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Mutagenicity of a new dimethyl nitroso compound in pea (*Pisum sativum* L.)

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Abstract

A new nitroso-urea compound (nitroso dimethyl urea, NDMU) with two methyl groups was tested in two pea varieties (Early Superb and Mahndofer) under seed treatment with 0.02% concentration applied for 6 hours and compared with its single methyl analogue, N-nitroso methyl urea (NMU). The variety Early Superb was less mutable (mutation rate 2.1%) than Mahndorfer (13.3%). NDMU had slightly superior mutagenicity (7.3% mutations) over NMU (6.1%), although the new mutagen is much less toxic than NMU. Thus, the mutation rates can be further enhanced with higher doses of NDMU, which makes it particularly suitable for mutagenesis in higher plants.

Key words : Nitroso dimethyl urea, NDMU, toxicity, mutagenicity, pea

Introduction

The chemical compounds belonging to the N-nitroso group are the most efficient mutagens known so far [1-3]. Considering their extremely high and universal mutagenicity, they were fancifully called "Supermutagens" by their discoverer [4]. Among the three groups of N-nitroso monoalkylating mutagens (urethanes, ureas and guanidines), the nitroso ureas are believed to be most efficient in all biological systems, including cultivated plants [5]. The supermutagenicity of N-nitroso compounds continues to remain an enigma although several reasons have been given to explain their exceptional properties such as slow hydrolysis and longer life in solution, nitrosylation, enhanced reactivity, mutagenic effects of immediate and distant products of degradation, and so on. N-nitroso alkyl ureas have been demonstrated to cause much fewer chromosomal damage while retaining high mutagenicity estimated on the basis of mutations appearing in the M_2 generation [6]. They are also much less damaging in terms of plant survival after seed treatment [7]. However, these mutagens reduce fertility of M_1 plants drastically even at the doses which may not reduce seed germination or plant survival noticeably.

Another chemical, N-nitroso dimethyl urea (NDMU), has been added to this group of distinguished mutagens. Although it carries two methyl groups, both attached to the same nucleus of nitrogen atom to which the nitroso group is bound, its bifunctional nature has not been demonstrated. It was therefore necessary to investigate the performance of this mutagen and determine its suitability in mutation breeding of crop plants.

The mutagen was received from the laboratory of Prof. I. A. Rapoport in Moscow, for which the author is grateful.

Materials and methods

The experiment was conducted in the fields of Division of Genetics, Indian Agricultural Research Institute, New Delhi.

Dry seeds of two pea varieties, Early Superb (early, dwarf, green seeded) and Mahndorfer (medium, tall, white seeded), were treated with 0.02% NMU and NDMU for 6 hours. The treated seeds (500 in each treatment) were directly sown, and M_1 plants harvested individually. Observations in the M_2 generation were recorded through the entire period of crop growth. Mutation frequency was calculated as percentage of mutated M_2 progenies as well as number of distinguishable mutational events per 100 M_2 progenies (% mutations), which takes into account multiple mutations of various kind in each mutated plant progeny.

Results and discussion

The results on toxic effects of mutagenic treatment, expressed as survival rate of the treated plants in the M_1 generation, are presented in Table 1. The severe toxicity of NMU is evident as only 7.6% plants of var. Early Superb and 15.4% of Mahndorfer lived up to seed setting stage. In contrast, plant survival improved manifold with the same dose of NDMU and 80.4% plants of Early Superb and 64.4% of Mahndorfer produced seeds.

Strong toxic effects of NMU are well established. It is surprising that addition of one more methyl group to its molecule conspicuously reduced its toxicity in both genotypes. Among the varieties, Early Superb was more sensitive to NMU than Mahndorfer, while NDMU was slightly more toxic to Mahndorfer (64.4% survival) than to Early Superb (80.4%). The overall response of the two varieties to mutagenic treatment is reduced (44.0% survival in Early Superb, 39.9% in Mahndorfer) because of the differential manifestation of toxicity in the two genotypes (Table 1).

