988. A limited number of reprints will be available.

As annual updates are made, changes and additions will be incorporated into the master files of the Catalogue. New references added during updates will be integrated into an alphabetical listing only periodically. However, references are held in a data base and can be organised alphabetically by author at any time.

This Catalogue is expected to be available in Microsoft Word for IBM and Apple Macintosh personal computers. Those with access to word processing programs can request disks which will be cheaper and faster to produce than hard copy. The manuscript is now approximately 120 pages.

The reference numbers used in this Supplement relate to the 1988 revision and not to earlier versions and supplements. As in past years, this supplement has been offered to the editors of Annual Wheat Newsletter, Cereal Research Communications and Wheat Information Service for inclusion in the respective journals.

Additions to Symbols List

<i>Ibf</i>	Iodine binding factor
<i>Mal</i>	Malic enzyme
<i>Wx</i>	Waxy
<i>Cxp</i>	Carboxypeptidase
<i>Pepc</i>	Phosphoenolpyruvate carboxylase
<i>Ss</i>	Sucrose synthase
<i>Su</i>	Substituted urea insensitivity
<i>X</i>	Prefix for all DNA markers

DNA Markers

The following list is an initial attempt to catalogue DNA loci in wheat. At present, the criteria for inclusion is the detection, by Southern hybridization, of restriction fragments which have been located to specific chromosomes in wheat. The nomenclature used is that agreed at the 7th IWGS at Cambridge, 1988 (Hart and Gale, Proc. 7IWGS, pp. 1215-1218).

The present list does not include the 5S-rRNA or the 18S-5.8S-26S rRNA (*Nor*) loci, which are included elsewhere in the catalogue. Also, no attempt has been made to list homoeoloci in related species although many have been noted. eg., 932, 934.

Locus Symbol	Identifying Probe
Group 1S	
<i>XGh-1A, B, D</i> [225].	pTag 544.
<i>Xpsr161-1A, B, D</i> (932).	PSR161.
<i>Nor-B1</i> (see Nucleolus Organiser Regions).	
<i>5S-Rna</i> (see Ribosomal RNA/5SRNA).	
Group 1L	
<i>XAdh-1A, B, D</i> [<i>Adh-A, B, D2</i> (936)].	p3NTR.
<i>XGlu-A, B, D1-1</i> [773, 226].	pTag1290(773).
<i>XGlu-A, B, D1-2</i> [773, 226].	pTag1290(773).
<i>Xpsr162-1A, B, D</i> (932).	PSR162.
Group 2S	
<i>Xpsr135-2A, B, D</i> (932).	PSR135.
<i>Xpsr108-2A, B, D</i> (937).	PSR108.
<i>Xpsr109-2A, B, D</i> (937).	PSR109.
<i>Xpsr122-2A, B, D</i> (937).	PSR122.
<i>Xpsr126-2A, B, D</i> (937).	PSR126.
<i>Xpsr130-2A, B, D</i> (937).	PSR130.
<i>Xpsr131-2A, B, D</i> (937).	PSR131.
<i>Xβ-Amy-2A, B, D</i> [<i>PSR1-2A, B, D</i> (934)]	pcβC51 (935).
Group 2L	
<i>Xpsr101-2A, B, D</i> (932).	PSR101.
<i>Xpsr102-2A, B, D</i> (937).	PSR102.
<i>Xpsr112-2A, B, D</i> (937).	PSR112.
Group 3S	
<i>Xpsr123-3A, B, D</i> (932).	PSR123.

Group 3L

Xpsr156-3A, B, D (932). PSR156.

Group 4S (Pre-7IWGS nomenclature)(4AS=4Aa:4BL:4DS)

XNra-4B (933, 919). bNRp10(918).

XWx-4B (933, 919). pcwx27(907).

Xpsr144-4A, B, D (932). PSR144.

Xpsr160-4B (933). PSR160.

Xpsr119-4B (773). PSR119.

Group 4L (4Aβ:4BS:4DL)

Xpsr163-4A,B,D (932). PSR163.

Xβ-Amy-A, D1. [PSR1-4A, D (934)]. pcβC51(935).

Group 5S

Xα-Amy-5A,B,D. [αAmy3 (50)]. λAmy33.

Xpsr118-5A,B,D (932). PSR118.

Group 5L

Xβ-Amy-B1. [PSR1-5A (934)]. pcβC51 (935).

Xspr128-5A,B,D (932). PSR128.

Group 6S

Xpsr167-6A,B,D (932). PSR167.

XNra-6A,B,D. [XNar-6A,B,D(917)]. bNRp10.

Nor-B2 (see Nucleous Organizer Regions).

XCxp-6A,B,D [916]. 2437(912).

XGli-6A,B,D [225]. pTag53.

Group 6L

Xα-Amy-6A,B,D. [αAmy1(915)]. 2119.

Xpsr154-6A,B,D (932). PSR154.

Group 7S

Xpsr160-7A,D (933, 919). PSR160.

Xpsr119-7A,D (919). PSR119.

Xpsr108-7A,B,D (933, 919). PSR108.

Xpsr103-7A,B,D (933,919). PSR103.

Xpsr150-7A,B,D (933,919). PSR150.

