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Reciprocal extrachromosomal inheritance in barley: the transmission of an infective element through male and female gametes

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My backcross pollinations of 53 *spontaneum* barleys recurrently with cv. 'Adorra' resulted in a maternally inherited, partial spike fertility case assigned "58". Abnormal kernels, embryos and pollen, triploidy, miniature and streaked plants were among other symptoms. The 58-element greatly enhanced expression of the P-43 multi-ovary mutant in cv. 'Paavo'. Cv. 'Sudan' was a dominant 58-sterility enhancer. Pollinating of lymegrass with 58-barley resulted in lymegrass monoploids, in which 58-specific DNA was not detected. The 58-Adorra usually showed increased RNA levels and RdRp activities. Germinating in ribavirin and in etoricoxib reduced the 58-sterility. Transfections of the shoot apexes of embryos with deproteinized 58-nucleic acids did not result in infection. Any plasmid or virus explaining the 58-symptoms could not be detected. Electrophoresed germ proteins showed a glycosylated protein in 58-Adorra (M_r approximately 78.000) not observed in Adorra. The 58-element has features of a prion. The fallout from the Soviet Chernobyl brought hot particle radioactivity to Finland before the 1986 spring sowing, and impaired this study intensively going on between 1982 and 1989.

Keywords: inherited barley disease, interspecific crosses, non-Mendelian, prion-like

After finding the first cytoplasmic male sterility *msm1* in barley (*Hordeum vulgare* ssp. *spontaneum*) in 1976 (Ahokas 2018), I started a backcross program with 53 *spontaneum* barleys of ecologically distinct origins with cv. 'Adorra' as the recurrent pollen parent. The aim was to see if there were other maternally inherited traits. The backcrosses soon lead to the find of the *msm2* cytoplasm (Ahokas 1982, 2018). One of the *spontaneum*s produced variable partial spike fertility. It was arbitrarily assigned "58". The only spike of the 58 founder was used in crosses and the original strain from Israel was lost. It was heterozygous for the *Black lemma* gene. The "58-element" lowered grain yield in the genetic Adorra background, while the stem height did not differ significantly (Tables 1 & 2).

The most intensive period of my studies of the 58-element was between 1982 and 1989. During approximately two months in spring 1986, the radioactive fallout from the explosion and fire of the 4th Chernobyl nuclear power plant in the Soviet Union heavily contaminated many regions of Finland, including Elimäki Municipality where my field experiments were taking place. With the air currents, parts of Finland receive the first fallout within 12 hours of the explosion in Chernobyl (Savolainen et al 1986, Pöllänen et al. 1997). Hot particles and many radioactive isotopes with different half-life times arrived (Arvela et al. 1987, Raunemaa et al. 1987, Sinkko et al. 1987, Toivonen et al. 1988). Over 10 000 hot particles per m^2 were estimated to have occurred in a studied site in Finland (Rytömaa et al. 1986, Toivonen et al. 1988). Hot particles concentrated on the soil surface and formed pointy radiation sources at germinating crops in the field in spring 1986, inducing mutations which caused odd phenotypes in subsequent generations of the plants (Ahokas 1989a, 2023). For biological research purposes, a hot ^{144}Ce particle with an activity of 1200 Bq was isolated from soil (Toivonen et al. 1988). The mutagenicity of the Chernobyl fallout is inevitable (Møller and Mousseau 2015).

The 58-element is inherited through both egg and pollen. Three plastid mutants show exclusively maternal inheritance in barley (Ahokas 1976, 1997a). Likewise, a yellow streak mutant appearing in 58/7*Adorra in 1985 was inherited with the cytoplasm. – The illustrating of pedigrees, backcrosses and crossing directions follows suggestions by Purdy et al. (1968), though using capital letters only in the cultivar initials. – This is the only article about my results concerning this 58-element.

Partial spike fertility, higher grain protein and lowered yield due to the 58-element

In 1983, yields of cv. Adorra and F_2 and F_3 generations of 58/7*Adorra were measured in four replications on three-row plots (0.45 × 1 m) sown with 30 á 33 seeds per row meter (Table 1). In the 1984 season, the 53 *spontaneum* barleys, each seven times recurrently pollinated with cv. Adorra (i.e. *H. vulgare* ssp. *spontaneum*/7*Adorra) were grown in the field of the Saarela Farm. Besides 58, no other Israeli wild barleys in the backcrossing program caused the 58-like phenomena. The other ssp. *spontaneum* strains crossed seven times were PI 282636,

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PI 282637, PI 282638, PI 282642, PI 282646, PI 284742, PI 284743, PI 284753, PI 284755, PI 296796, PI 296800, PI 296801, PI 296802, PI 296812, PI 296813, PI 296821, PI 296827, PI 296829, PI 296830, PI 296831, PI 296838, PI 296850-16/7, PI 296853, PI 296856, PI 296899, PI 296919, PI 296922, PI 296944, PI 354926, PI 354945, PI 354949, PI 391090, PI 391091, PI 391096, PI 391110, PI 391111, PI 391112, PI 391118, PI 391131, PI 391133, PI 391134, PI 391135, PI 391136, PI 391137, PI 405187, 79BS14-3, 79BS30-4, 79BS31-2, 79BS32-2, 79BS34-2, M79-1159-01, R79-1161-07, and R79-1163-04.

The yields, along with the 53 other *spontaneum* F_3 derivations, were tested randomized into four blocks in 1984. The yield of 58/7*Adorra calculated for 1 ha was 3996 kg, that of cv. Adorra, 4452 kg, and in mean of the 53 other *spontaneum*/7*Adorra's 4501 ± 40 kg, ranging from 3759 to 5057 kg at 15% moisture, showing significant differences (F = 1.539, $p < 0.05$). The partial sterility in 58/7*Adorra mostly explains its low grain yield. Partial spike fertility lowers grain yield and raises grain protein content (Ahokas 1979), which was also increased in 58/7*Adorra (Table 2). Line 79BS14-3 resulted in *msm2* cytoplasmic male sterility (Ahokas 1982, 2018) and was not evaluated for the yield.

Table 1. The yields of plots (0.45 m^2) replicated in 4 randomized blocks in the Saarela field in 1983. F2 and F3 refer to the generations after the last backcross.

Stock	Plant height (cm)[1]	Grain yield (corresponding to kg ha^{-1})[1]
Adorra	94.6 ± 1.2 a	3956 ± 243 b
58/7*Adorra, F2	95.4 ± 2.1 a	2686 ± 132 c (vs. Adorra, *p* = 0.006)
58/7*Adorra, F3	96.5 ± 1.8 a	3087 ± 232 c (vs. Adorra, *p* = 0.032)

[1]Mean ± SEM. The numbers followed by the same letters do not differ significantly.

Table 2. Results of cv. Adorra and 58-Adorra extracted from the trial of 53 backcrossed derivatives on the plots (0.15 m^2) and grain protein percentage and yields replicated in 4 randomized blocks in the Saarela field in 1984.

Stock	Grain yield (kg ha^{-1})[1]	Protein (6.25 × N%)[1,2]	Protein yield (kg ha^{-1})[1]
Adorra	4452 ± 516 a	9.7 ± 0·3 b	408 ± 56 d
58/7*Adorra, F_3	3996 ± 203 a	10.7 ± 0·2 c (*p* = 0.037)	428 ± 27 d

[1]Mean ± SEM. The numbers followed by the same letters do not differ significantly at $p \leq 0.05$. [2]Determinations of Kjeldahl N were purchased from Viljavuuspalvelu Oy, Helsinki.

The soil in 1983 and 1984 was fertilized with 450 kg ha^{-1} of the boron containing fertilizer 15-15-15 Y-lannos (Kemira). Soluble boron from 0.3 to 0.7 mg l^{-1} in the soil eliminates anther sterility caused by boron deficiency in barley (Simojoki 1991).

