

## A mutation breeding programme for sprouting resistance in bread wheat

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**Abstract.** A breeding programme involving  $^{60}\text{Co}$  mutagen treatment of the early maturing, hexaploid spring wheat variety Ruso, is described. The initial objective of the breeding work was to improve Ruso's resistance to sprouting in the ear. Treatment in the moist chamber followed by falling number tests were used for screening the mutants. Several very sprouting resistant but late maturing mutants were found. None the less efforts were made to retain also the earliness of the mother variety in the sprouting resistant mutants. In the  $M_{10}$  generation, there remained four usable early mutant lines showing a clear improvement in sprouting resistance. Two of them, in addition, have remarkably stiff straw, even when compared with the already very lodging resistant mother variety.

In 1967, the Hankkija Plant Breeding Institute released a new spring wheat variety, Ruso. Within a few years, this variety came to dominate the spring wheat area in Finland, and in 1973 occupied 52 per cent of the total spring wheat area (Anon. 1975). The most important characteristics of Ruso are its very stiff straw and good yielding ability, especially in view of the early maturity of the variety. Because of its earliness, Ruso can be grown throughout the Finnish spring wheat production region (KIVI 1970).

In order that this valuable new genotype could be utilized effectively in further breeding work, Ruso was subjected to  $^{60}\text{Co}$  mutagen treatment a year before its release. Since the main weakness of Ruso is its susceptibility to sprouting, the mutation breeding programme was directed at improving this character. It was also hoped that any mutants resistant to sprouting would retain the earliness of the mother variety. Our generally wet harvesting seasons have often led to large losses in the quality of wheat crops through sprouting before the crop is combined. The risk is highest with the late ripening varieties.

This paper deals with the main procedures of the breeding programme and with its product, the resulting material.

## Mutagen treatment and selection procedures

Evenly sized, dry seeds of Ruso spring wheat were irradiated with 15 resp. 20 Krad from the  $^{60}\text{Co}$  source of the Institute of Radiochemistry, University of Helsinki. Each seed lot contained approximately 2 500 grains.

The amount of material and its treatment in different generations after mutagen treatment (from  $M_0$  to  $M_{10}$ ) are described in Table 1.

Field trials and selection work were carried out at Tammisto and Anttila Experimental Farms in South Finland. The usual procedures worked out for mutation breeding were used. The large scale sprouting resistance screening performed in the  $M_3$  was described earlier (KIVI and RAMM-SCHMIDT 1969). In conjunction with the sprouting resistance investigations on later generations, the falling number test was used. For these tests, a moist chamber was built to incorporate a section with a fairly constant temperature (+11 – +14 °C). The higher humidity was maintained with a commercial air-moistener, and the ear samples were placed on the rotating shelves.

Extensive screening of the mutants was carried out also in the  $M_5$  (KURRI 1973). The protein analyses were made by the KJELDAHL method.

As well as the mother variety Ruso, Tähti spring wheat was used as a standard of comparison for the mutant lines. This variety, very resistant to sprouting but late maturing, was released by the Plant Breeding Institute of the Finnish Agric. Res. Centre in 1972 (MANNER 1972).

Table 1. The procedures of the breeding programme from  $^{60}\text{Co}$  treatment the the  $M_{10}$  generation.

Year	Generation	Material	Treatment/evaluation
1966	$M_0$	2 x approx. 2 500 seeds	$^{60}\text{Co}$ treatment, 15 and 20 Krad
1966	$M_1$	517 harvested plants	
1967	$M_2$	approx. 7 000 plants	growth rhythm test, selections for macromutants
1968	$M_3$	5 700 lines	sprouting res. screening, new single plant selections
1969	$M_4$	425 lines	sel. for agronomic characters, falling number tests <sup>1)</sup>
1970	$M_5$	216 lines	screening for e.g. yield/protein relationships
1971	$M_6$	76 lines	first actual yield test
1972	$M_7$	23 lines	comparative trials at different sites, yield,
1973	$M_8$	15 lines	agronomic characters, grain quality, baking properties
1974	$M_9$	12 lines	first lines for official evaluation
1975	$M_{10}$	6 lines	

<sup>1)</sup> After  $M_4$ , falling number tests were included in all generations.