Table 1. Toxic effects of NMU and NDMU in M_1 generation of pea

Treatment (0.02%-6h)	No. of seeds treated	No. of M_1 plants harvested	Plant survival %
Variety Early Superb			
NMU	500	38	7.6
NDMU	500	402	80.4
Variety Mahndorfer			
NMU	500	77	15.4
NDMU	500	322	64.4

The difference in the mutation inducing ability of the two analogous alkylating chemicals was unequivocal (Table 2). NMU did not cause any mutations in the variety Early Superb and mutated only 7.8% M_2 progenies of Mohndorfer with 9.1% mutation rate. NDMU, in contrast, mutated 2.2% progenies of Early Superb and 13.3% in Mahndorfer (14.3% mutations).

Table 2. Mutation frequency in M_2 generation of pea

Treatment (0.02%-6h)	M_2 progenies			Mutational events	
	total	mutated	% mutated	total	% mutation
Variety Early Superb					
NMU	38	0	0	0	0
NDMU	400	9	2.2	9	2.2
Variety Mahndorfer					
NMU	77	6	7.8	7	9.1
NDMU	322	43	13.3	46	14.3

A comparison of the two varieties, averaged over mutagenic treatments (Table 3), shows that Mahndorfer is six-fold more mutable with 13.3% mutations than Early Superb (2.1%).

Table 3. Toxic and mutagenic effects of NMU and NDMU on two genotypes of pea

Treatment (0.02%-6h)	No. of seeds treated	Plant survival in M_1 %	Mutation rate (%) on the basis of	
			M_2 progenies	mutational events
Varieties :				
Early Superb	1000	44.0	2.1	2.1
Mahndorfer	1000	39.9	12.3	13.3
Mutagens :				
NMU	1000	11.5	5.2	6.1
NDMU	1000	72.4	6.8	7.3

The mutation frequency, when averaged over the genotypes, did not reveal a large difference between the two mutagens (mutation rate 6.1% with NMU, 7.3% with NDMU). This was a consequence of the poor mutability of var. Early Superb. The impact of high mutagenicity of NDMU was apparent in the var. Mahndorfer in which 14.3% mutations were recorded as against the highest of 2.2% in Early Superb.

The comparison of the two related chemicals for their mutagenicity vis-a-vis toxic effects presents an interesting picture. As stated above, NMU did not induce very high mutation rates in spite of severe toxicity. NDMU, on the other hand, although much less toxic, especially in the var. Mahndorfer, induced mutations with several times greater efficiency.

It is known that toxicity and mutagenicity of mutagens do not always run parallely. Nevertheless, it has been demonstrated beyond doubt that within a series of analogous chemicals the toxicity increases and mutagenicity decreases as the molecule increases in size with addition of new chemical groups or polyfunctionality as a result of the same active group repeated in the enlarging molecule. Generally, chemical reactivity declines with reduced penetration and mobility of the heavier molecules.

Such a trend is not observed in the present case. NDMU with a larger molecule due to an additional methyl group, instead of showing higher toxicity, was less toxic (72.4% survival over both varieties) than its single-methyl analogue NMU (11.5% survival). This would increase the efficiency of NDMU by a large margin, which makes this mutagen particularly suitable for mutation breeding in crop improvement.

The lower toxicity of NDMU combined with high mutagenicity can be explained only by assuming that this mutagen causes less cytological and physiological damage. This is also reflected in higher fertility of the NDMU treated M_1 plants. High mutation rate with lower cytotoxicity was demonstrated for 1,4-bis diazoacetyl butane in Drosophila by [8]. For other N-nitroso compounds low rate of chromosomal aberrations and higher mutation frequencies compared to ionizing radiation in pea was reported earlier [6]. NDMU appears to be another striking example of a nitroso-compound with similar effect.