Grown in a greenhouse, Adorra*4//58/10*Adorra in the F_3 or F_4 generations of the last 14th cross showed significantly more floret sterility than cv. Adorra (Fig. 1). In a 58-Adorra spike, thin kernels likely carry triploid germs (Fig. 2). Very small embryos may mean a monoploid germ and shrivelled endosperm may suggest that the germ will result in a miniature rosette (Fig. 4).

Fig. 1. Cumulative sum distribution of spike fertility in cv. Adorra (75 spikes) and 58-Adorra (150 spikes) grown side-by-side in a greenhouse. Their statistical difference is highly significant. Fig. 2. Spikes of 58-Adorra, Adorra and triploid 58-Adorra grown in a greenhouse. The awns were cut off. Some thin grains (black dots) occur in the variably sterile 58-Adorra spikes. The thins have an increased probability of carrying a triploid embryo. The blue arrowhead points to a shortened rachis segment. Fig. 3. Primary spikes of 58-Adorra (Adorra*5//58/10*Adorra) with compressed grain rows caused by the shortened rachis segments (blue arrowheads) and other irregularities in plants from the germination in etoricoxib solution for 82 h. The etoricoxib treatment significantly reduced floret sterility but permitted rachis irregularities. Grown under open sky. Fig. 4. Miniature rosette variants of 58-Adorra (58/8*Adorra) having the diploid chromosome number (2n = 14). A shriveled kernel in 58-Adorra likely produces a miniature rosette upon germination. Breadth of the coral-pink stake is 1.6 cm. Fig. 5. An almost perfectly fertile spike of the P-43 mutant and highly sterile, nearly isogenic 58-derivative of P-43 (= P-43//58/5*P-43). The 58-element strongly enhances sterility in multi-ovary plants. Fig. 6. Multi-ovarism in a single floret: three stamens variably transformed towards pistil morphology and the proper pistil. Fig. 7. Spikes of P-43 grown in a greenhouse from seeds electrophoretically transfected with the nucleic acid of 58-Adorra. They mimic the sterilizing effect of the 58-element in P-43. This sterility usually vanished in the next generation.

Table 3. Spike fertility and occurrences of thin kernels in the primary spikes of 20 plants of Adorra*5//58/10*Adorra in the F_3 generation of the 15th cross. The last 5 backcrosses were with Adorra as the seed parent, demonstrating the transmission of the 58-element through pollen. Sown from the numbered F_2 plants in summer 1988 in the Saarela field, fertilized with boron containing Normaali Y-lannos (Kemira) 500 kg ha^{-1}.

Number of F_2 parent	Kernels/florets in total	Number of thin kernels	Seed set (%)
1.	29/117	14	24.8
2.	44/114	9	38.6
6.	42/81	4	51.9
11.	67/117	4	57.3
7.	62/103	2	60.2
16.	65/107	6	60.7
20.	73/113	7	64.6
13.	71/106	4	67.0
4.	87/119	3	73.1
5.	59/80	0	73.6
19.	107/142	3	75.4
14.	70/91	0	76.9
15.	111/143	1	77.6
8.	86/110	1	78.2
3.	97/121	4	80.2
12.	94/117	0	80.3
10.	85/104	0	81.7
17.	65/79	2	82.3
9.	94/114	0	82.5
18.	100/115	0	87.0

Spearman rank correlation between thin kernel numbers and seed set, $r_s = -0.772$, $p = 2 \times 10^{-7}$

Fertility of 58-descendants crossed with cv. Sudan, Bomi, and P-43 mutant of cv. Paavo

Nearly 100 strains or mutants of domesticated and *spontaneum* barley were crossed to test their sensitivity towards the 58-element, mostly in the F_1 generations only. Some results with 'Sudan' ("Egyptian Sudan from Portugal", CI 6489), 'Bomi' and P-43 are shown here. P-43 is a multi-ovary mutant found by me in 'Paavo'. A special sensitivity factor in Sudan caused partial sterility in crosses with 58-Adorra but not with cv. Adorra (Table 4). An F_2 segregation of the cross 58/7*Adorra//P-43 was 116 nearly fertile and 43 multi-ovary-sterile plants which fits a 3 : 1 ratio ($\chi^2 = 0.354$, $p = 0.552$): the P-43 mutant gene is recessive. Sixteen isolated spikes of P-43//58/5*P-43 grown in the field in 1989 gave 22 kernels in 726 florets (3% seed set) with a low germinability. The sterilizing multi-ovary expression of the P-43 mutant is greatly enhanced by 58-element (Figs. 5–7, Table 5). The increased RNA levels associated with 58 (see below), may have enhanced the expression of multi-ovarism in P-43. A microRNA pairing with the *APETALA2* RNA in *Arabidopsis thaliana* controlled the development of stamens and carpels (Chen 2004). P-43 is a recessive tester, while Sudan (Table 4) is a dominant tester for the presence of the 58-element.

Table 4. F_1 seed sets of six cv. Sudan plants as seed parents (♀) pollinated with Adorra and 58-Adorra (Adorra*4//58/10*Adorra). Grown in a greenhouse in spring 1988.

\♂:	Adorra		Adorra*4//58/10*Adorra	
♀ \ F_1 spikes:	Florets in total	Sterile (%)	Florets in total	Sterile (%)
Sudan (A)	184	1 (0.5)	139	32 (23.0)
Sudan (B)	331	2 (0.6)	184	39 (21.2)
Sudan (C)	202	4 (2.0)	61	21 (34.4)
Sudan (D)	290	0 (0.0)	304	70 (23.0)
Sudan (E)	333	1 (0.3)	319	103 (32.3)
Sudan (F)	340	0 (0.0)	251	98 (39.0)

Mann-Whitney rank sum test, U = 0.000, $p = 0.002$

Table 5. Seed sets of the multi-ovary mutant P-43 in cv. Paavo and in its 58-derivative of the seventh cross F_3 with P-43 grown under open sky in the 2020 season.

P-43			P-43 with 58 (= P-43//58/5*P-43/3/P-43)		
Spikes	Florets: fertile/total	Fertility	Spikes	Florets: fertile/total	Fertility
29	550/971	56.6%	56	191/1988	9.6%

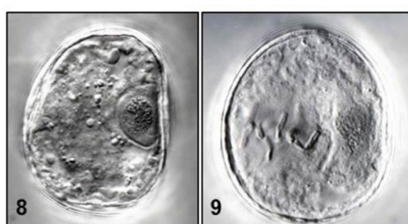
$\chi^2 = 418$, $p < 0.001$

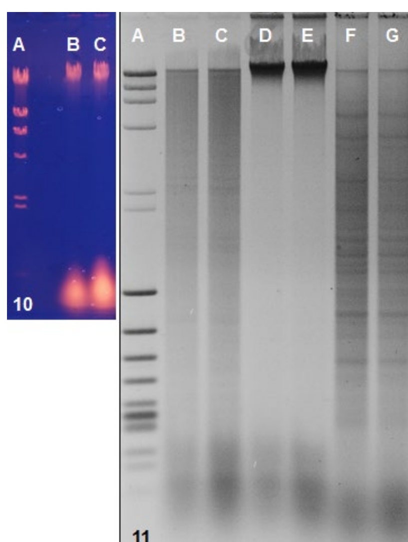
Bomi was a genotype with fairly high spike fertility in pedigrees involving the 58-element in the Saarela field in the 1986 season (Table 6). Bomi, however, lead to the occurrence of thin kernels. The thins, often leading to triploids, are commonly induced by the 58-element. On the contrary, among the 11 cultivars studied, Bomi produced the highest frequency, 0.23% of spontaneous triploids (Sandfaer 1973). In the 1986 trial on 0.375 m^2 plots with seven replications, 58/7*Adorra//3*Bomi F_2 produced a grain yield of 3723 ± 149 not significantly different from that of Bomi, 3605 ± 151 kg ha^{-1}; t = 0.560, $p = 0.586$.

Table 6. Seed sets in F_1 plants of the 58-hybrids of cv. Bomi backgrounds compared with Adorra.