Xpsr152-7A,B,D (932,933,919). PSR152.

Xpsr65-7A,B,D (933,919). PSR65.

XNra-7A,D (919). bNRp10 (918).

<i>XWx-7A, D</i> (919).	pcwx27 (907).
<i>XSs1-7A,B,D</i> . [<i>Ss1</i> (914)].	pST8.
<i>XSs2-7A,D</i> . [<i>Ss2</i> (914)].	pST3.

Group 7L

<i>Xpsr129-7A,B,D</i> (932, 933, 919).	PSR129.
<i>Xpsr105-7A, B,D</i> (933, 919).	PSR105.
<i>Xpsr165-7A,B,D</i> (933, 919).	PSR165.
<i>Xpsr169-7A,B,D</i> (933, 919).	PSR169.
<i>Xpsr72-7A, B, D</i> (933, 919).	PSR72.
<i>Xpsr117-7A,B,D</i> (933, 919).	PSR117.
<i>Xpsr129-7A,B,D</i> (933, 919).	PSR129.
<i>Xpsr121-7A,B,D</i> (933, 919).	PSR121.
<i>Xa-Amy-7A,B,D</i> (915,933,919). [<i>α-Amy2</i> (915)].	4868(912), <i>Amy2/46</i> (913).
<i>XPepc-7A,B,D</i> (933,919).	SORCO(911).

Glume Colour

Rg1 v : Highbury (1010).

Height

Rht1 v : Guardian (1029); Frontier (1029).

Temporary Designation: *Rht1S* (1017). v : Saitama 27 (1017). Occurs frequently in Italian and Yugoslavian wheats (1017): Argelato, Centauro, Chiarano, Etruria, Farnesse, Gallo, Gemini, Lario, Pandas, Produttore, Orlandi, Orso, Salvia, Sprint, Strampelli.

Rht1 and *Rht2* Genotype list (1030).

Rht8 v : Novasadska Rana 1 (1028).

Herbicide Response

1. Difenzoquat Insensitivity

dfq1. 2BL (1032) s : CS*7/Marquis 2B (1032).

3. Chlortoluron Insensitivity

Insensitivity/resistance is dominant. (1033).

Su1 (1033). 6B (1033). s : CS/Cappelle Deprez 6B

v : Cappelle-Deprez.

su1.

v : Chinese Spring; Poros.

su1 also controls insensitivity to metoxuron (1033).

Hybrid Weakness

1. Progressive Necrosis

Replace final paragraph: Rye line 1R136-2 carries *Ner1* (1016) that complements *Ne2* (650, 1016) and *Ner2* (1016) to produce necrosis. Rye lines L155 and L256 carry *Ner2* (1016) that complements *Ne1* (319, 1016) and *Ner1* (1016).

Nucleolus Organizer Regions

Nor-V1 (901). 1V (901). ad : CS/*D. villosum*.

Proteins

2. Enzymes

1. Acid phosphatase

AcpH-S^S1 (900). 4S^S(900). ad : CS/*T. searsii*.

III. Aminopeptidase

Amp-Ag^e1 (902). 6Ag^e(902). ad,su : Rescue/*Ag. elongatum*.

IV. α -Amylase

Delete last paragraph and replace with:

A further set of α -amylase genes, *X α -Amy-5* [*α -Amy3*], have been identified in 5A, 5B and 5D by cross-hybridization with *α -Amy-1* and *α -Amy-2* probes (50).

V. β -Amylase

Last paragraph, first sentence: . . . 2DS and designated the *X β -Amy-2* [*β -Amy-2* (682)] set.

VI. Endopeptidase

Ep-S^S1 (900). 7S^S (900). ad : CS/*T. searsii*.

VII. Esterase

Est-R5. [EstA].

A second gene encoding grain esterases, designated EstB, was located in 4RL in King II and Petkus and in 7RS in Imperial (903).

Est-R^m5 (8). [EstB (903)]. 6R^m (8), 6R^mL (903). ad : CS/*S. montanum*.

VIII. Glucosephosphate isomerase

Gpi-S^S1 (900). 1S^S (900). ad : CS/*T. searsii*.

Gpi-V1. IV (570,901).

IX. Glutamate oxaloacetate transminase

<i>Got-Ag^e2</i> (902).	6Ag ^e (902).	ad,su : Rescue/ <i>Ag. elongatum</i> .
<i>Got-S^s3</i> (900).	3S ^s (900).	ad : CS/ <i>T. searsii</i> .
<i>Got-V3</i> (905).	3VL (905).	tv, ad : CS/ <i>D. villosum</i> .
<i>Got-R4</i> . [<i>Got1/7R</i> (906)].	7RL (906).	al : <i>S. cereale</i> .

XI. Lipoxygenase

<i>Lpx-H1</i> (904).	4H (904).	ad : CS/Betzes.
<i>Lpx-H2</i> (904).	5H (904).	ad : CS/Betzes.
<i>Lpx-S^s2</i> (900).	5S ^s (900).	ad : CS/ <i>T. searsii</i> .

XII. Malate dehydrogenase

<i>Mdh-S^s1</i> (900).	1S ^s (900).	ad : CS/ <i>T. searsii</i> .
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A third set of dimeric MDH-3 isozymes identified in mature grain, are separable from MDH-1 and MDH-2 by their higher pI's in IEF (929).