The range of mutation types (spectrum) induced by NMU and NDMU in the two varieties is presented in Table 4. As would be expected, depending on the total number of mutations induced, the mutation spectrum was narrower (6 types) in the NMU treatments, and only 5 types of mutations were induced in var. Early Superb. NDMU induced 16 different types of

Table 4. Spectrum of mutations induced by NMU and NDMU in two varieties of pea

Mutagen or variety	Total mutational events	No. of mutation types	Albina	Xantha	Chlorina	Viridis (fertile)	Viridis (sterile)	Golden green	Bluish green	Xantho-viridis	Xa- maculata	Al-terminalis	Light yellow with crumpled leaf	Fasciata	Waxless	Heavy wax	Laciniata	Sterile	Late	Wrinkled seed
Mutagens :																				
NMU	7	6			+			+		+				+				+	+	
NDMU	55	16	+	+	+	+	+	+	+		+	+	+		+	+	+	+	+	+
Varieties :																				
Early Superb	9	5			+	+		+									+	+		,
Mahndorfer	53	17	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+

mutations, and the more mutable var. Mahndorfer responded by producing mutations in 17 loci. It was possibly only a matter of chance that *xantho-viridis* and *fasciata* mutations were not induced by NDMU although many other types were isolated.

The dependence of mutation spectrum on the total number of mutations induced in a particular treatment has been demonstrated [9]. A rigid specificity of mutagenesis in qualitative terms has not been established as mutation induction, by and large, remains a random process. This fact has been again emphasized by the results of this study. Thus, inducing mutations in high frequencies appears to be the only way to ensure a wider spectrum and recovery of even rare mutation types. These requirements make the less toxic and highly mutagenic agents like NDMU particularly suitable for applied uses of induced mutagenesis.

The highest mutation rate observed with 0.02%-6h NDMU treatment was 14.3% in var. Mahndorfer. However, this was at 64.4% M_1 survival. NMU induced 9.1% mutations in the same genotype at 15.4% survival. Thus, a clear possibility exists for increasing mutation rate with much wider spectrum by increasing the dose of NDMU to the extent that M_1 survival is brought down to 25-30%.

References

1. **Rapoport I. A.** 1961. Chemical mutations in the sex chromosome at a rate higher than 50% with increased proportion of semilethals [in Russian]. Dokl. Akad. Nauk SSSR, **141**: 1476-1479.
2. **Rapoport I. A.** 1961. 85% mutations in the sex chromosome under treatment with nitrosoethyl urea [in Russian]. Dokl. Akad. Nauk SSSR, **146**: 1418-1421.
3. **Rapoport I. A.** 1963. Crossing the universal mutational barrier with more than 100% mutations of X chromosome [in Russian]. Dokl. Akad. Nauk SSSR, **148**: 696-699.
4. **Rapoport I. A.** 1966. Peculiarities and mechanism of action of supermutagens [in Russian]. *In*: Supermutagens (ed. I. A. Rapoport). Nauka Publishers, Moscow: 9-23.
5. **Sharma B.** 1966. Influence of some physical and chemical agents on the mutation process of pea (*Pisum sativum*) [in Russian]. Genetika (Soviet Genetics), **2**: 45-52.
6. **Sharma B.** 1968. On the diverse aberration-mutation ratio induced by NMU. Curr. Sci., **37**: 675-676.
7. **Sharma B.** 1965. Physical and Chemical Mutagenesis in Pea [in Russian]. Ph. D. Thesis. Timiryazev Agricultural Academy, Moscow (USSR).
8. **Rapoport I. A.** 1960. Mutational effect of 1,4-bis diazoacetyl butane [in Russian]. Dokl. Akad. Nauk SSSR, **130**: 1134-1137.
9. **Sharma B. and Bansal H. C.** 1972. The multiple-cluster mutation complex in mutagenesis with higher plants. Theor. Appl. Genet., **42**: 25-31.