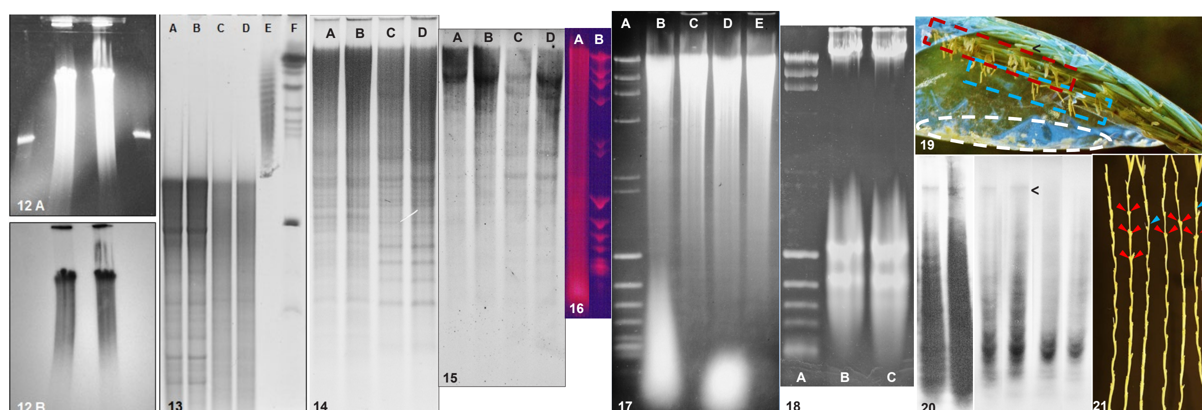
Cv. or pedigree	58-element present	Sterile/fertile (% fertile)	Thin kernels	Rachis deformed
Cv. Bomi	No	20/1219 (98.4)	0	0
58/7*Adorra//4*Bomi, F_1	Yes	3/292 (99.0)	0	0
58/8*Adorra//Bomi, F_1	Yes	9/526 (98.3)	1	0
58/11*Adorra//Bomi Risø mut. 13, F_1	Yes	5/331 (98.5)	0	0
58/11*Adorra//Bomi Risø mut. 1508, F_1	Yes	6/575 (99.0)	1	0
Cv. Adorra	No	9/971 (99.1)	0	0
58/12*Adorra, F_1	Yes	53/1110 (95.4)	7	5
Adorra*4//58/10*Adorra, F_1	Yes	94/776 (89.2)	0	1

Fertility of cv. Adorra vs. cv. Bomi: $\chi^2 = 1.550$, $p = 0.213$. Fertility of 58-Adorra vs. 58-Bomi: Mann-Whitney rank sum test, U = 0.000, $p<0.001$





Figs. 8 & 9: Aberrant pollen grains of 58-Adorra with DIC optics. Fig. 8. A pollen grain with a single interphase nucleus in its cell compartment. Fig. 9. A pollen grain with a delayed haploid mitotic metaphase showing chromatids of the seven chromosomes and the vegetative interphase nucleus in the common cytoplasm. Figs. 10 & 11. Nucleic acids of barley and size markers electrophoresed on agarose gels, EtBr stained and illuminated with UV. Fig. 10, A–C: The leaf tissue of seedlings showed 58/10*Adorra (C) to have a higher proportion of RNA than cv. Adorra (B). Markers, λ DNA HindIII (A). Ektachrome film. Fig. 11, A–G: Nucleic acids of Adorra (B, D & F) and 58-Adorra (C, E & G) digested with MspI (B & C), HpaII (D & E) and BstNI (F & G). 58-Adorra evidently has higher proportions of RNA than Adorra in the isolated nucleic acids. Markers, λ DNA-HindIII/ΦX-174 DNA-HincII (A). Polaroid 667 negative.



Figs. 12 & 13: Pulsed-field electrophoretic runs of DNA on agarose gels stained with EtBr and illuminated with UV. No plasmid in the barley DNA is discernible. Figs. 12, A & B: Long Adorra and 58-Adorra DNA labelled with ^{32}P in vivo. The main mass of DNA moved 20 á 26 mm and unlabelled λ DNA markers on both sides advanced 52 á 55 mm (A). The dried gel exposed to X-ray film through Elmu plastic wrap for 57 h (B). Fig. 13: Barley DNA of 58-Adorra (A & D), and of Adorra (B & C) digested with restriction endonucleases, with MspI (A & B) and with BglII (C & D). Markers, concatemeric λ DNA (E) and Saccharomyces cerevisiae chromosomes (F). Polaroid 667 negative. Fig. 14: DNA restrict digested with EcoRI (A & B) and with XbaI (C & D) and fractionated on agarose gel. Adorra (A & C), and 58-Adorra (B & D). EtBr stained and illuminated with UV. Polaroid 667 negative. Fig. 15: Fluorograph of the radioactivity of the tracts in Fig. 14 after capillary blotting to a GeneScreenPlus membrane and hybridization with *S-labelled probe of DNA isolated from Ustilago nuda smut sori from the diseased 58-Adorra plants. No difference revealed between Adorra and 58-Adorra. Fig. 16: The nucleic acids of U. nuda purified with Qiagen-tube 100 (A). Markers, λ DNA HindIII/ΦX174 DNA HincII (B). EtBr stained, photography under UV illumination with Agfa CT200 film. Figs. 17 & 18: Agarose gel fractionated nucleic acids stained with EtBr and illuminated with UV. Markers, λ DNA HindIII/ΦX174 DNA HincII (A). Figs. 17, B–E: Nucleic acids of the miniature rosette variant of 58-Adorra (Fig. 4) without any RNase treatment (B), digested with ribonucleases A (C), with RNase H (D), and with both ribonuclease A and RNase H (E). RNase H removed about half of the RNA suggesting them to be RNA-DNA hybrids after the isolation of the nucleic acids. Figs. 18, B–C: Alike RNA distributions of 58-Adorra (Adorra*4//58/10*Adorra) (B), and of Adorra (C). Isolation of the RNA started with 16 g stem tops of the pre-booting stage, removing the leaves in contact with the air. Fractionating with CF-11 columns (5 g), the first fractions were eluted with 80 ml of 1 × STE containing 15% ethanol, and the second fractions were eluted with 20 ml STE buffer, and precipitated with ethanol for the samples. Figs. 19–21: Monoploids and a diploid revertant from the pollinations of Leymus arenarius with Adorra*4//58/10*Adorra/3/2*Koral, and two barley references. Fig. 19: Two spikes of the descendant no. 16-1 enclosed in a single glassine bag. The upper spike has short (3.5 á 4 mm) but fertile and dehiscing anthers (purple frame) of the stem of a spontaneously diploidized revertant; sterile, rudimentary anthers of the sterile, monoploid spike (blue frame); shed pollen inside the isolation bag (white ellipse). Kirkkonummi. July 26, 2003. Fig. 20: Southern hybridisation of TaqI restricted and electrophoresed DNAs using labelled 58-Adorra DNA as the probe. Tracks from the left: Adorra, 58-Adorra, monoploid descendants nos. 24-1, 17-2, 10, and 17-1. The arrowhead points to the 25 kbp zone with a diffuse signal in the tracks of the barleys and the monoploids nos. 24-1 and 17-2. Fig. 21: Spikes of monoploid no. 17-2 prepared to show the rachii with radical, juxtapositional irregularities (red arrowheads) and segment shortenings (blue arrowheads) in 5 spikes and with a regular rachis in the spike to the left. Scale 5 × 5 mm. Compare with the irregular rachii in 58-Adorra spikes in Fig. 3.

Diploid miniature rosette, monoploid and triploid progenies due to the 58-element

Feulgen staining of chromosomes was used. Ten germinants of 58/7*Adorra from thin seeds pretreated for cytology with a 12.5-hour chilling at +0 °C were counted to have 21 chromosomes (triploidy), and one germinant ≥20 chromosomes (cytology by Ms. Silja Miikkulainen in 1985). I counted the root tips of miniature rosette plants of 58/8*Adorra (Fig. 4) to have 14 chromosomes and 2, 3 or 4 nucleoli per cell.