<i>Mdh-A3</i> (929).	5AS.	v : CS.
<i>Mdh-A3a</i> (929).		v : CS.
<i>Mdh-A3b</i> (930).		v : Bersee.
<i>Mdh-B3</i> (929).	5BS.	v : CS.
<i>Mdh-B3a</i> (929).		v : CS.
<i>Mdh-B3B</i> (929).		v : Hope.
<i>Mdh-D3</i> (929).	5DS.	v : CS.
<i>Mdh-D3a</i> (929).		v : CS.
<i>Mdh-D3b</i> (929).		v : Sears' Synthetic.
<i>Mdh-E3</i> (929).	5ES.	ad : CS/ <i>Ag. elongatum</i> .
<i>Mdh-H3</i> (930).	5H.	ad : CS/Betzes.
<i>Mdh-U3</i> (930).	5U.	ad : CS/ <i>T. umbellulatum</i> .

XIII. Peroxidase

<i>Per-V1</i> (901).	1V (901).	tv, ad : Cresco/ <i>D. villosum</i> .
<i>Per-H2</i> . [<i>Per-5</i> (908)]		

XIV. Phosphodiesterase

<i>Pde-V1</i> (905).	3VS (905).	ad : CS/ <i>D. villosum</i> .
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XVII. Shikimate dehydrogenase

<i>Skdh-S^s1</i> (900).	5S ^s (900).	ad : CS/ <i>T. searsii</i> .
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XVIII. Superoxide dismutase

<i>Sod-H1</i> (904).	2H (904).	ad : CS/Betzes.
<i>Sod-S^s1</i> (900).	2S ^s (900).	ad : CS/ <i>T. searsii</i> .

XX. Aromatic alcohol dehydrogenase

<i>Aadh-Ag^e2</i> (902).	6Ag ^e (902).	ad,su : Rescue/ <i>Ag. elongatum</i> .
<i>Aadh-V2</i> (901).	6V (901).	ad : CS/ <i>D. villosum</i> .

XXI. Aconitase

<i>Aco-Ag^e1</i> (902).	6Ag ^e (902).	ad,su : Rescue/ <i>Ag. elongatum</i> .
<i>Aco-S^s1</i> (900).	6S ^s (900).	ad : CS/ <i>T. searsii</i> .
<i>Aco-S^s2</i> (900).	5S ^s (900).	ad : CS/ <i>T. searsii</i> .

XXII. NADH dehydrogenase

<i>Ndh-V1</i> (901).	4V (901).	ad : CS/ <i>D. villosum</i> .
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XXIV. Malic enzyme

A dimeric enzyme extractable from mature grains.

<i>Mal-A1</i> (929).	3AL.	v : CS.
<i>Mal-B1</i> (929).	3BL.	v : CS.
<i>Mal-B1a</i> (929).		v : CS.
<i>Mal-B1b</i> (929).		s : CS/ <i>T. spelta</i> 3B.
		v : <i>T. spelta</i> PBI line 1.
<i>Mal-B1c</i> (929).		s : CS/Synthetic 3B.
		v : Sears' Synthetic.
<i>Mal-D1</i> (929).	3DL.	v : CS.
<i>Mal-E1</i> (929).	3E.	ad : CS/ <i>Ag. elongatum</i> .
<i>Mal-H1</i> (929).	3H.	ad : CS/Betzes.
<i>Mal-R1</i> (929).	3R.	ad : CS/Imperial.

3. Endosperm Storage Proteins

I. Glutenins

After preamble: An attempt will be made to prepare and include a list cross-referencing the *Glu-1* alleles, glutenin and sub-unit numbers, and *Glu-1-1* and *Glu-1-2* alleles.

Glu-A1.

<i>Glu-A1h</i> .	tv: ... USSR, <i>T. dicoccum</i> .
<i>Glu-A1i</i> .	tv: ... India, <i>T. dicoccum</i> .
<i>Glu-A1j</i> .	tv: ... Germany, <i>T. dicoccum</i> ; Lambro (924).
<i>Glu-A1l</i> (924).	tv: ... Chinook Canada, <i>T. durum</i> .
<i>Glu-A1m</i> . [<i>Glu-A1-IV</i> (926)].	tv: ... Corado, Portugal, <i>T. durum</i> .

Glu-A1n. [*Glu-A1-V* (926)]. tv: ... PI 61189, USSR, *T. durum*.

There is a possibility that *Glu-A1* alleles *i*, *j* (803) and *k* (220) may correspond to alleles *d*, *e*, *f* or *g* (808) which were published shortly earlier.