## Variability in the $M_2$ — $M_5$ generations

Of the seed material treated, about 10 per cent yielded viable  $M_1$  plants capable of setting seed. This survival rate is in accordance with the recommendations for dose — efficiency relationships.

In the  $M_2$  and  $M_3$  generations, numerous and sometimes very drastic mutants occurred for all conceivable characters e.g. morphological ear characters. This kind of variability has been thoroughly investigated e.g. by MACKEY (1954) and TAVČAR (1964). The number of chlorophyll deviations in the  $M_2$  and  $M_3$  was extremely low, which is typical of polyploid crop species.

In the  $M_2$  generation, the growth rhythm of approximately 4 000 individual plants was observed (Fig. 1). This generation is characterized by a significant trend towards late ripening, a phenomenon especially noticeable when the mother genotype is early.

The single plant and pedigree selections made and the testing of selected lines in successive generations in the field and in the moist chamber, led to an altered earliness distribution by the  $M_5$  generation (Fig. 1). The general tendency

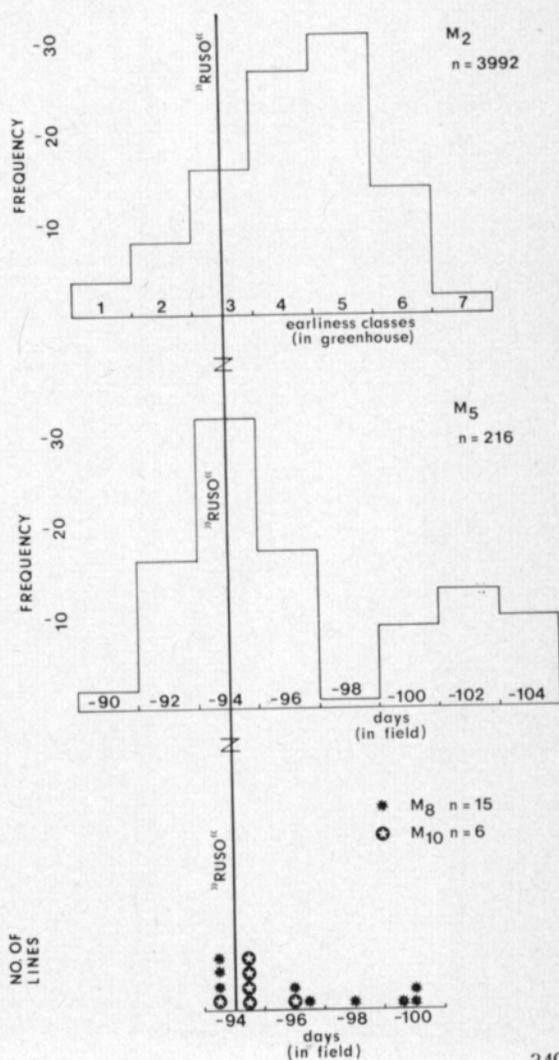


Fig. 1. Distribution of earliness in different  $M$  generations. Figures have been drawn in which the date of maturity of Ruso has been placed along the same linear regression. In delimiting the «earliness classes» in the  $M_2$ , the time scale was started at a point corresponding to the time of maturity of the earliest lines (class 1). In all generations, two days class intervals are used.

for the level of earliness of the mother variety to persist shifted the major part of the distribution towards the earlier classes. The late-peak in the two-peak distribution curve of the  $M_5$  is a result of visual selection for high yielding lines and also of screening for resistance to sprouting in  $M_3$ . In the  $M_3$  generation it was not possible to take into account the degree of maturity of lines comprising the samples for the sprouting chamber. The test, therefore, favoured the late maturing part of the material. In the moist chamber treatments preceding the falling number tests in later generations, this failure was corrected.