The triploids induced by the 58-element may be caused by the atypical lack of the second chromosome division in microspores, resulting in pollen with two nuclei, the other with a diploid set of chromosomes. Aberrant pollen grains with one (Fig. 8) and two nuclei, instead of three, were observed. An intermediate may have been the delayed generative metaphase observed in mature pollen grain (Fig. 9). Cell divisions in a normal barley anther are synchronous (Charzyńska and Lenart 1989), while synchrony is not quite realized in 58-Adorra. Fertilization of the polar nuclei only may result in a monoploid, while a lack thereof may result in diploid endosperm causing a thin kernel (Fig. 2). I have found two non-allelic mutants which induce triploids in barley. The *tri* mutant in cv. Paavo (Ahokas 1977a) was found to partly have omissions of the second meiotic divisions in EMCs (Finch and Bennet 1979). Different aberrant microspore divisions result in diploid pollen in barley as shown with the *semi-minute* genotype (Prasad et al. 1983). Male mitochondria and plastids are excluded during syngamy in barley (Mogensen 1988) and are unlikely to mediate the transmission of the 58-element through pollen making nucleus or cytoplasm the transmitting media.

Among 278 *spontaneum* barley strains crossed with Adorra, I found 16 showing apparent chromosomal interchanges leading to partial fertility (Ahokas 1999). Yu and Hockett (1979) found five genetic mechanisms leading to partial fertility in barley including the chromosomal interchanges. The type of sterility caused by the 58-element does not fit into their five genetic mechanisms.

Failure to find DNA markers and plasmids for 58-element

Digestion with restriction endonucleases of isolated DNA of 58-Adorra did not reveal any deviating fragment polymorphism compared with Adorra. On various isolated DNA samples, different restriction endonucleases (*Alu*I, *Bgl*II, *Bst*NI, *Cla*I, *Dpn*I, *Eco*RI, *Eco*RII, *Eco*RV, *Hin*dIII, *Hpa*II, *Msp*I, *Pst*I, *Sal*I, *Sma*I, *Sal*I + *Sma*I, *Taq*I, *Xba*I) were used without finding specific fragments for the 58-element in gel electrophoresis, pulsed-field gel electrophoresis (PFGE) or after blotting to a membrane subsequently hybridized using DNA probes labelled either with Nick translation, Random primed DNA labelling or Oligolabelling (Figs. 10–16). The probe DNA was usually genomic 58-Adorra – some experiments used the DNA of *Hordeum bulbosum*, *Oryza sativa* or of fungal tissue of two endoparasites, *Ustilago nuda* sori or *Claviceps purpurea* sclerotia collected from diseased 58-Adorra plants (Figs. 15 & 16). The labelled DNAs were usually purified with Nensorb 20, Nap-5 or Nick columns before the use as the probe. DNA of normal Adorra was also used as a blocking agent in many of the prehybridization solutions to enrich the putative specific DNA sequence of 58-Adorra along with denatured salmon sperm DNA as the blocking agent. Chloroplast DNA isolated from Adorra, 58-Adorra and deformed seedlings of 58-Adorra crossed with the *awned palea* (*adp*, syn. *adp1*) mutant did not differ with agarose gel electrophoresis (results not shown). The expression of the *adp* mutant deforming the floral organs of barley (Ahokas 1977b, Bossinger 1990) also appeared to be enhanced by the 58-element as that of P-43. The detected plasmids in eukaryotes were located in nuclei, mitochondria or chloroplasts (Esser et al. 1986).

Proportions of RNA in the nucleic acids and RNA-dependent RNA polymerase activities

The reproducibly higher content of RNA in 58-Adorra (Figs. 10 & 11) raised the question of increased RNA-dependent RNA polymerase (RdRp) activity associated with the 58-element. The *in vitro* RdRp activity appeared to be raised in the spike primordia tissue in 58-Adorra (Table 7), being on about the level of Adorra in the preparation of immature embryos plus scutella (Table 8). At this early yellow-maturity stage in the embryos plus scutella consistently higher RdRp activity of 58-Adorra appeared only using poly(rC) as the added template. The RdRp reactions “at +0 °C” (Table 8) may have actually taken place on the DE-81 paper at room temperature during drying and became well preserved from endogenous RNase activities.

The RNA-dependent DNA polymerase (RdDp, reverse transcriptase) activity assayed from two tissues of Adorra and 58-Adorra, with and without RNasin ribonuclease inhibitor, gave inconsistent results. The floral extract, i.e., including primordial reproductive tissue, assayed with added templates and RNasin was higher in 58- Adorra than Adorra. The used assay proved to be adequate, *per se*, with the positive control using added AMV reverse transcriptase (25 units per vial) which raised the RdDp activity ≥910 times. The RdDp may be involved in the reverse recombination, or mutagenesis frequently occurring in 58-barleys.

RNase H ribonuclease digests RNA in RNA-DNA hybrids. RNase H-removable RNA formed about half of the RNA in the isolated nucleic acids of 58-Adorra. RNase H especially reduced the long RNAs (Fig. 17) and made more or wider single strand DNA (ssDNA) sequencies in 58-Adorra than Adorra. The wider ssDNA of 58-Adorra digested with RNase H made the incorporation of label with Klenow polymerase several times more intense in 58-Adorra than in Adorra DNA. Subsequent RQ1 DNase digestion coherently liberated more dpm (63, 68 and 69%) from the DNA of 58-Adorra than from the DNA of Adorra (50, 51 and 59%) labelled with Klenow polymerase (Table 9).

CF-11 chromatographed RNA did not show differences between Adorra and 58-Adorra when electrophoresed on agarose gels (Fig. 18). Possible double-stranded RNA (dsRNA) was isolated from 10 g leaf tissues of Adorra and 58-Adorra according to the described method (Morris & Dodds 1979). The preparations were electrophoresed on 7.5% acrylamide gel in the continuous buffer (40 mM Tris, 20 mM Na acetate, 1 mM Na EDTA, pH set to 7.2 with acetic acid, autoclaved) until the bromophenol blue front started to run out. Under UV the gels stained with EtBr (ethidium bromide) showed two bands near the start, one clear and another faint, where Adorra and 58-Adorra did not differ from each other. The dsRNA carrying barley cv. ‘Nakano Wase’ was introduced in 1911 from Japan to the USA, where dsRNA occurs in its pedigrees being transmitted both through pollen and egg (Zabalgogeazcoa et al. 1993), like the present 58-element.

Table 7. Assaying RNA-dependent RNA polymerase (RdRp) activity in spike primordia extracts with 2.33 µM GTPα^{35}S as the label, including unlabelled ATP or ATP, CTP plus UTP, with or without RNasin ribonuclease inhibitor and added template. The mass ratio of the original samples of Adorra and 58-Adorra was 1 : 0.84, leading to the figures underestimating the activity of 58-Adorra. The 58-Adorra line was crossed 11 times with Adorra as the recurrent parent and with the Adorra cytoplasm (Adorra*3//58/8*Adorra).