Glu-B1

Glu-B1m. tv: ... Iran, *T. dicoccum*.
Blu-B1n. tv: ... Germany, 1 *T. dicoccum*.
Blu-B1o. tv: ... Ethiopia, *T. dicoccum*.
Glu-B1p. tv: ... Ethiopia, *T. dicoccum*; Dritto (925).
Glu-B1q. tv: ... Morocco, *T. dicoccum*.
Glu-B1r. tv: ... Bulgaria, *T. dicoccum* Lambro (924).
Glu-B1t. [*Glu-B1-VII* (926)]. tv: ... Canoco de Grao Escuso, Portugal, *T. turgidum*.
Glu-B1u. [*Glu-B1-VIII* (926)]. tv: ... Tremez Mollez, Portugal, *T. durum*.
Glu-B1v. [*Glu-B1-IX* (925)]. tv: ... Roccia, Italy, *T. durum*.
Glu-B1w. [*Glu-B1-X* (925)]. tv: ... Quaduró, Italy, *T. durum*.
Glu-B1x. [*Glu-B1-XI* (925)]. tv: ... Athena, Italy, *T. durum*.

Glu-3

In preamble replace 701 with 698.

Add below *Glu-3* list: Multiple alleles at each of the three *Glu-3* loci were observed (921) and effects of allelic differences on bread making quality noted (922).

II. Gliadins

Gli-1

Add to text following listing for *Gli-D1*: Three alleles at each of the *Gli-1-1* (omega gliadings) loci were noted (698). The complexity of the *Gli-1* compound loci is further emphasized by reports of individual genes being separable by recombination, where *Gid-1A* (a block of gamma and omega genes) is separable by 0.3% from *Gid-4-1A* (omega gliadins) which is in turn separable by 1.5% from *Gid-3-1A* (omega gliadins) (550).

III. Other endosperm storage proteins

Triticin proteins (920). [Triplet proteins (702)].

Replace entire section:

Storage globulins with homology to pea legumins and related proteins in oats, rice and several dicotyledonous species (920).

Tri-A1 [702] (698). 1AS (702). v : CS.
Tri-A1a. [cs(698)]. v : CS.
Tri-A1b. [h(698)]. v : Hope.

<i>Tri-D1</i> [702] (361, 698).	1DS (702).	v : CS.
<i>Tri-D1a</i> . [cs (698)].		v : CS.
<i>Tri-D1b</i> . [i (698)].		v : India 115.

4. Protease inhibition

At the end of the paragraph following the listing for *Isal* add: Also, genes encoding inhibitors of insect α -amylases were located in *H. chilense* chromosomes 4H^{ch} and 7^{ch} (910).

III. Iodine binding factor

A monomeric water soluble protein from mature grain which preferentially binds iodine (928).

<i>Ibf-A1</i>	5AL (928).	v : CS.
<i>Ibf-A1a</i> (928).		v : CS.
<i>Ibf-A1b</i> (928).		v : Cappelle-Desprez.
<i>Ibf-A1c</i> (928).		v : Hope.
<i>Ibf-A1d</i> (928).		v : Chris.
<i>Ibf-A1e</i> (928).		v : Sears' Synthetic.
<i>Ibf-B1</i> (928).	5BL (928).	v : CS.
<i>Ibf-B1a</i> (928).		v : CS.
<i>Ibf-B1b</i> (928).		v : Cappelle-Desprez.
<i>Ibf-B1c</i> (928).		v : Ciano 67.
<i>Ibf-B1d</i> (928).		v : Sears' Synthetic.
<i>Ibf-D1</i> (928).	5DL (928).	v : CS.
<i>Ibf-D1a</i> (928).		v : CS.
<i>Ibf-D1b</i> (928).		v : Cappelle-Desprez.
<i>Ibf-D1c</i> (928).		v : Purple Pericarp.
<i>Ibf-D1d</i> (928).		v : Sears' Synthetic.
<i>Ibf-Agⁱ1</i> (928).	5Ag ⁱ (928).	ad : Vilmorin/ <i>Ag. intermedium</i> .
<i>Ibf-E1</i> (928).	5EL (928).	ad : CS/ <i>Ag. elongatum</i> .
<i>Ibf-R1</i> (928).	5RL (928).	ad : CS/Imperial, CS/KingII.
<i>Ibf-S¹1</i> (928).	5S ¹ . (928).	ad : CS/ <i>Ae. sharonensis</i> .
<i>Ibf-U1</i> (928).	5U (928).	ad : CS/ <i>Ae. umbellulata</i> .
<i>Ibf-H1</i> (928).	4H (928).	ad : CS/Betzes.
<i>Ibf-S¹1</i> (928).	4S ¹ (928).	ad : CS/ <i>Ae. sharonensis</i> .

Reaction to *Erysiphe graminis*

- Pm1.* v : Sappo *Pm2 Pm4b* (Carries *Lr20* (1021)).
Pm2. v : Sappo *Pm1 Pm4b* (1002); Halle stamm 13471 *M1d* (1002).
Pm3a. v : Hadden (1002).
Pm4a. tv : Valgerado (1002).
Pm4b. v : VPM1 (1002). Sappo *Pm1 Pm2* (1002).
Pm5. v : Redcoat (1002).
Pm6. v : Abe (1002); Arthur (1002); Mengavi (1002).
Pm8. v : Halle Stamm 1444 (1002).

A single gene transferred from Insave FA rye to Amigo wheat (1003) is apparently not *Pm8* (1002).

- Pm13* 3B (1006), 3D (1006). v : *T. longissimum* derivatives (1007).
al : *T. longissimum* (1007).
M1d. 4B (1002). v : Maris Dove (1002).
Halle Stamm 13471 *Pm2* (1002).