The variability of raw protein content was significantly winder among the 216  $M_5$  lines investigated than in the 79 samples of the mother cultivar ( $t$ -value = 3.56\*\*\*,  $F$  = 2.67\*\*\*). The correlation coefficient between protein content and yield in the mutant lines was  $r = -0.82$ , while the corresponding figure for Ruso was only  $r = -0.20$  (KURRI 1973).

The average yield of the  $M_5$  lines was only 82 per cent of the yield of the mother variety. This trend is usual when a high yielding genotype is subjected to mutagen treatment (cf GAUL 1964, KIVI 1965).

### The basis for the selection of mutants

After intensive screening of the  $M_5$ , the number of lines was decreased to 76 in the  $M_6$  (1971), this figure representing a good one per cent of the lines selected or taken in the  $M_2$  and  $M_3$  generations. These mutants were selected on four different bases: resistance to sprouting (falling number), high protein content, the appearance of a high yield potential and morphological deviation (Table 2). Nearly half of the mutants in the  $M_6$  belonged to the last group.

The six «protein mutants» in the  $M_6$  were all discarded by the  $M_8$ . Their poor agronomic characters became apparent as soon as the material was put into yield trials.

Among the lines still present in the trials in the  $M_{10}$  (1975), the basic aim of the breeding program could be resolved: four of the six early maturing mutant lines had been originally selected as sprouting resistant mutants.

Table 2. The distribution of mutant lines in advanced M generations as affected by the basis for selection in  $M_2$  or  $M_3$ .

Base of selection	Symbol <sup>1)</sup>	Mutant lines in generation (year)		
		$M_6$ (1971)	$M_8$ (1973)	$M_{10}$ (1975)
Morphological deviation .....	m	31	4	1
Sprouting resistance .....	fn	22	6	4
Appearance of high yield .....	y	17	5	1
High protein .....	pr	6	—	—
		<u>76</u>	<u>15</u>	<u>6</u>

<sup>1)</sup> The appropriate symbol is used as a subscript to the mutant line number, and shows the basis upon which each line was selected.

## Sprouting resistance mutants

The falling number made on the mutants treated in the moist chamber showed that several positive mutations had occurred and mutants were selected in the  $M_3$ - $M_5$  generations (Fig. 2). The best mutants seemed to exceed in their resistance even the late ripening variety Tähti (Table 3). Nevertheless, in accordance with the initial idea of the breeding programme, most of the attention was focused on the mutants with a similar growing time to that of Ruso wheat.



Fig. 2. Three sprouting resistant mutant lines (below) selected in the moist chamber in  $M_3$  generation. Above them three badly sprouted samples of the mother variety Ruso.

Table 3. The three most sprouting resistant mutants compared with the mother variety Ruso and the late maturing Tähti.

Mutant, Variety	Falling number in five tests		Growing time d	Straw- stiffness 0-100	Yield (Ruso = 100)
	aver.	lowest			
642 <sub>y</sub>	237	132	98	50	103
684 <sub>m</sub>	210	124	100	38	103
523 <sub>fn</sub>	177	114	96	40	90
Tähti	198	109	100	64	103
Ruso	104	60	95	50	3 780 kg/ha

The six mutant lines of the  $M_{10}$  generation had been tested in 12 falling number tests from 1972-74 (cf. Fig. 3). In six tests, the falling number of Ruso was low (under 125), indicating that its starch was already strongly damaged. In those six tests, the mutant lines, with the exception of line 713<sub>fn</sub>, showed some tendency towards better sprouting resistance (Table 4). Not one of these mutants reached, however, the level of the very resistant Tähti wheat.

The statistical analysis of the material shows that only the mutant 734<sub>m</sub> was significantly more resistant to sprouting than Ruso (Table 5). Neither this mutant line nor also line 552<sub>fn</sub> were significantly weaker than Tähti. In the half of the test results which included samples whose starch was undamaged or only slightly damaged, no statistically significant differences occurred.