Genotype	The activity as dpm in the extract, % and dpm per µg protein [in bracts]	
	With RNasin (36 units per vial)	Without RNasin
	With 0.2 A_{260} units of twenty:one poly(rC)·oligo(dG) per vial as the template. 1 mM ATP:	
Adorra	35 290 dpm 39% [473 dpm]	
58-Adorra	89 460 dpm 100% [756 dpm]	
Adorra		40 250 dpm 62% [539 dpm]
58-Adorra		64 750 dpm 100% [547 dpm]
	With brome mosaic virus RNA 0.75 µg per vial as the template. Precursors ATP, CTP, UTP, 1 mM each:	
Adorra		9 460 dpm 15% [126 dpm]
58-Adorra		37 180 dpm 57% [314 dpm]
	Without added template. Precursors ATP, CTP, UTP, 1 mM each:	
Adorra	8 890 dpm 10% [119 dpm]	9 510 dpm 15% [127 dpm]
58-Adorra	34 360 dpm 38% [290 dpm]	31 100 dpm 48% [263 dpm]

Spike primordia, 823.7 mg of Adorra and 692.1 mg of 58-Adorra at the stage of flag leaf visibility were sampled in chilled conditions. Grinding in 2.5 ml of extraction buffer, 50 mM Tris-HCl pH 8.0, 100 mM NH_4Cl_2, after autoclaving, 150 mM ß-mercaptoethanol and dodecyl-ß-D-maltoside (25 mg ml^{-1}). The supernatant (10 min 3000 rpm in Sorval SS-34 rotor) was concentrated to 1.85 ml with Centricon-30. Then the extract was precipitated with equal volume of saturated $(NH_4)_2SO_4$ and kept in ice (10 min). Precipitate (10 min centrifugation at 10 000 rpm in SS-34 rotor) was dissolved in 1.85 ml of the activity buffer (50 mM Tris-HCl pH 8.0, 10 mM $MgCl_2$, after autoclaving, 10 mM DTT). After the reaction at 20.7 °C for 120 min, the cocktails were pipetted in 5 spots on a DE-81 chromatographic paper and allowed to dry. Then the paper was washed six times for 5 min in 0.5 M Na_2HPO_4, twice for 1 min in 94% ethanol, twice for 1 min in water and twice for 1 min in 94% ethanol and allowed to dry. The polymerase activities were determined as the dpm from ^{35}S-labelled GTP incorporated and retained by the DE-81 paper, which was exposed to X-ray film to delineate the radioactive spots. The spots were cut off, inserted into scintillation tubes, immersed and mixed in 4.5 ml of Econofluor-2 with 3% v/v Protosol and measured with a Wallac beta scintillation counter. The dpm of the control discs lacking templates, RNasin and extracts were subtracted. The protein in the extracts were determined with the Lowry method using BSA as the standard.

Table 8. Activity of RNA-dependent RNA polymerase (RdRp) in extracts of embryos plus scutella of early yellow-mature grains assayed with RNasin ribonuclease inhibitor, with or without added template and actinomycin D (0.75 µg per reaction vial), incubated at +0 °C or +20 °C for 120 min.

Genotype	Activity cpm per 20 µl extract, per 1 µg protein [in bracts], and % of the maximum for protein		
	Template	With/Without actinomycin	Added precursor(s) - Label(s); °C
	Poly(rA) 30 µg per vial		ATP - UTPα^{35}S:
Adorra	5 889 dpm [57] 5%	With actinomycin D	+20 °C
58-Adorra	7 219 dpm [66] 6%	With actinomycin D	+20 °C
Adorra	7 176 dpm [70] 6%	With actinomycin D	+0 °C
58-Adorra	7 488 dpm [69] 6%	With actinomycin D	+0 °C
	Poly(rAC) 30 µg per vial		ATP - GTPα^{35}S, UTPα^{35}S:
Adorra	89 787 dpm [873] 78%	With actinomycin D	+20 °C
58-Adorra	94 763 dpm [870] 78%	With actinomycin D	+20 °C
Adorra	103 394 dpm [1005] 90%	Without actinomycin D	+20 °C
58-Adorra	84 725 dpm [778] 70%	Without actinomycin D	+20 °C
Adorra	114 659 dpm [1114] 100%	With actinomycin D	+0 °C
58-Adorra	113 698 dpm [1044] 93%	With actinomycin D	+0 °C
	Poly(rC) 30 µg per vial		ATP - GTPα^{35}S:
Adorra	77 807 dpm [756] 68%	With actinomycin D	+20 °C
58-Adorra	106 449 dpm [978] 88%	With actinomycin D	+20 °C
Adorra	94 137 dpm [915] 82%	With actinomycin D	+20 °C
58-Adorra	116 195 dpm [1067] 96%	With actinomycin D	+20 °C

	Brome mosaic virus RNA 0.75 µg per vial		ATP, CTP - GTPα^{35}S, UTPα^{35}S:
Adorra	54 527 dpm [530] 48%	With actinomycin D	+20 °C
58-Adorra	67 702 dpm [622] 56%	With actinomycin D	+20 °C
Adorra	89 051 dpm [865] 78%	With actinomycin D	+0 °C
58-Adorra	75 929 dpm [679] 63%	With actinomycin D	+0 °C
	Without added template		
Adorra	38 843 dpm [377] 34%	With actinomycin D	+20 °C; ATP, CTP, UTP - GTPα^{35}S
58-Adorra	49 340 dpm [453] 41%	With actinomycin D	+20 °C; ATP, CTP, UTP - GTPα^{35}S
Adorra	6 701 dpm [65] 6%	With actinomycin D	+20 °C; ATP, CTP, GTP - UTPα^{35}S
58-Adorra	7 015 dpm [64] 6%	With actinomycin D	+20 °C; ATP, CTP, GTP - UTPα^{35}S

Embryos plus scutella of 217.5 mg fresh weight of Adorra (mean mass 1087.5 µg) and 191.8 mg of 58-Adorra (mean mass 959 µg) were worked in chilled conditions: grinding in 1.74 (Adorra) and 1.534 ml (58-Adorra) of extraction buffer (50 mM Tris-HCl pH 8.0, 100 mM NH_4Cl_2, after autoclaving 150 mM ß-mercaptoethanol), and the mortars were rinsed three times with buffer aliquots of 160 (Adorra) and 140 µl (58-Adorra). After vortexing, 5 × 15 s, supernatants were collected (10 min 3000 rpm in Sorval SS-34 rotor). The supernatants were precipitated with equal volumes of saturated $(NH_4)_2SO_4$ and kept in ice (10 min). Precipitate (after 10 min centrifugation at 10 000 rpm in a SS-34 rotor) was dissolved in 1087.5 µl and 959 µl of the activity buffer (50 mM Tris-HCl pH 8.0, 10 mM $MgCl_2$, 40 mM KCl, after autoclaving, 10 mM DTT). The reaction mixture contained 1 mM each, ATP, and the needed precursors CTP, GTP, UTP, and labels, 2.33 µM of GTPα^{35}S and/or UTPα^{35}S. After incubation at +20 °C or +0 °C (in ice) for 120 min, the reaction cocktails (30 µl) were pipetted as microdroplets on four spots on a DE-81 chromatographic paper and the procedure was as presented in the footnote for Table 7.

Table 9. Enzymatic *in vitro* labelling intensities of ribonuclease H (RNase H) digested nucleic acids of Adorra and 58-Adorra in three experiments.