Reaction to *Mayetiola destructor*

- H3* v : Becker (1024); Cardinal (1025); 1GR855 (1026).
H18 v : Shield (1027).

Reaction to *Pseudocercospora herpotrichoides*

- Pch.* 7D (1008); 7DL (1009). s : Hobbit Sib*/VPM1 7D (1008).
v : Rendevouz (1009). Roazon (1008).

Reaction to *Puccinia graminis*

- Sr6* v : Shield (1027).
Sr24 v : Collin (1019).
Sr27 v : Widespread in triticales (478, 1022).
Sr30 v : Various unnamed accessions (1014, 1015).

Reaction to *Puccinia recondita*

- Lr2a* v : Shield *Lr3 Lr10* (1027).
Lr3 v : Shield *Lr2a Lr10* (1027). See also (1020).
Lr10 v : Shield *Lr2a Lr3* (1027).
Lr20 v : Sicco (1021). *Lr20* in Sicco appears to differ from that in Sappo, Timmo and Maris Halberd (1021).
Lr26 v : See also (1021).
Temporary designation: *LrW* (1005). v : V618 *Lr33* (1005). V336 *Lr33 LrB* (1005).

Reaction to *Puccinia striiformis*

<i>Yr1</i>	v: Regina <i>Yr2</i> (1001), Odra (1001).
<i>Yr2</i>	v: Hana (1001); Zdar <i>Yr4a</i> (1001); Slavia (1001); Viginta <i>Yr3a, Yr4a</i> (1001)
<i>Yr3a</i>	v: Zdar <i>Yr4a</i> (1001); Viginta <i>Yr2 Yr4a</i> (1001).
<i>Yr4a</i>	v: Zdar <i>Yr3c</i> (1001); Viginta <i>Yr2 Yr3a</i> (1001).
<i>Yr16</i>	v: Bersee (1028).

Temporary Designation: *YrA*. Refers to a phenotype specificity that appears to be controlled by complementary genes (1018). v: Avocet*; Anza = Karamu = Mexicani = T4 = WW15; Banks;* Condor, Cocamba; Egret*; Inia 66; Lerma Rojo 64; Lerma Rojo 64A; Nainari 60; Nuri 70; Sandal 73; Sonalika; Zaminder 80. Condor selection P44 *Yr6**; Cajime 71 *Yr6*; Pari 73 *Yr6*; Saric 70 *Yr6*; Yecora 70 *Yr6* (1018).

Reaction to *Tilletia* spp.

<i>Bt1</i>	v: Tyee <i>Bt4</i> (1023).
<i>Bt4</i>	v: Tyee <i>Bt1</i> (1023).

Resistance to Colonization by *Eriophyes tulipae*

Cmc2 (1022). 6D (902). v: Rescue 6DL, 6AgS (902). su : Cadet 6Ag (6A); Cadet 6Ag (6D) (1013). Rescue 6Ag (6A), 6Ag (6B), 6Ag (6D) (1013).
ad: Cadet + mono-6Ag (1013); Rescue + 6Ag (1013).

Genetic Linkages

Chromosome

1AS	<i>Gli-A1/Gli-A3</i>	—	<i>Tri-A1</i>	55.2 ± 8.2 cM	698
			<i>Tri-A1</i> — cent	11.2 ± 2.0 cM	698
1AL	cent	—	<i>Glu-A1</i>	33.9 ± 5.4 cM	909
				10.2 ± 3.5 cM	909
1BS	<i>Yr10</i>	—	<i>Gli-B1</i>	5.0 ± 2.18%	1010
			<i>Glu-B1</i>	47.0 ± 4.99%	1010
	<i>Rg1</i>	—	<i>Gli-B1</i>	1.8 ± 0.82%	1010
	<i>Rg1</i>	—	<i>Glu-B1</i>	44.3 ± 3.06%	1010
		<i>Gli-B1</i> —	<i>Glu-B3</i>	1.7 ± 0.8 cM	698
		<i>Gli-B1</i> —	cent	59.7 ± 7.1 cM	698
				56.1 ± 10.3 cM	698
		<i>Gli-B1</i> —	<i>Glu-B1</i>	42.0 ± 4.94%	1010

			42.5 ± 3.04%	1010	
	<i>Glu-B3</i>	—	<i>Glu-B1</i>	35.4 ± 4.8 cM	698
1BL	<i>Glu-B1</i>	—	cent	28.1 ± 2.8 cM	909
Gene order <i>Yr10/Rg1</i> — <i>Gli-B1</i> — <i>Rf3</i> — <i>Glu-B3</i> — <i>Nor-B1</i> — cent — <i>Glu</i> — <i>B1</i> (1010).					
1DS	<i>Gli-D1</i>	—	<i>Tri-D1</i>	I	1031
	<i>Gli-D1/Glu-D3</i>	—	<i>Tri-D1</i>	55.8 ± 7.4 cM	698
	<i>Gli-D1</i>		cent	I	1031
	<i>Gli-D1</i>	—	<i>Glu-D1</i>	63.5 cM	1004
	<i>Tri-D1</i>	—	cent	15.4 ± 2.1 cM	698
				14.1 ± 2.5%	1031
1DL	cent	—	<i>Glu-D1</i>	30.9 ± 2.7 cM	909
				22.0 ± 3.5 cM	909
				7.7 to 9.7 cM	1004
7BL	cent (5B.7B)	—	<i>Ep-B1</i>	I	359
	<i>α-Amy-B2</i>	—	<i>Ep-B1</i>	I	359
	<i>Lr14a</i>	—	<i>Ep-B1</i>	9.6 ± 3.0%	359
7DS	<i>Rc3</i>	—	<i>α-Amy-D2</i>	4 ± 5 cM	1009
		—	<i>Lr</i>	37 ± 5 cM	1009
		—	<i>Pch/Ep-D1b</i>	I	1009
7DL	<i>α-Amy-D2</i>	—	<i>Lr</i>	42 ± 6 cM	1009
		—	<i>Pch</i>	I	197, 1009
	<i>Lr</i>	—	<i>Pch</i>	18 ± 4 cM	1009
	<i>Pch</i>	—	<i>Ep-D1b</i>	0	1009

