An alternative index for mutagenic efficiency

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ABSTRACT

Mutagenic efficiency was defined by Konzak et al. in 1965 as the ratio mutation/biological damage factor. His formula considers that mutagens always causes damage, but it has been shown that low doses of gamma-rays stimulate seedling growth. In this situation a negative value for damage is obtained with a negative mutagenic efficiency rate. This has no biological interpretation and the notion of range for mutagenic efficiency variation is also lost. An alternative index for mutagenic efficiency, which is a modification of the Konzak et al. 1965 formula, is proposed [mutation factor (1-damage/100)]. This index is more general, with known limits and positive results, thus overcoming some of the limitations found in the original formula.

KEY WORDS: Rice, mutation, mutagenic efficiency.

INTRODUCTION

According to Gustafsson and Gadd (1966), De Vries was, in 1901, the first scientist to use artificial mutation in genetic breeding. Since then, the artificial mutation technique has been used to develop several cultivars (Micke et al., 1985). However, the technique used is labor intensive and produces a low frequency of mutation which is difficult to detect. The increase in the dosage of the mutant agents in order to increase the mutation frequency often results in more than proportionally unfavorable effects in the target populations.

Ehrenberg et al. (1958), among others, introduced the terminology mutation efficiency to compare the effects of ionized radiation and mutant chemical agents. The efficiency is evaluated by the induced mutation rate relative to other undesirable biological effects such as lethality and sterility which cause mutant elimination.

Konzak et al. (1965) and Nilan et al. (1965) defined mutation efficiency as the "mutation factor"/"biological damage" ratio. The criteria used to evaluate the biological damage were seedling height, survival, meiotic and mitotic chromosomal aberrations and, specially, the "mutant factor/ sterility" ratio.

The mutant efficiency can be evaluated by the ratios Msp/L, Msp/I and Msp/S (Konzak et al., 1965), where:

Msp = Mutations / 100 M1 spikes;
L = % of survival reduction;
I = % of seedling height reduction;
S = % of reduction in fertility.

Konzak et al. (1965) formulas always consider mutagenic substances as damage-causing agents and, consequently, the percentage of loss will be always positive. However, low doses of gamma radiation frequently cause stimuli, resulting in a negative "I" value and negative and meaningless efficiency values.

In the present work, a modification to the formulas of Konzak et al. (1965), is presented to make them more general and always positive.

MATERIAL AND METHODS

An example of the derivation will be given for the Msp/I efficiency because the "I" damage is sometimes involved in negative efficiencies.
From Konzak et al. (1965) original formula:

$$Msp = \frac{Nm}{Np} \cdot 100$$

$$I = \frac{To - Tr}{To} \cdot 100$$

Where:
Msp = number of mutations per 100 M_1 spikes
I = % of seedling height reduction
Nm = number of mutations
Np = number of M_2 seedlings
To = height of the seedling control
Tr = height of seedling due to mutagenic treatment

$$\frac{Msp}{I} = \frac{Nm}{Np} \cdot 100 \left(\frac{1}{\frac{To - Tr}{To} \cdot 100} \right) = \frac{Nm}{Np} \cdot \frac{To}{To - Tr}$$

Proposed index:

$$\frac{Nm}{Np} \cdot \frac{Tr}{To} \cdot 100 = \frac{Nm}{Np} \cdot 100 \cdot \frac{Tr}{To}$$

$$= \frac{Nm}{Np} \cdot 100 \; \frac{To - To + Tr}{To} = \frac{Nm}{Np} \cdot 100 \left(1 - \frac{To - Tr}{To} \right)$$

$$= Msp \left(1 - \frac{I}{100} \right)$$

The theoretical limits of these indexes are shown below for comparison:

The index of Konzak et al. (1965) gives rise to values without interpretation when Tr = To (the efficiency has an infinite value) or when Tr > To (causing a negative efficiency), whereas the proposed index always has a positive value.

Situation	Konzak et al.(1965)	Proposed
Tr < To	$a \frac{Nm}{Np}$	$+b \frac{Nm}{Np} . 100$
Tr = To	infinite	$\frac{Nm}{Np} . 100$
Tr > To	$-a \frac{Nm}{Np}$	$+b \frac{Nm}{Np} . 100$

$a = \frac{To}{To - Tr} = \frac{100}{I}$, $b = \frac{Tr}{To}$

Results from a study of mutagenic efficiency combining gamma rays and sodium azide (Montalván, 1995) were used to illustrate the application of the proposed efficiency index.