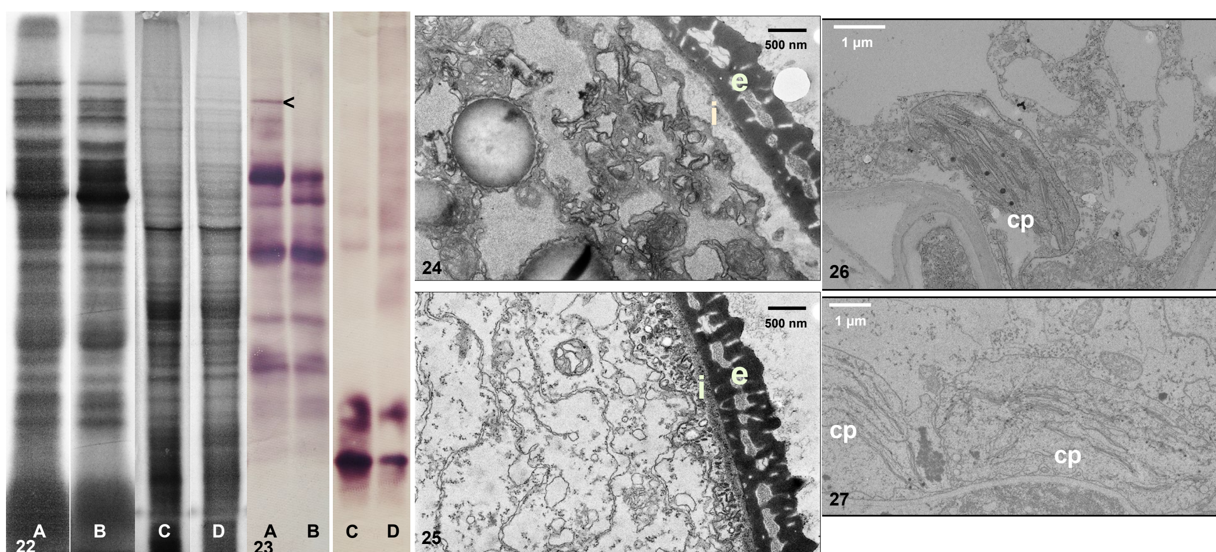
Nucleic acids	After RNase H digestion labelled as dpm. Removal as % and as dpm [in bracts]		
	Label	Incorporated with Klenow	Removed with RQ1 DNase
April 26, 1986: Adorra	dTTPα^{35}S	7 473 dpm	59% [= 4 414 dpm]
58-Adorra	dTTPα^{35}S	23 335 dpm	68% [= 15 928 dpm]
June 27, 1986: Adorra	dTTPα^{35}S	3 778 dpm	50% [= 1 907 dpm]
58-Adorra	dTTPα^{35}S	52 846 dpm	69% [= 36 446 dpm]
March 15, 1987: Adorra	dCTPα^{35}S	13 695 dpm	51% [= 6 968 dpm]
58-Adorra	dCTPα^{35}S	16 308 dpm	63% [= 10 270 dpm]

DNA of the genotypes was isolated from equal amounts (16, 35 and 52 g fresh mass) of upper leaves and early booting stem tops, ground in liquid nitrogen with mortar, proteinase K (20 mg) was added to the frozen tissue powder and the nucleic acids prepared with somewhat differing methods, principally following that of Wienand (1983). Each preparation contained two phenol extractions and one with chloroform containing isoamyl alcohol, and precipitation with –10 °C or –20 °C ethanol overnight and pelleting at 5000 rpm. The pellet was washed with 70% ethanol at +4 °C. The dried pellet was dissolved in the activity buffer: 40 mM Tris-HCl pH 7.6, 6 mM $MgCl_2$, 0.4% glycerol, after autoclaving, 1 mM ß-mercaptoethanol. 30 µl samples were digested with 2 units of RNase H for 30 min at 37 °C. Klenow polymerase (6 units) labelled the mixture in the presence of 1 mM each, three unlabelled dNTPs, and 2.33 µM of the fourth dNTP as a label for 30 or 55 min at 30 or 37 °C. The cocktail was divided into two equal portions, to one of which 6 µl of 0.1 M EDTA was added, while the other was digested with 6 units of RQ1 DNase at 37 °C for 45 min after which stopped with 1 µl of 0.5 M EDTA. Five spots on DE-81 paper were allowed to dry and the paper was washed and subjected to scintillation measurement as given in the footnote of Table 7.

Efforts to transfer the 58-element to lymegrass by interspecific pollinating with a 58-barley

As the crossing of lymegrass (*Leymus* [syn. *Elymus*] *arenarius*) with the pollen of barley was known to result in a low frequency of lymegrass monoploids (Ahokas 1970, 1973, 1997b), the crossing was an experiment to see if the 58-element could be transferred from barley into lymegrass. I pollinated 1386 emasculated lymegrass florets with 58-barley pollen. A fluid vesicle in the place of endosperm in the pollinated florets usually had a small embryo, which I axenically dissected and cultured *in vitro* on the Barley Medium II (Norstog 1973) 15 á 21 days after the pollination. This resulted in 10 lymegrass monoploids and an unclassified plant that were grown further in soil pots. After years, three of the 10 monoploids produced spontaneously reverted diploid stems showing fertility. The subsequent generation of the self-pollinated, spontaneously diploidized 16-1 (Fig. 19) proved to be fertile. Under glassine bags, 13 spikes of lymegrass plants of the population used in the crosses were tested in their native beech habitat in Kirkkonummi in 1993 for self-fertility. The mean was 71.3 ± 3.5% and varied from 46.3 to 84.7%, thus, the lymegrass is self-compatible but the partial seed set could not have revealed the putative partial sterility caused by the 58-element in a lymegrass specimen.

DNAs extracted from the monoploid lymegrasses were restricted with *Eco*RI and *Taq*I, electrophoresed, blotted to a membrane and hybridized with labelled 58-Adorra DNA as the probe. Two of the monoploids (nos. 17-2 and 24-1) showed *Taq*I fragments of 25 kbp similar to those in the DNA of Adorra and 58-Adorra (Fig. 20); nos. 17-2 and 24-1 might have traces of the barley genome. Any DNA fragment specific to 58 could not be shown in the lymegrass monoploids. Sixty-three spikes of seven monoploid clones grown in Kirkkonummi in the 2003 season were studied. Four of them had only spikes with irregular rachis segments, two had spikes with both irregular and regular segments (Fig. 21), and one (no. 24-2) had 11 spikes with only regular segments. Since 5 of 23 of the lymegrasses in the original population studied in 2023 had irregular rachii, the property should only be taken as tentative proof of the presence of the 58-element in the monoploids, though only in the monoploids did radical, juxtapositional irregularities occur (Fig. 21). Though a barley ♀ × lymegrass ♂ cross rarely results in a monoploid barley (Ahokas 1973, 1997b), such crossing provides a way to test the putative presence of the 58-element in the diploidized revertants of the lymegrass by using them as the pollen parent (♂) for a barley without the 58-element as the seed parent (♀). The real hybrids of barley ♀ × lymegrass ♂ do not have set spikes (Ahokas 1970, 1997b).



Figs. 22 & 23: Proteins electrophoresed on SDS-PA gels and detected with autoradiography from in vitro translations (Fig. 22), and glycosylated proteins after blotting to a membrane (Fig. 23). Figs. 22, A & B: No difference detectable between 58-Adorra (A) and Adorra (B) after in vitro translation of RNA isolated from spike primordia (fresh mass: 58-Adorra 6.4 g; Adorra 6.2 g). Preparation of the RNA contained grinding in liquid nitrogen, presence of proteinase K (10 mg) in buffer (0.1 M Tris-HCl pH 7.4, 0.4 M NaCl, 0.2% SDS), three extractions with phenol : chloroform : isoamyl alcohol (50 : 49 : 1) and one chloroform extraction. After ethanol precipitation and washing, the buffered, dissolved preparation involved digestion with RQ1 DNase and addition of RNasin. The RNA was purified with Nensorb 20 column collecting 20, 10 and 10 drops effluents (Johnson et al. 1986), which were used in the in vitro translation for 120 min at 30 °C with Lysate Nuclease Treated (Rabbit Reticulocyte) from Promega labelling the proteins with *S-Met. Figs. 22, C & D: No difference detectable between 58-Adorra (C) and Adorra (D) prepared from 57 and 50 axenic germs, respectively, growing in darkness with the first leaves emerging. The preparation of nucleic acids followed that given above for A and B, but the mRNA was caught with 1.8 cm^2 of Messenger affinity paper (Hybond-mAP) according to the protocol (Anon. 1987). In vitro translated for 105 min at 25–27.6 °C with Wheat Germ Extract (Nuclease Treated) from BRL labelling the proteins with *S-Met. Fig. 23: The purple alkaline phosphatase reaction products show the sites of glycosylated proteins on the blotted membrane. The germinants of 58-Adorra (Adorra*4/3/58-//10*Adorra) (A) had a glycosylated protein of molar mass approximately 78 000 (arrowhead) not observed in the normal Adorra germinants (B). The dormant embryos plus scutella proteins were not qualitatively different between 58-Adorra and Adorra (C & D). Figs. 24 & 25: TEM photographs of thin sections of a normal-looking 58-Adorra pollen grain (24), and of one showing delay in development (25) with polyribosomes in the still immature cytoplasmic structures and a thin intine (i). The dark-looking sporopollenin layers of the exines (e) secreted from the tapetum appear normal. Inclusions which could be regarded as viruses were not found. Fixed with 2% OsO_4 in 100 mM phosphate buffer (pH 7.2) and embedded in LX-112 resin. Examined with JEOL 1200 EX. Figs. 26 & 27: TEM photographs of thin sections with chloroplasts (cp). Greenhouse grown leaf tissue pieces (area approximately 0.7 × 3 mm) were fixed, treated and examined as given in the legend for Figs. 24 & 25. Fig. 26: Normal chloroplast and tissue without signs of viral inclusions in 58-Adorra (Adorra*4//58/10*Adorra). Thin, partly broken section. Fig. 27: From yellowish tissue of a streak mutant apparently induced by the 58-element in 58/7*Adorra. Flaccid, large chloroplasts, practically lacking grana. No signs of viral inclusions.