References

53. 26: 287-301
220. 'eBT-2288'. Genome 30: 442-445.
359. 30: 186-192
365. 30: 229-233
404. 61: 63-72
545. Obanni et al. (1988). Crop Sci 28: 223-226.
552. Patterson et al. (1988). Crop Sci 28: 652-654.
698. Delete reference and replace with: Singh NK, Shepherd KW (1988) Linkage mapping of genes controlling endosperm storage proteins in wheat. 1. Genes on the short arms of group 1 chromosomes. Theor Appl Genet 75: 628-641
703. Delete reference.

900. Pietro ME, Tuleen NA, Hart GE (1988) Development of wheat-*Triticum searsii* disomic chromosome addition lines. Proc 7th Int Wheat Genet Symp Camb UK (TE Miller and RMD Koebner eds): 409-414
901. DePace C, Benedettelli S, Qualset C, Hart GE, Scarascia Mugnosa GT, Delre V, Vittori D (1988) Biochemical markers in *Triticum* × *Dasypyrum* amphiploids and derived disomic addition lines. Proc 7th Int Wheat Genet Symp Camb UK (Te Miller and RMD Koebner eds): 503-510
903. Kudryakov NV (1987) Mapping of EstA and EstB genes controlling the synthesis of esterase isozyme in rye grains. Soviet genetics 23: 1139-1145
904. König S (1988) Nachweis von biochemischen markerfaktoren für chromosomen von *Hordeum vulgare* L. Biochem Physiol Pflanzen 183: 345-349
905. Urbano M, Resta P, Benedettelli S, Bianco A (1989) A *Dasypyrum villosum* (L.) Candargy chromosome related to homoeologous group 3 of wheat. Proc 7th Int Wheat Genet Symp Camb UK (TE Miller and RMD Koebner eds): 169-173
906. Rebordinos L, Perez de la Vega M (1988) Gene duplication in the structural gene for a glutamate oxaloacetate transaminase zone (GOT1) in *Secale*. J Hered 79: 78-80
907. Rohde W, Becker D, Salamini F (1988) Structural analysis of the waxy locus from *Hordeum vulgare*. Nucleic Acids Res 16: 7185-7186
908. Benito MC, Sanchez M, Shin JS, Blake T (1988) A map of barley chromosome 2 using isozymic and morphological markers. Biochem Genet 26: 387-394
909. Singh NK, Shepherd KW (1988) Linkage mapping of genes controlling endosperm storage proteins in wheat. 2. Genes on the long arm of the group 1 chromosomes. Theor Appl Genet 75: 642-650
910. Sanchez-Monge R, Fernandez JA, Salcedo G (1987) Subunits of tetrameric α -amylase inhibitors of *Hordeum chilense* are encoded by genes located in chromosomes 4H^{ch} and 7H^{ch}. Theor Appl Genet 74: 811-816
911. Westhoff P. (Personal Communication)
912. Baulcombe DC, Bufford D (1983) Gibberellic-acid-regulated expression of α -amylase and six other genes in wheat aleurone layers. Planta 157: 493-501
913. Huttly AK, Martienssen RA, Baulcombe DC (1988) Sequence heterogeneity and differential expression of the α -Amy2 gene family in wheat. Mol Gen Genet 214: 232-240
914. Marana C, Garcia-Olmedo F, Carbonero P (1988) Linked sucrose synthase genes in group-7 chromosomes in hexaploid wheat (*Triticum aestivum* L.). Gene 63: 253-260
915. Lazarus CM, Baulcombe DC, Martionssen RA (1985) Amylase genes of wheat are two multigene families which are differentially expressed. Plant Mol Biol 5: 13-24
916. Baulcombe DC, Barker RF, Jarvis MG (1987) A gibberellin response wheat gene has homology to yeast carboxypeptidase Y. J Biol Chem 262: 13726-13735
917. Kleinhofs A, Chao S, Sharp PJ (1988) Mapping of nitrate reductase genes in barley and wheat. Proc 7th Int Wheat Genet Symp, Camb. UK (TE Miller and RMD Koebner eds): 541-546
918. Cheng CL, Dewdney J, Kleinhofs J, Goodman HM (1986) Cloning and nitrate induction of nitrate reductase mRNA. Proc Natl Acad Sci USA 83: 6825-6828
919. Chao S, Koebner RMD, Sharp PJ, Gale MD (1989) (In preparation)
920. Singh NK, Shepherd KW, Langridge P, Clem-Gruen L, Skerritt JH, Wrigley CW (1988) Identification of legumin-like proteins in wheat. Plant Mol Biol 11: 633-639
921. Gupta RB, Shepherd KW (1988) Low-molecular weight glutenin subunits in wheat: their variation, inheritance and association with bread-making quality. Proc 7th Int Wheat Genet Symp Camb UK (TE Miller and RMD Koebner eds): 943-949
922. Gupta RB, Singh NK, Shepherd KW (1988) The cumulative effect of allelic variation in LMW and HMW glutenin subunits on dough properties in the progeny of two bread wheats. Theor Appl Genet 77: 57-64
924. Lukow OM, Payne PI, Tkachuk R (1989) The HMW glutenin subunit composition of Canadian wheat cultivars and their association with bread-making quality. J Sci Food Agric 46: 451-460
925. Vallega V (1986) High-molecular-weight glutenin subunit composition of Italian *Triticum durum* cultivars and spaghetti cooking quality. Cér Res Comm 14: 251-257
926. Vallega V, Mello-Sampayo T (1987) Variation in high-molecular-weight glutenin subunits amongst cultivars of *Triticum turgidum* L. from Portugal. Euphytica 36: 755-762

