

Pollen irradiation and variability in plant breeding materials.

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Introduction.

In a conventional breeding programme, all F₁ plants, from homozygous self-fertilizing parents, are identically heterozygous. The F₂ is produced, and the character combination selected for, is carried on further into daughter generations. In case the pollen was irradiated before pollination it would be investigated whether genetic changes had taken place which influence the variability in the progeny, for instance by partial homozygosity and/or partial parthenogenesis. In both cases genetic stabilisation might be enhanced.

In this material, however, only in the F₁ generation an intended combination of desirable parental traits can be distinctly noted.

Mainly two reasons inspired this work: firstly the investigations of pollen irradiation by Brewbaker, J.L. and G.C. Emery, 1962, and secondly the author's interest in the interrelationship between autonomously genetically variable populations, (Denward 1963, 1967, and 1970). In a preliminary study aimed at investigating the potentiality of pollen irradiation in plant breeding, three experiments were carried out with materials that were available in the current research in my laboratory.

I. The first experiment:

Species used: Potato, *Solanum tuberosum* (2n=48).

Problem: Creation of poly-haploids by induced parthenogenesis.

Method used: Pollination with genetically inactivated pollen. Studies of the possibility of fragmentation, healing and reorganisation of the chromosomes.

Pollen: dried pollen from the deep freeze storage.

High dose used: X-irradiation of 20.000 rad.

Results obtained: Crosses were nearly sterile, less than 1 % of the seeds germinated.

Mitosis studies: Carnois root tip fixations of first seedling roots, Orcein squash, metaphase chromosome counts. The first root tip counts resulted in 24 chromosomes and many fragments were obtained. Four days later a peak of about 36 chromosomes and many fragments were observed. After one more week metaphase plates with different numbers, some with 48 chromosomes were observed.

Conclusions: Unexpected observations were heavy disturbances on chromosomes and genomes. Reconditioning by healing was indicated.

II. The second experiment:

Species used: Winter wheat, *Triticum aestivum* (2n=42).

Problem: poly-haploidization as described above.

Method used: a) pollen grains with high dose irradiation of 20.000 rad. b) pollen grains with moderate dose irradiation of 800 rad.

Results obtained: When a high dose of pollen irradiation was applied the crosses were completely sterile. When a moderate dose of irradiation was applied a few seemingly normal seeds were obtained and F₁ plants were grown from reciprocal crosses. F₂ progenies from different F₁ plants were distinctly different in field observations, thereby indicating differences in the genetic makeup in the F₁ plants from crosses with irradiated pollen.

Conclusions: No chromosome number reduction was observed. The differences between individual F₁ plants were proven by the F₂ populations of each F₁ plant.

Comments on experiments I and II as described above: The use of irradiated pollen in the crosses causes disturbances in the zygote formation and the embryo development.

For the reproducibility of the results, the continued experimentation called for a standardisation of the pollen population to be irradiated. The equivalence of the pollen population was carefully considered and the decision was made to carry out the irradiations at the time of anthesis. Then, all pollen grains carrying the male gametes to take part in the fecundation and embryogenesis would be of similar physiological status and having been protected from the variation in environmental climate factors as humidity, temperature etc. during formation and maturation.

III. The third experiment:

This experiment was chosen to study the estimation of an optimum dose of irradiation for variability studies.

Species used: Winter wheat, *Triticum aestivum* (2n=42).

Problem: Studies of effects of increasing irradiation doses.

Method used: Crosses with irradiated pollen with succeeding doubled doses (rad).

Results obtained: Tables 1 and 2 show the seed set results and performances of the F₁ generation respectively. The intersection between the curves for seed set and expression of aberrations (lethality, sterility) lies at about 1.250 rad.

After complementary tests the doses listed below were estimated for four different plant species to perform variability studies:

1. wheat: 1.250 rad
 2. barley: 1.250 rad
- and tentatively for:
3. pea: 500 rad

4. rape: 5.000 rad

Table1. Seed set after pollination with irradiated pollen.

Dos rad	Pollinat. spikes	Aborted kernels		Sown kernels		Germ.
		No	%	No	%	%
10 000	107	841	39.3	24	1.12	1.03
5 000	41	327	39.9	32	3.90	2.07
2 500	15	82	27.3	88	29.30	4.00
1 250	46	1	0.1	793	86.20	52.50
625	54	0	0.0	741	68.60	60.83

20 flowers per spikes were pollinated. Per cent (%) calculated on no of spikes * 20

Table 2. Performance of F₁ plants from crosses with irradiated pollen.