Cell-free in vitro protein translation experiments and a glycosylated protein in 58-germinants

Several experiments of the *in vitro* translation of isolated RNA preparations of Adorra and 58-Adorra of different source tissues were done using Nuclease treated lysate of rabbit reticulocyte (Promega) and Nuclease-treated

wheat germ in vitro translation system (BRL) or Wheat germ translation system (Promega) in the presence of RNasin. No differences in the *in vitro* translated polypeptides could be detected between Adorra and 58-Adorra with autoradiography of SDS-PAGE fractionated proteins labelled either with amino acids of ^{14}C-protein hydrolysate, ^{14}C-leucine or ^{35}S methionine (Fig. 22).

Meal of dormant embryos plus scutella of Adorra and Adorra*4//58/10*Adorra were extracted in a 100 mM acetate buffer (pH 5.5), including 0.25% SDS by grinding in a 1.5-ml Eppendorf tube. In another sample, three axenically grown germinants of approximately 14 mg mass were homogenized in the buffer, kept on ice for one hour, spined 15 seconds, and treated at 92 °C for two minutes. DIG-labelling of the proteins in the samples was done according to the protocol of the Glycan detection kit (Anon. 1989). The proteins were fractionated on electrophoretic SDS-PA gels and transferred with a semi-dry electroblotter (Multiphor II) to an Immobilon membrane for 55 minutes at 0.8 mA per cm^2. The glycans were detection on the membrane with the alkaline phosphatase reaction (Anon. 1989). Differences in the glycosylated protein patterns of dormant embryos plus scutella were not found, but in the germinants, 58-Adorra had a glycosylated protein of molar mass approximately 78 000 not observed in the normal Adorra (Fig. 23).

Effects of treatments with ribavirin and etoricoxib at germination on 58-sterility

Ribavirin is *in vivo* phosphorylated to ribavirin triphosphate which serves as an inhibitor of RNA capping and of some polymerases, including RdRp (Sidwell et al. 1979, Lerch 1987, Graci and Cameron 2005). Ribavirin treatment probably decreased the excessive production of RNA in cells carrying the 58-element and explains the curing effect of the treatment at the germination of Adorra*5//58/10*Adorra – seeds were dehusked with fingers, surface sterilized in 0.05% $HgCl_2$ for 20 minutes, and washed in sterile water with three changes. Lots of 10 kernels were germinated in 250-ml Erlenmayer flasks on 100 ml of autoclaved agar (1% final concentration) in tap-water with 10 mg $CaSO_4$, and with ribavirin added in 20 ml of water through ultra filter before solidification of the agar. The plants were grown under continuous light for 288 hour before being planting in soil in a greenhouse, alternating with the control seedlings grown alike without ribavirin. The ribavirin-treated seedlings had a slight chlorosis. All the spikes were counted at maturity. The spike sterility was reduced by 9.5 percentage points ($p = 0.085$) in the 0.01 mg ribavirin ml^{-1} treatment and by 13.3 percentage points ($p = 0.001$) in the 0.1 mg ml^{-1} treatment over the control fertility 69.5%.

The anti-inflammatory drug etoricoxib controls the production of prostaglandins. Prostaglandins play a role in plants (Groenewald and v. d. Westhuizen 1997, Mueller 1998). Prescribed to me, Etoricoxib Krka had ill-making, painful and long-lasting side-effects; I stopped administering it and tested the drug with germinating barley. Seeds were germinated in Petri dishes on filter paper with etoricoxib 18 mg ml^{-1} of sterile tap water for 85 hours, during which the germinants started to stunt in comparison with the water controls and they were grown further in soil. Only the primary spikes, whose primordia were subjected to the treatment, were counted. Etoricoxib reduced sterility in Adorra*5//58/10*Adorra by 16.3, 15.1 and 1.4 percentage points (significances at $p \le 0.045$) in comparison with the fertilities of 74.8, 77.4, and 89.8 percent in the water controls, respectively, in trials in the seasons of 2021, 2022 and 2023 under open sky. However, etoricoxib lead to the manifestation of rachis abnormalities (Fig. 3).

Subcellular inclusions associated with 58 were not found with electron microscopy

Thin sections of either microspore, pollen, stamen vascular bundle, or various leaf tissues studied under transmission electron microscopy (TEM) did not show subcellular particles associated with the 58-element. Pollen grains from a plant grown from a thin seed of 58-Adorra partly had variably immature cellular structures compared with the pollen of Adorra or normal pollen in 58-Adorra. The deviating pollen grain seemed to have abnormal long-lasting protein synthetic activity expressed as many polyribosomes (Figs. 24 & 25). None of the subcellular structure observed under TEM could be thought to be inclusions associated with viruses or phytoplasmas (cf. Mayhew and Carroll 1974, Ghosh et al. 1988, Hiruki 1992, Edwardson and Christie 1996, Boberg et al. 2010). The TEM samples also included the yellowish tissue of a streak plant: several new chlorotic streak mutants in barley have been observed under the presence of the 58-element (Figs. 26 & 27).

Pipetted (TR) and electrophoretic (ETR) transfection trials of barley embryos

Deproteinized nucleic acids of 58-Adorra, of singly DNA (= RNase H + A treated) or RNA (= RQ1 DNase treated), or DNA + RNA together, were used in electrophoretic (Ahokas 1989b) and pipetted transfections. With the hypothesis that the transmitting agent comprises either DNA or RNA, the nucleic acids were isolated with a method

involving proteinase K treatment and two or three phenol extractions to remove proteins ("phenol" = phenol : chloroform : isoamyl alcohol, 25 : 24 : 1, stored under 0.1 m Tris-HCl buffer, pH 8). The treated seed material was de-husked and surface sterilized kernels, on which a needle puncture was made under a microscope on the embryo towards its shoot apex (Ahokas 1989b). In the TR series, a 3 µl droplet of the nucleic acid solution was pipetted on the punctured embryo of a dormant kernel, and, after three hours, allowed to wet in a refrigerator at +4 °C on 1% agar containing $CaSO_4$ (0.1 g l^{-1}), and after 120 hours, planted in a greenhouse. Alternatively, after wetting the kernels at +0 °C, the nucleic acid in mild buffer was run with electrophoresis (ETR series) into the punctured embryo as detailed in Ahokas (1989b). Altogether 392 survived punctured Adorra and P-43 kernels were transfected in the TR series, and 86 survived punctured P-43 kernels in the ETR series. The putatively transfected TR and ETR plants lost their 58-like symptoms, at least in the fourth successive generations. The P-43 mutant responds strongly to the presence of the 58-element with sterility (Fig. 5), and when used as the ETR recipient, P-43 mimicked the 58 effect (Fig. 7).