On the basis of the falling number tests, the mutant lines 734<sub>m</sub> and 552<sub>fn</sub> appear to be the most promising types (Fig. 3). Lines 569<sub>y</sub> and 713<sub>fn</sub> have been dropped from further investigations, the former because of a weakness in its baking characteristics and the latter on account of a peculiar yearly variability in sprouting resistance.

Thus, the final results of this 10 year breeding programme are, in fact, four reliable mutant lines. Regarding their sprouting resistance they can be put in descending order as follows: 734<sub>m</sub>, 552<sub>fn</sub>, 689<sub>fn</sub>, and 426<sub>fn</sub>. They are all at least as high yielding as the mother variety (Table 6), but not one of the seems to be a significantly high yielding mutant. Except for the line 734<sub>m</sub>, the mutants have stiffer straw than does Ruso. On this basis alone, the lines 689<sub>fn</sub> and 552<sub>fn</sub> at least appear to be a worthwhile outcome of the breeding effort. The four mutant lines are at least as early maturing as Ruso. All the investigations of baking quality show that the mutants do not differ significantly from the mother variety.

Table 4. The falling numbers for six mutant lines, the mother variety Ruso, and the variety Tähti in 12 sprouting tests from 1972–75.

	Average for 12 test results	Average of the six best results (nd)	Average of the six poorest results (bd)
Ruso .....	213	347	80
426 <sub>fn</sub> .....	231	353	109
552 <sub>fn</sub> .....	222	321	122
569 <sub>y</sub> .....	197	300	94
689 <sub>fn</sub> .....	216	322	110
713 <sub>fn</sub> .....	188	293	83
734 <sub>m</sub> .....	233	323	143
Tähti .....	259	342	176
LSD <sub>5</sub> % .....		94	58
LSD <sub>1</sub> % .....		126	78

Best and poorest groups formed on the basis of Ruso's falling number.

Table 5. The t-test analysis for the badly damaged group (bd) of falling number tests represented in Table 4.

	Ruso	Tähti	426 <sub>fn</sub>	552 <sub>fn</sub>	569 <sub>y</sub>	689 <sub>fn</sub>	713 <sub>fn</sub>	734 <sub>m</sub>
Ruso	—	96.50**	ns	ns	ns	ns	ns	63.0*
Tähti			67.33*	ns	82.50**	66.33*	93.17**	ns
426 <sub>fn</sub>				ns	ns	ns	ns	ns
552 <sub>fn</sub>					ns	ns	ns	ns
569 <sub>y</sub>						ns	ns	ns
689 <sub>fn</sub>							ns	ns
713 <sub>fn</sub>								59.67*
734 <sub>m</sub>								—
LSD <sub>5</sub> %	57.96*							
LSD <sub>1</sub> %	77.55**							