RESULTS AND DISCUSSION

Table 1 shows the estimates of Msp, I, Msp/I and Msp(1-I/100) reported by Montalván (1995). The highest and the lowest points correspond to the Msp/I from treatment (pH 3 and 15Kr) with the value 6.33/3.18 and treatment (pH 7 and 15 Kr) with the value 4.6/-2.68, respectively. At the lowest point the effect of the stimulus of a negative I is observed. This situation precludes an understanding of the range of efficiency variation.

Table 1 - Frequency of mutation at spikes level (Msp), injury (I), and Msp/I and Msp (1-I/100) efficiencies.

Treatments Gamma rays (KR) + SA (mM)	Msp	I	Msp/I	Msp [1/]-I/100)
Control, distilled water	0.40	0.00	IC*	0.40
10 + distilled water	7.26	14.04	0.52	6.24
15 + distilled water	4.60	-2.68	-1.72	5.83
20 + distilled water	5.43	7.92	0.69	5.00
30 + distilled water	5.84	25.81	0.23	4.33
Control (pH 3)	0.42	5.12	0.08	0.40
15 + pH 3	6.33	3.18	1.99	6.13
30 + pH 3	8.63	31.09	0.28	5.95
0 + 0.5 (pH 3)	14.04	14.99	0.94	11.93
10 + 0.5	11.52	19.24	0.60	9.30
20 + 0.5	15.23	31.54	0.48	10.43
0 + 1,0	58.92	57.89	1.02	24.81
10 + 1.0	29.08	27.91	1.04	20.98
15 + 1.0	22.11	57.47	0.38	9.38
20 + 1.0	15.93	54.17	0.29	7.30
30 + 1.0	17.86	85.59	0.21	2.57
0 + 5.0 (pH 3)	60.63	76.92	0.79	13.99
15 + 0.5	27.15	80.06	0.341	5.41
30 + 5.0	14.81	89.39	0.17	1.57

[1/] IC = incalculable

On the other hand, when the control treatment (Treatment 1, Table 1) is considered as a reference to the calculation of the damage caused by the mutagenic treatments, it assumes the value zero, and therefore the calculation of its efficiency is impossible. Generally the controls, however, have a value for the factor of mutations originating from spontaneous mutation. Therefore, the control has an efficiency value which could be considered as a reference when analyzing the efficiency of mutagenic treatments. In the alternative proposed index, it is possible to calculate the efficiency of the controls. Depending on the value of the spontaneous mutation measured by the control, a more exact efficiency index will reduce this effect in the mutation treatment factor. This index would be:

E. M. = Msp_{corr} x (1 - i/100), where Msp_{corr} = [Msp(Tr) - Msp (To)] and Msp(Tr) and Msp(To) are the Msp of the mutagenic treatment and control, respectively.

By generalizing, the proposed formulas of mutagenic efficiency become:

E. Mut.(1) = F.M. x (1 - Damage/100)

E. Mut.(2) = $F.M._{corr.}$ x (1-Damage/100)

F.M. refers to the mutation factor (Msp, Msd, etc.) and the damage refers to injury, lethality, etc. The corrected mutation factor is $F.M._{corr.}$ = F.M.(Tr) - F.M.(To), where F.M.(Tr) and F.M.(To) are the mutation factor of the mutagenic treatment and control, respectively.

Konzak et al. (1965) established that the mutagenic efficiency is directly related to the mutation factor and inversely related to damage. However, as the mutation factor, based on the panicles (Msp) or the seedlings (Msd) has much lower values than the damage, the efficiency calculations are largely affected by the damage values. Thus, the possibility of differentiating the efficiency of the treatments is reduced.

In the proposed index, the efficiency is calculated as being directly related to the mutations factor (corrected or not) and to the prevented or avoided damage effect (1-I/100). Thus, negative values are avoided in the efficiencies, and the damage is magnified without reducing its importance.

The oversensitivity of the Konzak et al.(1965) index is due to low values of I, as can be seen in Tables 2 and 3 that show the efficiency values calculated from simulations, and also in Figure 1 that shows data from the diagonals, with a negative slope of Tables 2 and 3. Figure 1 shows, in a single graph, the multiple calculation of both indexes with the use of these diagonals. It can be seen that the index of Konzak et al. (1965) acquires large values when Msp = 2.51 and I = 0.15. The proposed index has a value of 2.506 which supports the findings of other simulated situations.