927. Vallega V (1988) Comparative analysis of high-molecular-weight glutenin subunit composition in various *Triticum* species. *Plant Breeding* 100: 241-246
928. Liu CJ, Gale MD (1988) *Ibf-1* (Iodine binding factor), a highly variable marker system in the *Triticeae*. *Theor Appl Genet* 77: 233-240
929. Liu CJ, Gale MD (1988) Three new marker systems, iodine binding factor (*Ibf-1*), malic enzyme (*Mal-1*) and malate dehydrogenase (*Mdh-3*) in wheat and related species. *Proc 7th Int Wheat Genet Symp Camb UK* (TE Miller and RMD Koebner eds): 555-560
930. Liu CJ, Gale MD (Personal communication)
932. Sharp PJ, Desai S, Chao S, Gale MD (1988) Isolation, characterization and applications of a set of 14 RFLP probes identifying each homoeologous chromosome arm in the *Triticeae*. *Proc 7th Int Wheat Genet Symp Camb UK* (TE Miller and RMD Koebner eds): 639-646
933. Chao S, Sharp PJ, Gale MD (1988) A linkage map of wheat group 7 chromosomes using RFLP markers. *Proc 7th Int Wheat Genet Symp Camb UK* (TE Miller and RMD Koebner eds): 493-498
934. Sharp PJ, Desai S, Gale MD (1988) Isozyme variation and RFLPs at the β -amylase loci in wheat. *Theor Appl Genet* 76: 691-699
935. Kreis M, Williamson MS, Buxton B, Pyrell J, Hejgard J, Svendsen I (1987) Primary structure and differential expression of α -amylase in normal and mutant barleys. *Eur J Biochem* 169: 517-525
936. Mitchell LE, Dennis ES, Peacock WJ (1989) Molecular analysis of an alcohol dehydrogenase (*Adh*) gene from chromosome 1 of wheat *Genome* 32: 349-358
937. Sharp PJ, Soltes-Rak E (1988) Homoeologous relationships between wheat group 2 chromosome arms as determined by RFLP analysis *Proc 7th Int Wheat Genet Symp Camb, UK* (TE Miller and RMD Koebner): 635-637
1001. Bartos P, Johnson R, Stubbs RW (1987) Postulated genes for resistance to yellow rust in Czechoslovakian wheat cultivars. *Cereal Rusts Bull* 15: 79-84
1002. Bennett, FGA (1984) Resistance to powdery mildew in wheat: a review of its use in agriculture and breeding programmes. *Plant Pathology* 33: 279-300
1003. Lowry JR, Sammons, DJ, Baenziger PS, Moseman JG (1984) Identification and characterization of the gene conditioning powdery mildew resistance in 'Amigo' wheat. *Crop Sci* 24: 129-132
1004. Lagudah ES, Halloran GM (1988) Phylogenetic relationships of *Triticum tauschii*, the D genome donor to hexaploid wheat. 2. Inheritance and chromosomal mapping of the HMW subunits of glutenin and gliadin gene loci of *T. tauschii*. *Theor. Appl Genet* 75: 599-605
1005. Dyck PL, Jedel PE Genetics of resistance to leaf rust in two accessions of common wheat. (In manuscript)
1006. Ceoloni C (1988) (Personal communication)
1007. Ceoloni C, Del Signore G, Pasquini M, Testa A (1988) Transfer of mildew resistance from *Triticum longisistimum* into wheat by *ph1* induced homoeologous recombination. *Proc 7th Int Wheat Genet Symp Camb, UK* (TE Miller and RMD Koebner eds): 221-226
1008. Jahier J, Doussinault G, Dosba F, Bourgeois E (1979). Monosomic analysis of resistance to eyespot in the variety "Roazon". *Proc 5th Int Wheat Genet Symp New Delhi, India*, (S. Ramanujam ed): 437-440
1009. Worland AJ, Law CN, Hollins TW, Koebner RMD, Guira A (1988) Location of a gene for resistance to eyespot (*Pseudocercospora herpotrichoides*) on chromosome 7D of bread wheat, *Plant Breeding* 101: 43-51
1010. Payne PI, Holt LM, Johnson R, Snape JW (1986) Linkage mapping of four gene loci, *Glu-B1*, *Glu-B1*, *Rg1* and *Yr10* on chromosome 1B of bread wheat. *Genet Agr* 40: 231-242
1011. Whelan EDP (1988) (Personal communication)
1013. Whelan EDP (1988) Transmission of a chromosome from decaploid *Agropyron elongatum* that confers resistance to the wheat curl mite in common wheat. *Genome* 30: 293-298
1014. Claude PP, Dyck PL, Evans LE (1986) An evaluation of 391 spring wheat introduction for resistance to stem rust and leaf rust. *Can J Plant Pathol* 8: 132-139
1015. Shang HS, Dyck PL, Martens JW (1988) Inheritance of resistance to *Puccinia graminis tritici* in eight resistant accessions of common wheat. *Can J Plant Pathol* 10: 36-40