Dose	No Kernels	Dead No - %	Ster. Pl. No - %	Vitality			Remains No - %
				1	2	3	
				No - %	No - %	No - %	
10 000	24	2 8.3	1 4.2	11 45.8	7 29.2	1 4.2	2 8.3
5 000	32	15 46.9	2 6.3	5 15.6	9 28.1	1 3.1	0 0
2 500	88	76 86.4	5 5.7	0 0	1 1.1	5 5.7	1 1.1
1 250	61	15 24.6	23 37.7	7 11.5	9 14.8	5 8.2	2 3.3
625	194	21 10.8	7 3.6	79 40.7	71 36.6	3 1.5	13 6.7

Legend for table 2: Ster. Pl. = Sterile plants; 1. = Viable plants; 2. = Semi-viable plants; 3. = Aberrant plants.

Under the pretext of plant breeding, selections were made in the experiments II and III as shown above. The selections were distinctly aimed at consolidating the combination of the desired traits of the parents in the original cross plus a low interplant variability in the progeny tests. The combination of traits can be expected to be fully identified only in the F₁ generation. The low interplant variability expectation is so far hypothetical but is based on the assumption that fragmentation and healing has produced partial homozygosity and/or partial parthenogenesis among the F₁ plants which will enhance stabilisation. Consequently the recognized selected plants were few and the discarded ones many. With the mentioned selection strategy the breeding population retained a high breeding potential at a relatively low volume.

After selection in three generations F₁, F₂ and F₃, lines in the fourth generation were entered to be tested for the official cultivar list. Lines of all four species passed the official Distinctness Uniformity and Stability (DUS) tests readily for cultivar list recognition.

The observations in the three experiments are supposed to be only an orientation regarding the radiation effect per se, and it seems to be interesting enough to suggest pollen irradiation among

the experimental techniques in plant breeding. In a discussion of the irradiation effects on the pollen genome (Pandey 1980, 1986; Snape *et al.* 1983; Borrino *et.al.* 1985; Chyi and Sanford 1985) it was argued if fragmentation of chromosomes gave “new” recombination expressions or if egg cell transformation could be accepted as an explanation of the recorded recombination results (Pandey 1980, 1986). The one does not seem to necessarily exclude the other. Therefore the question arose: Does pollen irradiation influence on the variability of the crossing progeny?

According to these results and conclusions, the major experiment in barley is presented as follows.

Variability in Barley after Irradiation of Pollen with dominant Barley Genes.

The idea of this investigation was to estimate the hereditary effect on mutant pollen and the F₁ generation by irradiating pollen prior to pollination. With the observations received the used methods could be applied for the experiment reported below.

Four dominant homozygous mutants of two-rowed spring barley were chosen as pollen donors for the studies, and material was received from Professor Jerry Franckowiak, North Dakota State University, Fargo, ND, USA as demonstrated in table 3. The nomenclature was used according to international adopted rules (Franckowiak and Lundqvist, 2010).

Table 3. The different dominant mutant types.

Mutant	Gene symbol	BGS no	Seed source	Indication
Erectoides-r	Ert-r	332	94 FGH 622	Erectoides spike (dense spike)
Zeocriton 1	Zeo 1	82	94 FGH 437	Zeocriton spike (very dense spike)
Red lemma and Pericarp 2	Pre2	76	94 FGH 513	Anthocyanin pigmentation
Black lemma 1	Blp 1	203	94 FGH 203	Purple pigmentation

The cultivar ‘Alexis’ was used as female parent. ‘Alexis’ is homozygous recessive in the respective mutant loci. The mutants are dominant and with distinct phenotypic expression as homo- and heterozygots. The mutants were crossed with ‘Alexis’. All the crosses with untreated pollen were fertile with full seed set and normal vigorous F₁-plants.

In the preliminary experiments the LD50-dose of pollen irradiation was estimated at 1.250 rad with the Gamma Caesium Source of the Wallenberg Laboratory, Lund University, Sweden. In the same experiments the optimal irradiation technique was carried out as follows: The irradiation was made on whole spikes at anthesis with the earliest anthers in dehiscence. Then the pollen grains were – at the pollination stage – mature and as far as possible unaffected by variations in the environment and not disturbed by environmental conditions. As a standard self-pollinated ‘Alexis’ seeds were germinated and grown into normal plants.