The index of Konzak et al. (1965) also has a tendency to rise after the observed peak and decline slightly when a high I value (I = 90) is used. The decrease is proportionally greater in the case of the proposed index, indicating that it emphasizes damage, showing sharper falls in the efficiency (from 16.3 to 4.8 in Figure 1).

The oversensitivity of the Konzak et al. (1965) index, in relation to extreme damage values and negative efficiencies, leads to the reference loss of the extremes of the mutation efficiency index. This can be considered a limitation of the method.

Table 2 - Mutagenic efficiency evaluated through Msp/I for simulated values of Msp and I, the numbers in the first column on the left are Msp values; and the first line at the top corresponds to I.

	-3	-2	0	0.01	0.1	0.15	2	8	18	23	28	33	38	43	48	53	58	63	90
0,00	0,00	0,0	N.C.	0,0	0,0	0,00	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000
0,009	-0,00	-0,00	N.C.	0,9	0,09	0,06	0,005	0,001	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000
0,01	-0,00	00000	N.C.	1,0	0,1	0,07	0,005	0,001	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000
0,51	-0,05	-0,08	N.C.	51	0,1	0,07	0,005	0,001	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,006
1,00	-0,33	-0,5	N.C.	100	10,0	6,67	0,500	0,125	0,056	0,043	0,036	0,030	0,026	0,023	0,021	0,019	0,017	0,016	0,011
2,51	-0,84	-1,26	N.C.	251	25,1	16,73	1,255	0,314	0,139	0,109	0,090	0,076	0,066	0,058	0,052	0,047	0,043	0,040	0,028
3,01	-1,00	-1,51	N.C.	301	30,1	20,07	1,505	0,376	0,167	0,131	0,108	0,091	0,079	0,070	0,063	0,057	0,052	0,048	0,033
4,01	-1,34	-2,01	N.C.	401	40,1	26,73	2,005	0,501	0,223	0,174	0,143	0,122	0,106	0,093	0,084	0,076	0,069	0,064	0,044
8,01	-2,67	-4,01	N.C.	801	80,1	53,4	4,005	1,001	0,445	0,348	0,286	0,243	0.211	0,186	0,167	0,151	0,138	0,127	0,089
12,01	-4,00	-6,01	N.C.	1201	120,1	80,07	6,005	1,501	0,667	0,522	0,429	0,364	0,316	0,279	0,250	0,227	0,207	0,191	0,133
16,01	-5,34	-8,01	N.C.	1601	160,1	106,7	8,005	2,001	0,889	0,696	0,572	0,485	0,421	0,372	0,334	0,302	0,276	0,254	0,178
20,01	-6,67	-10	N.C.	2001	200,1	133,4	10,01	2,501	1,112	0,870	0,715	0,606	0,527	0,465	0,417	0,378	0,345	0,318	0,222
24,01	-8,00	-15	N.C.	2401	240,1	160,1	12,01	3,001	1,334	1,044	0,858	0,728	0,632	0,558	0,500	0,453	0,414	0,381	0,267
28,01	9,34	-14	N.C.	2801	280,1	186,7	14,01	3,501	1,556	1,218	1,000	0,849	0,737	0,651	0,584	0,528	0,483	0,445	0,311
32,01	-10,7	-16	N.C.	3201	320,1	213,4	16,01	4,001	1,778	1,392	1,143	0,970	0,842	0,744	0,667	0,604	0,552	0,508	0,356
36,01	-12,00	-18	N.C.	3601	360,1	240,1	18,01	4,501	2,001	1,566	1,286	1,091	0,948	0,837	0,750	0,679	0,621	0,572	0,400
40,01	-13,3	-20	N.C.	4001	400,1	266,7	20,01	5,001	2,223	1,740	1,429	1,212	1,053	0,930	0,834	0,755	0,690	0,635	0,444
44,01	-14,7	-22	N.C.	4401	440,1	293,4	22,01	5,501	2,445	1,913	1,572	1,334	1,158	1,023	0,917	0,830	0,759	0,699	0,489
48,01	-16,00	-24	N.C.	4801	480,1	320,1	24,01	6,001	2,667	2,087	1,715	1,455	1,263	1,117	1,000	0,906	0,828	0,762	0,533

Table 3 - Mutagenic efficiency evaluated through Msp(1-/100) for simulated values of Msp and I, the numbers in the first column on the left are Msp values; and the first line at the top corresponds to I.