1016. Ren ZL, Lelley T (1988) Genetics of hybrid necrosis in rye. *Plant Breeding* 100: 173-180
1017. Worland AJ, Petrovic S (1988) The gibberellic acid insensitive dwarfing gene from the variety Saitama 27, *Euphytica* 38: 55-63
1018. Wellings CR, McIntosh RA, Hussain M (1988) A new source of resistance to *Puccinia striiformis* f.sp. *tritici* in spring wheats (*Triticum aestivum*). *Plant Breeding* 100: 88-96
1019. Marshall D, Gardenshire JH, Gilmore EC, McDaniel ME, Erikson CA (1988) Registration of 'Collin' wheat. *Crop Sci* 28: 868
1020. Bartos P, Stuchlikova E (1988) Genes for leaf rust resistance in productive wheats. Proc 7th European and Medit Cereal Rusts Conf Vienna (B Zwatz ed): 85-87
1021. Dyck PL, Johnson R (1988) Resistance to the leaf rust or brown rust pathogen (*Puccinia recondita*) due to *Lr20* and its temperature sensitivity in European spring wheats. Proc 7th European and Medit Cereal Rusts Conf, Vienna (B Zwatz ed): 91-93
1022. Singh SJ, McIntosh RA (1988) Allelism of two genes for stem rust resistance in triticale. *Euphytica* 38: 185-189
1023. Allan RE, Peterson CJ, Line RF, George DW, Rubenthaler GL (1980) Registration of Tyee wheat. *Crop Sci* 20: 829-830
1024. Lafever HN (1988) Registration of 'Becker' wheat. *Crop Sci* 28: 376
1025. Lafever HN (1988) Registration of 'Cardinal' wheat. *Crop Sci* 28: 377
1026. Lafever HN (1988) Registration of 'GR855' wheat; *Crop Sci* 28: 378-379
1027. Cholick FA, Hatchett JH, Steiger DK, Buchanan GW, Sellers KM (1988) Registration of 'Shield' wheat. *Crop Sci* 28: 720-721
1028. Worland AJ, Petrovic S, Law CN (1988) Genetic analysis of chromosome 2D of wheat II. The importance of this chromosome to Yugoslavian varieties. *Plant Breeding* 100: 247-259
1029. Worland AJ, Law CN (1985) Aneuploidy in semidwarf wheat varieties. *Euphytica* 34: 317-327
1030. Gale MD, King RW (1988) Semi-dwarf genes in Australian wheats. *Agric Sci* 1: 18-20
1031. Gupta RB, Shepherd KW (1987) Interaction between genes controlling a new group of glutenin subunits in bread wheat. *Theor Appl Genet* 74: 459-465
1032. Leckie D, Snape JW, Parker BB (1988) Intrachromosomal mapping of the herbicide resistance gene *Dfq1* in hexaploid wheat. Proc 7th Int Wheat Genet Symp Camb UK (TE Miller and RMD Koebner eds): 551-554
1033. Snape J, Parker B, Leckie D, Rosati-Colaireti G and Bozorgipour R (1988) Differential responses to herbicides in wheat: uses as genetic markers and target genes for genetic manipulation. In Proc Int Cong EUCARPIA: Genetic manipulation in plant breeding, Biotechnology for the breeder, Helsingor, Denmark (In press)

IV. Editorial Remarks

Announcement for Future Issues

WIS Nos. 70 and 71 will be planned for publication in March and September, 1990, respectively. Manuscripts for No. 71 will be accepted anytime not later than July, 1990. Lists of genetic stocks and records of genetic resources are mostly welcome.

Manuscripts should be typewritten (double-space) in English, and submitted with duplicates. One article should not exceed five printed pages (about 10 typewriting pages). Lists of stocks are exempted from this page limit.

Off-prints could be printed by order at cost price. Back numbers are available by order at cost price.

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Explanation of the Picture on the Cover

Callus screening against toxins against *Helminthosporium sativum* to establish the resistant plant in common wheat. See the article by H.C. Chawla and G. Wenzel in this volume for the details.

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