A single deviating transfected Adorra, number TR23, showed partial fertility, rachis segment shortening, and e.g., in the third Tr_3 generation after the transfection, segregated one fully and six partially fertile plants. The F_1 generation of the test-cross with cv. Sudan of the partially fertile TR23 contained four partially fertile (with floret sterilities of 25.9, 31.7, 33.3 and 38,9%) and two normally fertile plants (0.0 and 1.3% sterilities); the result should be compared with those in Table 4. Thus, TR23 is inherited as a mutant causing partial fertility, an explanation also supported by additional crosses. On June 20, 1986, I planted a set of 50 transfected germinants including TR23, in soil boxes in a greenhouse where the thermostat-controlled roof windows mostly stayed open in the summer permitting, e.g., the radioactivity from Chernobyl fallout to make it to the seedlings via rainwater. The forgotten, open note-book beside the box of the just planted TR germinants transferred from the refrigerator has wetted marks as proof of rain inside the greenhouse within a few hours. The precipitation was also recorded at weather stations. Hot particles from the fallout radioactivity washing onto the TR seedlings (see introduction) is a putative reason for the TR23 mutation, since the Adorra used in this experiment originated from a single plant two generations earlier and was thereafter keenly observed to be devoid of visible mutations while using it in the *msm1*, *msm2* and 58 backcrosses. The field grown plants in Elimäki in 1986 also resulted in a number of variants in the following years, the reason for which was rather the radioactivity in the field tops soil which the seeds encountered during sowing in May 1986 (cf. Ahokas 1989a).

Hot 2–10 µm sized particles in Chernobyl fallout were isolated from Scots pine needles collected on April 28 and 29, 1986 in SW Finland. These hot, airborne particles showed total gamma activities between 15 and 560 Bq (Raunemaa et al. 1987). Hot particles of activities up to 1200 Bq were isolated from soil (Toivonen et al. 1988). These are strong enough to induce mutations in contact with plant tissues. Cell death and malignancy were experimentally induced in cultured mammalian cells with hot particles from Chernobyl (Toivonen et al. 1988, Servomaa 1991).

Prion-like protein as a putative 58-element

Altogether, 23 or 24 virus, viroid and virus-like diseases and aster yellows phytoplasma were listed as known barley diseases (Mathre 1982, Anon. 2023). Aster yellows phytoplasma capable of infecting barley was detected in Finland in the 1960s (Murtomaa 1966, Boberg et al. 2010). The symptoms of the phytoplasma are distinct from those caused by the 58-element. Barley mosaic and barley stripe mosaic virus (BSMV) are seed transmitted (Mayhew and Carroll 1974, Mathre 1982). Particles of the latter were shown with TEM in barley egg cells (Mayhew and Carroll 1974) and were found to be transmitted by contact (Jackson et al. 1983). Though the effect of ribavirin and the increased RdRp activity in 58-Adorra might suggest a virus, the lack of transfer of the 58 symptoms with natural vectors in the experimental environments encountered over many years and the lack of transfections with nucleic acids (see above) or with approach node grafting do not suggest that the 58-element is a virus. In the approach node grafting, when the first stem node emerged, surfaces of nodes were whittled away and the wounds immediately stabilized against each other with Parafilm using Adorra as "the recipient" and 58-Adorra as "the donor". In 1986, in the second generation succeeding the approach node grafting of 44 recipient plants, all the plants of the grafted generation with suspected infections proved to normal without 58-symptoms.

Moreover, the hypothetical virus in Adorra and 58-Adorra was extracted from 200 g leaf samples homogenized with a Waring Blender in a 350 ml buffer according to the method of Hull et al. (1976). The prepared samples were ultracentrifuged after loading onto a 10 to 40% sucrose density gradient in a 10 mM phosphate buffer of pH 7.2 (SW41Ti rotor, 23 000 rpm, 2 h). The absorbances at 260 nm of the gradients and their fluorescence with EtBr did not detect any proof of a virus comparable with that by Hull et al. (1976).

Antisera to BSMV were tested: lyophilized antisera PVAS-43 and PVAS-130 (purchased from ATCC) were dissolved in 0.85% NaCl. They were tested in Petri dishes in 1% agarose in 0.85% NaCl and 0.1% Na azide. Holes of Ø 4 mm were filled with 26 µl of the antisera diluted from 0 down to 1/256 parts. Pressed sap of 58-Adorra and Adorra leaf tissue were tested in the sample holes. No indication of BSMV antigenicity in Adorra or 58-Adorra was found with the double diffusion test.

The PFGE using carefully isolated, "long" DNA of Adorra and 58-Adorra, or DNA from their fresh or frozen pollen grain samples, did not reveal any plasmid-like DNA after EtBr staining (Figs. 12–13) or after Southern hybridization on a GeneScreen*Plus* membrane using labelled DNA of 58-Adorra as the probe. DNA samples of 58-Adorra labelled *in vivo* with either ^{3}H or ^{32}P and run with PFGE and plotted to a GeneScreen*Plus* membrane did not show evidence of a plasmid-like element by autoradiography (Figs. 12 A & B).

The glycosylated protein in the germinating 58-Adorra might suggest the presence of a prion protein; the protein of scrapie PrP 27-30 and Creutzfeldt-Jakob disease (CJD) are sialoglycoproteins (Bolton et al. 1987, Kingsbury 1987). "Prions are defined as proteinaceous infectious particles that lack nucleic acids" (Prusiner 1998); a definition which would be in accordance with the present knowledge of the 58-element. Prions in plants have emerged as a serious topic (Rasmussen et al. 2014, Pritzkow et al. 2015, Dixson and Azad 2022, Carlson et al. 2023, Blagojevic et al. 2024). As to the higher RNA levels in 58-tissues, higher yields of RNA in scrapie infected hamster brains were also observed, and both infected hamster and mouse brains had modified RNAs (German et al. 1985). Moreover, the prion protein PrP can interact with modelled RNAs (Weiss et al. 1997, Mercey et al. 2006). Proteinase K reduces prion protein alone or in combination with other chemicals *in vitro* (McKinley et al. 1987, Harm et al. 2022). Note that in the transfection experiments in the shoot apexes of embryos (previous section), the nucleic acids were deproteinized with proteinase K plus phenol extractions because the 58-element was expected to be of nucleic acid. Phenol deactivates CJD and scrapie prion (Kingsbury 1987).

Some general information about material and methods

I sowed the material spacing the seeds by hands and likewise weeded the field by hands avoiding herbicides, at the Saarela Farm, Elimäki Municipality. The greenhouse growing in the 1980s took place in the Viikki Experimental Farm, University of Helsinki, where 6 × 9 plants usually grew in white plastic (PE) 40 × 60 cm boxes (Perstorp) with perforated bottoms. The soil (20 cm deep) in the boxes was limed and fertilized B2 peat (Satoturve Oy) with an additional fertilization including micronutrients during early growth. High pressure sodium vapor lamps (400 W) and 500-W blended-light lamps (Airam, Osram or Philips) guaranteed sufficient long-day illumination. Organic soil, Puutarhurin Musta multa (Biolan), in the white boxes was used for growing under open sky in the summers of the 2010s in Kirkkonummi Municipality.

The suppliers of chemicals, biochemicals, absorbing matrices, filters or fertilizers were Amersham, Amicon, ATCC, BDH, Bio 101, Biometra, Bio-Rad, Boehringer Mannheim, BRL, Calbiochem, Diagen, Difco, Fluka, FMC, Hoefer, Invitrogen, Kemira, LKB, Merck, NEB, NEN, Oriola, Pharmacia, P-L Biochemicals, Prolabo, Promega, Serva, Sigma, Stratagene, Whatman, and YA-Kemia. Radioactively labelled precursors and protein size markers were purchased from Amersham and NEN (Du Pont). X- and beta-ray films from Amersham and Kodak were used with Kodak X-Omatic regular intensifying screens. A Wallac beta scintillation counter was used to measure radioactivity. DNA was quantitated with EtBr fluorescence or with Hoechst 33258 using TKO 100 fluorometer (Hoefer).

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