	-3	-2	0	0.01	0.1	0.15	2	8	18	23	28	33	38	43	48	53	58	63	90
0,00	0,000	0,000	0,00	0,00	0,0	0,00	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000
0,009	0,009	0,009	0,009	0,009	0,009	0,009	0,009	0,008	0,007	0,007	0,006	0,006	0,006	0,005	0,005	0,004	0,004	0,003	0,000
0,01	0,010	0,010	0,01	0,01	0,010	0,010	0,010	0,009	0,008	0,008	0,007	0,007	0,006	0,006	0,005	0,005	0,004	0,004	0,001
0,51	0,153	0,153	0,15	0,15	0,150	0,150	0,147	0,138	0,123	0,116	0,108	0,101	0,093	0,086	0,078	0,071	0,063	0,056	0,051
1,00	1,030	1,020	1,00	1,00	0,999	0,999	0,980	0,920	0,820	0,770	0,720	0,670	0,620	0,570	0,520	0,470	0,420	0,37	0,100
2,51	2,585	2,560	2,51	2,51	2,507	2,506	2,460	2,309	2,058	1,933	1,807	1,682	1,556	1,431	1,305	1,180	1,054	0,929	0,251
3,01	3,100	3,070	3,01	3,01	3,007	3,050	2,950	2,769	2,468	2,318	2,167	2,017	1,866	1,716	1,565	1,415	1,264	1,114	0,301
4,01	4,130	4,090	4,01	4,01	4,006	4,004	3,930	3,689	3,288	3,088	2,887	2,687	2,486	2,286	2,085	1,885	1,684	1,484	0.401
8,01	8,250	8,170	8,01	8,009	8,002	7,998	7,850	7,369	6,568	6,168	5,767	5,367	4,966	4,566	4,165	3,765	3,364	2,964	0.801
12,01	12,37	12,25	12,01	12,01	12,00	11,99	11,77	11,05	9,848	9,248	8,647	8,047	7,446	6,846	6,245	5,645	5,044	4,444	1.201
16,01	16,49	16,33	16,01	16,01	15,99	15,99	15,69	14,73	13,13	12,33	11,53	10,73	9,926	9,126	8,325	7,525	6,724	5,924	1.601
20,01	20,61	20,41	20,01	20,01	19,99	19,98	19,61	18,41	16,41	15,41	14,41	13,41	12,41	11,41	10,41	9,405	8,404	7,404	2.010
24,01	24,73	24,49	24,01	24,01	23,99	23,97	23,53	22,09	19,69	18,49	17,29	16,09	14,89	13,69	12,49	11,28	10,08	8,884	2.401
28,01	28.85	28,57	28,01	28,01	27,98	27,97	27,45	25,77	22,97	21,57	20,17	18,77	17,37	15,97	14,57	13,16	11,76	10,36	2.801
32,01	32,97	32,65	32,01	32,01	31,98	31,96	31,37	29,45	26,25	24,65	23,05	21,45	19,85	18,25	16,65	15,04	13,44	11,84	3.201
36,01	37,09	36,73	36,01	36,01	35,97	35,96	35,29	33,13	29,53	27,73	25,93	24,13	22,33	20,53	18,73	16,92	15,12	13,32	3.601
40,01	41,21	40,81	40,01	40,01	39,97	39,95	39,21	36,81	31,81	30,81	28,81	26,81	24,81	22,81	20,81	18,80	16,8	14,8	4.001
44,01	45,33	44,89	44,01	44,01	43,97	43,94	43,13	40,49	36,09	33,89	31,69	29,49	27,29	25,09	22,89	20,68	18,48	16,28	4.401
48,01	49,45	48,97	48,01	48,01	47,96	47,94	47,05	44,17	39,37	36,97	34,57	32,17	29,77	27,37	24,97	22,56	20,16	17,76	4.801

Theoretically, this index goes from negative to positive values, both without known limits. The proposed index has established theoretical limits. The ideal mutagenic index happens with a 100% mutation factor and 0% damage factor. This index cannot be calculated using Konzak et al. (1965) formulas, and a damage value near zero efficiency could suddenly rise. This does not happen with the proposed index. Zero, or near zero damage values result in similar values for a determined mutation factor.