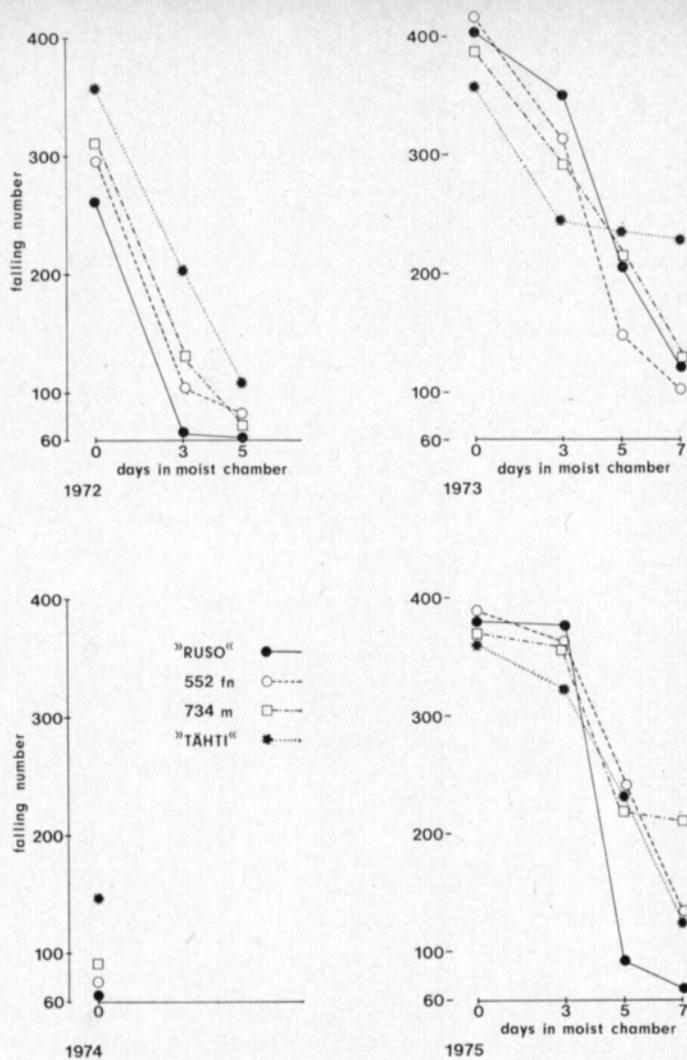


Fig. 3. Falling numbers of the two mutant lines 552<sub>fn</sub> and 734<sub>m</sub> in twelve tests from 1972–75, compared with the mother variety Ruso and with Tähti wheat. Those 12 tests form the basis for the data given in Tables 4 and 5. In 1974, no sprouting tests were made in the moist chamber because wet conditions in the field had already caused widespread sprouting.

Table 6. Characteristics of the «usable mutant lines» compared with those of the mother variety Ruso and the late maturing, sprouting resistant Tähti. Results are from the trials in the breeders' trial grounds.

Mutant line/variety	No of trials	Relative grain yield (Ruso = 100)	1 000 g.wt g	Zeleny value	Test value (Pelshenke)	Earliness + earlier - later d	Straw	
							Length cm	Stiffness 0–100
Ruso	20	3 780 kg	40.9	48	138	102	89	80
426 <sub>fn</sub>	9	103	-0.6	-2	-5	±0	-4	+5
552 <sub>fn</sub>	8	102	±0.0	+2	-8	+1	-8	+9
689 <sub>fn</sub>	14	102	-0.3	+6	-5	±0	-5	+10
734 <sub>m</sub>	11	102	+0.8	-4	-10	±0	-2	-4
Tähti	20	106	-3.4	+7	-9	-5	+3	+4

## Discussion of the breeding programme

In this mutation breeding programme, an especially valuable genotype was chosen as the subject of the mutagen treatment. At the time of its release, Ruso was a variety advanced with respect to straw stiffness, yielding ability and earliness.

The mutation breeding programme was begun as soon as we were convinced of the value of the Ruso genotype, in this case one year before its release. Selection after the mutagen treatment was based, primarily on morphologically deviating macromutants and secondarily on randomly chosen samples of individual plants whose pedigrees were tested in the moist chamber for sprouting resistance (physiological mutants, micromutants) (cf. GAUL 1964).

It is quite evident that the  $^{60}\text{Co}$  treatment induced sprouting resistant mutations, since such mutants could be isolated. Some of them exceed significantly the level of the mother variety and even that of the cultivar Tähti, most resistant to sprouting. In Norway, FROGNER ((1969) has obtained positive results in his mutation breeding programme for sprouting resistance in wheat.

In an earlier study, it was also shown that more than one sprouting resistant mutant frequently originates from a single ear of the  $M_1$  plant, behaviour typical of a mutagen like  $^{60}\text{Co}$  (KIVI and RAMM-SCHMIDT 1969).

During the execution of a breeding programme also the practical aspect of changes in the variety situation must be considered. The handling of the latest generations of this programme was largely influenced by the release of the late maturing, very sprouting resistant wheat Tähti in 1972. This variety ripens five days later than Ruso. Its release reduced the practical value of a large group of the most sprouting resistant but late ripening mutant lines. This can be seen also in the distribution of earliness (cf. Fig. 1). While in the  $M_8$  (1973) there were still mutants with a growing time several days longer than that of Ruso, nevertheless by the  $M_{10}$  (1975), all the late lines had disappeared and the growing times of the remaining mutants were clustered around that of the mother variety.