Results of seed set when irradiated pollen of the mutants was used on the ‘Alexis’ pistils are presented in Table 4. The presentation is separated in Table 4a comprising the seed color mutants

which are supposed to be physiological in nature and Table 4b comprising the morphological mutants. The distribution of the numerical seed set results of the pollination with irradiated mutant pollen on the 'Alexis' pistils was chi square tested for homogeneity over the complete contents of Table 4a and b. As indicated at the bottom of the tables the distribution of the seed set values was heterogeneous at the one per cent level, which means that the seed set results were not random. The reason seems to be the difference between the mutation types in the production of viable cross seed.

Table 4a. Seed set in crosses on 'Alexis' by irradiated physiological pollen mutants. No of seeds.

Mutant type	Normal seeds	Aberrant seeds	Dead seeds	Sum
Pre2 (Red lemma and Pericarp 2)	24	7	32	63
Blp1 (Black lemma 1)	40	7	28	75
Sum	64	14	60	138

Table 4b. Seed set in Alexis by irradiated pollen of morphological mutants. No of seeds.

Mutant type	Normal seeds	Aberrant seeds	Dead seeds	Sum
Ert-r (Erectoides-r)	12	4	40	56
Zeo1 (Zeocriton 1)	7	1	21	29
Sum	19	5	61	85

Chi square: 20.8274**, Df 6 (degrees of freedom), heterogeneous at the one per cent level

Significant heterogeneity was also shown in the "four field chi square analysis" of vigorous to dead seeds in Table 5, i.e. physiological versus morphological mutants. The progenies of the pollinations with irradiated physiological mutant pollen were superior to those pollinated with irradiated morphological mutant pollen. It is interesting to note the difference between the types of mutations in their respective pollen reactions to the irradiation.

Table 5. Number of normal and dead seeds after pollination of 'Alexis' pistils with irradiated physiological and morphological mutant pollen, respectively.

Type of pollen	Normal seeds	Dead seeds	Sum
Physiol. mutant pollen	64	60	124
Morph. mutant pollen	19	61	80
Sum	83	121	204

Chi square: 14.5***, Df 1, significantly heterogeneous.

Description of the F₁ generation.

According to the observations received above the methods for the following experiment could be described as follows.

All F₁- seeds were germinated and grown into F₁-plants in the greenhouse without selection. Observations and notes were made for all combinations of the individual plants and variables and are presented in Table 6. ‘Alexis’ was the female parent of all F₁-crosses as shown in Table 4a and b. The germination was 100 per cent. Therefore, the number of F₁ plants in the different populations in Table 6 are the same as the seed numbers in the crosses given in Table 4a and b. The columns in Table 6 indicate in the top rows the means and variances of Alexis self-pollinated standard population. Further down the columns, the means and variances of the crossing populations with the four different mutant pollinators, are given in groups of four rows with non-irradiated and with irradiated mutant pollen for production of the F₁- populations. There are no differences between means for the use of irradiated versus non irradiated pollen but, on the other hand, there are strikingly different variances in plant variability within the F₁s, which is increased by pollen irradiation before pollination.

Table 6. Means and Variances in F₁ Populations.

	No/spks	No/fl	No/gr	Spike-length	Straw-length	KWGT	TKW gr
Alexis, not irradi.	41.0	38.5	44.8	16.6	237.2	146.4	287.2
Mean	6.5	30.7	30.4	10.9	98.8	9.4	62.2
Ert-r, not irradi.	12.9	54.4	54.4	6.9	271.6	93.0	337.6
Mean	4.9	30.4	29.6	7.1	96.8	11.3	68.8
Ert-r, irradi.	36.0	160.0	1188.5	50.5	990.9	228.9	386.2
Mean	7.0	30.0	20.4	7.4	90.9	9.5	57.3
Zeol, not irradi.	8.9	241.6	308.9	12.1	89.6	84.0	382.4
Mean	5.1	28.8	27.9	6.3	86.2	10.0	68.4
Zeol, irradi.	31.5	24.0	438.8	4.0	333.3	234.3	259.3
Mean	7.5	28.0	18.8	6.0	81.7	11.4	63.7
Pre2, not irradi.	24.9	19.6	19.6	8.5	40.0	84.0	105.8
Mean	5.9	27.8	27.8	9.5	114.0	10.0	69.2
Pre2, irradi.	76.6	196.9	1563.8	34.6	961.8	234.3	1286.6
Mean	8.1	26.7	13.9	9.1	110.1	11.4	60.9
Blp1, not irradi.	78.0	104.4	104.4	26.4	1795.6	408.7	2316.9
Mean	6.0	29.4	29.4	9.4	102.2	11.0	55.9
Blp1, irradi.	124.4	348.8	3221.2	49.7	3988.6	1066.1	1270.7
Mean	8.1	27.9	21.3	9.8	106.6	12.8	61.3

Legend for Table 6: No/spks: number of spikes per plant; No/fl: number of flowers per spike; No/gr: number of grains per spike; Spike length and straw length in cm; KWGT: kernel weight (gr); TKW: thousand kernel weight (gr).