RESUMO

Um índice alternativo para eficiência mutagênica

A eficiência mutagênica foi definida por Konzak et al. em 1965 como a proporção dos fatores taxa de mutação/dano biológico. A fórmula por estes autores considera que o mutagênico sempre causa dano, todavia, tem sido verificado que baixas dosagens de raios gama estimulam o crescimento

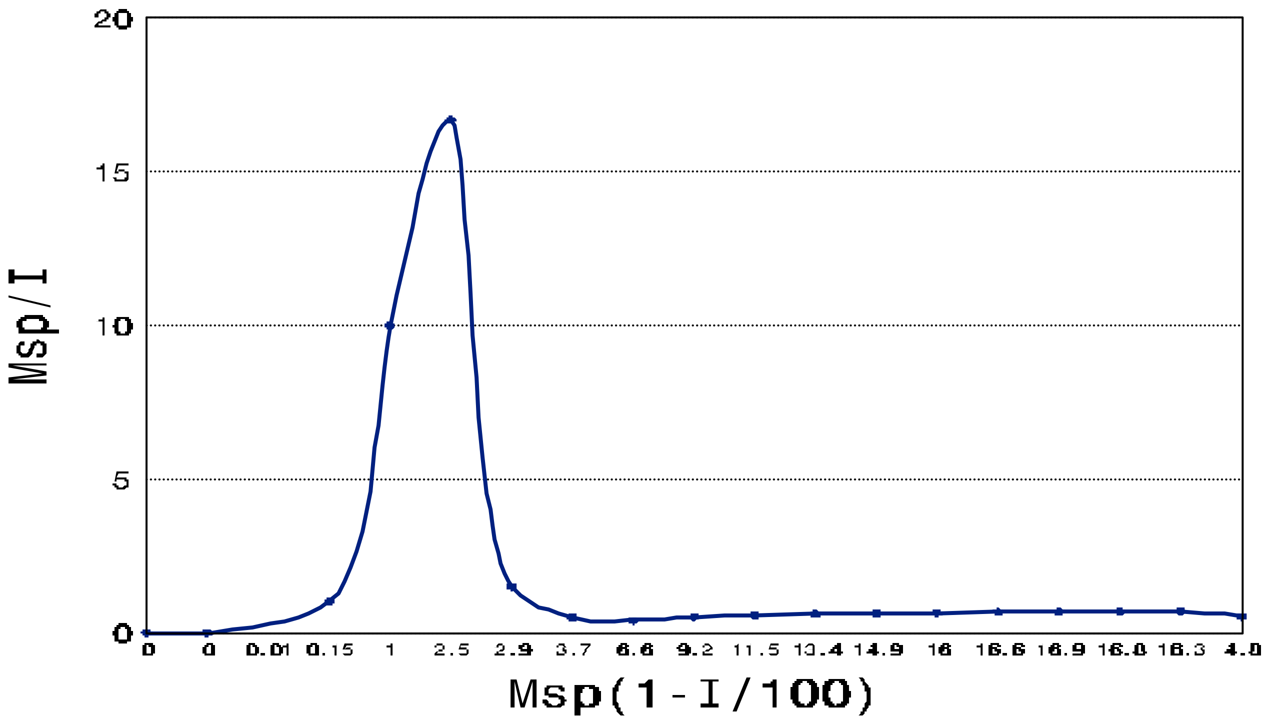

Figure 1 - Comparison among the values of the indexes of mutagenic efficiency Msp/I and Msp(1-I/100) for simulated values taken from the diagonal with a negative slope from Tables 2 and 3.

de plântulas. Nesta situação, um valor negativo para dano origina uma eficiência mutagênica negativa, que não têm interpretação biológica e mais ainda, a noção de intervalo de variação deste é também perdida. Um índice alternativo para eficiência mutagênica, que é uma modificação da fórmula de Konzak et al. 1965, é proposto neste estudo: [fator de mutação (1 – dano / 100)]. Este índice é mais geral com limites conhecidos e sempre positivos, superando desse modo algumas das limitações encontradas na fórmula original.

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Received: February 27, 2001;
Accepted: July 12, 2001.