## STUDIES ON MUTATIONS IN PEAS; COMBINED TREATMENT BY NF AND NEU

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Our mutation studies at Wiatrowo, using combined doses Nf + NEU, are aimed at finding and characterizing new mutants of pea. We seek genes which not only are new, but also are of some importance for breeding and cultivation. An hypothesis about the influence of the joint action of genotype x mutagen on the spectrum of induced mutations was also tested, the main points of which are summarized here.

Though the combined action of mutagens in Ml was stronger than the same dose of each mutagen used separately, it was less than additive. The effect of a combined treatment with Nf and NEU in Ml was strongly dependent on which mutagen was administered first. Increasing doses of Nf administered after one and the same dose of NEU caused an increase of Ml damage whereas increasing doses of NEU after one and the same dose of Nf increased the Ml damage less or, for certain characters, not at all.

On the basis of preliminary investigations (4, 5) the optimum dose of Nf + NEU has been chosen for line Wt 3527 (200r + 0.014% - 25% reduction of M1 seedling length) and Wt 4042 (500r + 0.014% - 50% reduction of seedling length) and, for comparison, Nf and NEU separately.

The results indicated that lower doses, causing less Ml damage, could, depending on genotype of the treated material, be the most effective. The analysis of mutation frequency and spectrum in M2 confirmed this (6, 7).

From a population of 30,000 M2 plants (5,000/dose = 500 families x 10 plants) 1314 mutation events have been selected (Table 1). Comparisons of the mutagens used, viz.Nf., NEU, Nf + NEU, based on the kinds and frequency of mutations in M2, showed that NEU was more effective than Nf and that the combined treatments gave higher values still. Again the effect of combined treatments was less than additive. The mutagenic effectiveness of the combined dose used for Wt 3527 was very high: 432 mutation cases found in 500 M2 families. Thus, for a given genotype, a dose can be chosen to yield almost one mutation for each treated seed. The number of mutation events calculated as a percent of M, families seems to be a more suitable expression than the number of mutants per 100 or 1,000 M2 plants as is commonly reported in the literature. Combined doses also induced a higher number of multi-mutant homozygotes (Fig. 1).

Mutants were classified and named according to PGA gene rules and guidelines (see Induced mutation spectrum based on 1314 mutations [8]). Interestingly, the results indicate that the combined treatments in comparison to the mutagens used separately acted as a different, third mutagen. This is evident from the distribution of mutation types. Some types were, at least in this experiment, specific for a given mutagen, i.e. types induced by only one of the mutagens in a treated genotype. Some can even be said to be doubly specific, i.e. they appear only after treatment with "one" of the mutagens (combined treatment here regarded as a separate mutagen) in one of the genotypes (numbers in brackets, Table 2). Such specific types were induced by each mutagen, with the greatest number appearing in Wt 3527 after combined treatments. Nf, which was clearly less effective with regard to number of mutations, induced twice as many specific mutations per induced mutant than the combined treatments and three times as many as NFL. Among the specific mutation types, hitherto unknown genes, e.g. orange pod, necrosis, were

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recovered. Moreover, the spectrum of induced mutations was found to be influenced by genotype x mutagen interactions.

The induced mutants resulting from these investigations are preserved in gene banks. Almost immediately after some of them were described they became the subject of investigation in various laboratories (1, 2, 3) as well as in our own breeding projects. After the locus identity test crosses had been performed the new mutants were used in mapping studies (8, 9).

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Fig. 1. Induction of multiple mutants. A-Percent of families with more than one mutation; B-number of double mutants.

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Table 1. Number of mutations and mutation rates in my.	rab.	le .		Number	of	mutations	and	mutation	rates	in	M2 .	
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			%		%		% M <sub>2</sub>
Line	Dose	No.	Sown	No.	Sown	No.	families
Wt 3527	200r	71	14.2	127	2.54	77	15.4
	200r + 0.014%	281	56.2	653	13.06	432	86.4
	0.014%	260	52.0	590	11.80	406	81.2
Wt 4042	500r	77	15.4	149	2.98	90	18.0
	500r + 0.014%	119	23.8	272	5.44	159	31.8
	0.014%	109	21.8	247	4.94	150	30.0

Table 2. Number of mutation types specific for a given mutagen and genotype.

Mutagen	Wt	3527	of mutation cases	Wt 4042
Nf	7	(5)	1/11	8 (3)
Nf + NEU	24	(18)	1/18	7 (3)
NEU	14	(7)	1/29	14 (6)