The lack of truly high yielding mutants in this material is not surprising, since Ruso itself was at the time of its release a remarkable advance in yielding ability, quite apart from its earliness. Breakage of the negative correlation between the protein content and yield potential seems to be extremely difficult in highly bred wheat crop (DENIĆ et al. 1976).

A most surprising result of the study is the isolation of stiffness mutants, since as regards resistance to lodging Ruso itself was a great step forward.

In any highly bred crop species, as is hexaploid bread wheat, the progress which mutant cultivars or elites can confer directly upon production is normally rather small — although not without significance, as is evidenced by the final results of this breeding programme. The more drastic mutants include normally harmful pleiotropic and/or linkage relationships; the elimination of those warrants further breeding efforts.

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## REFERENCES

- ANON. 1975. Kylvösiemenotanta 1973. Res. Reports, Reports, Res. Lab. of Grain Res. Comm. and State Granary 2/75: 1–59.
- DENIĆ, M., DUMANOVIĆ, J., KONSTANTINOV, K. & SIMIĆ, R. 1976. Some characteristics of induced variation in protein quantity and quality in wheat. *Genetika* (Yugoslavia) 1976, (in press).
- FROGNER, S. 1969. Avkastning og kvalitet hos vårhvete. State Exp. Sta. Møystad, Rep. No 63: 67–94.
- GAUL, H. 1964. Induced mutations in plant breeding. *Genetics Today*, Pergamon Press. p. 689–709.
- KIVI, E. I. 1965. The environmental influence on the incidence and selection of mutations. *Proc. Symp. on the Mutational Process*, Prague, p. 153–158.
- \* — 1970. Ruso ja Veka — uudet kevätvehnälajikkeet. *Hankk. Siemenjulk.* p. 151–157.
- \* — & RAMM-SCHMIDT, C. 1969. Selection for resistance to sprouting in  $^{60}\text{Co}$ -irradiated wheat. *Induced Mutations in Plants*, IAEA Vienna. p. 535–540.
- KURRI, P. 1973.  $^{60}\text{Co}$ -säteililytetyn kevätvehnän  $X_5$ - ja  $X_6$ -populaatioiden muuntelusta. Thesis for M.Sc. Agr., Institute of Plant Breeding Res., Univ. Helsinki. p. 1–27 (stenciled copy).
- MACKEY, J. 1954. Neutron and X-ray experiments in wheat and a revision of the speltoid problem. *Hereditas* 40: 65–180.
- MANNER, R. 1972. Tähti-kevätvehnä. *Koetoin. ja Käytäntö* 29, 6: 22–23.
- TAVČAR, A. 1964. Gamma-ray irradiation of seeds of wheat, barley and inbreds of maize, and the formation of some useful point mutations. *The Use of Ind. Mutations in Plant Breeding*, Pergamon Press. p. 159–174.

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## SELOSTUS

### Tähkäidännänkestävyys vehnän mutaatiojalostusohjelman tavoitteena

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Ruso-kevätvehnän tähkäidännänkestävyyden parantamiseksi suoritettiin sen mutagenssi-käsittely radiokoboltilla ( $^{60}\text{Co}$ ) vuonna 1966. Käyttäen ns. kostean kammion menetelmää ja sakoluvunmääritystä löydettiin useita tähkäidännänkestäviä mutaatiolinjoja, joista parhaimmat kuitenkin ovat selvästi Rusoa myöhäisempiä.

Käytännön jalostustavoitteeksi oli asetettu Ruson aikaisuutta olevien tähkäidännänkestävien mutanttien tavoittaminen. Aineiston  $M_{10}$ -polvessa (1975) oli jäljellä neljä käyttökelpoista linjaa, jotka aikaisuudeltaan ja satoisuudeltaan ovat Ruson veroisia ja sakoluvultaan sitä varmempia. Kaksi näistä linjoista on selvästi Rusoa lujakortisempia.