Conclusions.

As demonstrated in Table 6, irradiation of pollen before pollination increases the variability in the majority of the F₁-populations.

In the Tables 7a and b it is shown that the averages for the variables are significantly different for the different mutants. A similar behaviour was shown by the pollen incompatible genotypes of red clover (Denward, 1963) and in the biotype specific resistance genotypes in potato (Denward 1967). The phenomenon is called associate inheritance and is supposed to depend on genes associated to the sites of the mutants. The fact that old observations in clover and potato are now supported by a similar case in barley, are very challenging for further studies. The idea that pleiotropic effects by the barley mutants under study in this paper is not at priority for the present although not disregarded entirely yet.

Table 7a. Comparison between different mutant averages for the seven variables.

F₂ plant populations in greenhouse

	N/n	N/sp	N/fl	N/k	Sp/l	St/l	Kw	Pw
Sum total	300	1892	7670	7465	2733	18537	1531	3744
General subtraction*	299	11932	196096	185754	24898	1145401	7816	46725
Sum square between mut	4	12015	196406	186139	25210	1156489	7875	47094
Sum square within mut	295	12546	199220	190067	25783	1187449	8284	49092
Within square deviation		531	2814	3928	573	30960	409	1998
Between square deviation		83	310	385	312	11088	59	369
Mean square between mut		21	77	96	78	2772	15	92
Mean square within mut		2	19	13	2	105	1	7
Quotients		11	4	7	40	26	11	14
P<2,40*; 3,38**; 4,8***		***	**	***	***	***	***	***

Legend for table 7a: * General subtraction term (degrees of freedom; df 299); N/n: No of plants, N/sp: No of spikes, N/fl: No of flowers per spike, N/k: No of kernels per spike, Sp/l: spike length (cm), St/l: straw length (cm), Kw: kernel weight (gr), Pw: plant weight (gr).

Table 7b. Comparison between different mutant averages for the eight variables.

F₃ plant progeny rows in the field

	St/l	ldg1	ldg2	grain	ml	Rph	var	uni
Sum total	24280	2623	2464	2129	2891	2230	1077	9733
General subtraction*	1965061	22934	20238	15109	27860	16576	3866	315771
Sum square between mut	1976546	23247	20698	15276	27872	17173	4692	350219
Sum square within mut	2001600	23955	21714	19319	28173	17920	5741	379519
Within square deviation	25054	708	1016	4043	301	747	1049	29300
Between square deviation	11485	314	460	167	12	597	826	34448
Mean square between mut	2871	78	115	42	3	149	207	8612
Mean square within mut	85	2	3	14	1	3	4	99
Quotients	34	33	33	3	3	59	58	87
P<2.4*; 3.3.38**, 4.8***	***	***	***	*	*	***	***	***

Legend for table 7b: * General subtraction term (degrees of freedom; df 299); St/l = straw length (cm); ldg1 = Resistance to lodging 1; ldg2 = Resistance to lodging 2 (lodging data are taken at two different occasions); grain = filled grain; ml = mildew resistance; Rph = Barley rust resistance, var = visual variability; uni = uniformity.

The calculations were performed according to Bonnier and Tedin, 1940. They were made in one operation over the four mutants plus 'Alexis' and 15 variables. The results were presented in one original table that however for printing technical reasons are divided into Table 7a and 7b. The contents are identical with the original table.

A couple of more observations during the course of the experimental work above should be mentioned here. It is evident that the irradiation at the unicellular stage of the development cycle of the organism, in this case the barley pollen, does reveal an almost unlimited recombination. It is not only a question of a physical reshuffling of parts of the genetical structure. The phenomenon seems rather to be dependant on a chemical melting down to the minute parts of the DNA. If that is the case, then, theoretically the recombination could be unlimited. Could the organism retain its genetic basis of varietal characteristics by a rigid structure of phospholipidholding lamella and microtubules? Some cytological observations point in that direction (Denward, unpublished). Furthermore, no identical male gametes (pollen) as shown by F₁-plants were revealed. All of the individual pollen grains of the pollen population were affected by the irradiation at anthesis as far as can be judged for the present. To substantiate these two latter possibilities requires the technique of molecular cytogenetics.

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The reader who requires a complete presentation of basic figures in this investigation is advised to: www.t.d.breeding.se