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The Use of Induced Micro-Mutations for Quantitative Characters after EMS and Gamma Ray Treatments in Durum Wheat Breeding

Mehmet Ali Sakin
Faculty of Agriculture, Department of Agronomy, University of Gaziosmanpasa
60240, Tokat, Turkey

Abstract: Genetic variability resulted from micro-mutations allows breeding of quantitative characters and remains through next generations. The aim of the study was to: (i) determine induced variability of yield and the other quantitative characters such as plant height, number of fertile tiller, spike length, after selection in single plant grain yield and (ii) evaluate the applicability of micro-mutations in durum wheat breeding. Test populations were M_2 and M_3 generations of durum wheat variety called "Sofu" resulting from EMS (Ethyl Methane Sulphonate) for 8 hours at 24 °C without presoaking or gamma ray treatments. Base populations were constructed by selecting normal appearing single plants from the M_2 generation. Plants, selected based on the micro-mutation procedures, were grown as M_3 generation. The experiments were designed in the randomized complete blocks with 3 replications. Each micro-mutant population was grown separately from each other. Estimated variations of segregating generations increased depending on the character investigated and the mutagen used. The high heritability estimates in response to selection demonstrated that the part of the induced variability remains in generations. As a result of expected genetic advances in the M_3 generation that obtaining plants with higher yield by selection is possible. Micro-mutations related to yield and quantitative characters can potentially be used in plant breeding.

Key Words: Durum Wheat, Micro-Mutation, Yield, Selection, Heritability, Genetic Advance

Introduction

Micro-mutations are more important for direct use in plant breeding than as compared to the macro-mutations (Gaul *et al.*, 1969). Micro-mutations are defined through a plant progeny test and difficult to evaluate and handle. The genetic variability resulted from micro-mutations allows breeding of quantitative characters (Borojevic, 1965). Induced mutations of quantitative characters are obtained by treating with mutagens. The segregating populations are being used to achieve micro-mutants with good yielding properties. Gaul *et al.*, (1969) reported that micro-mutant selected in barley had 10 % higher yield potential than that of the initial line.

Breeders' efforts led to improvement of durum varieties, which were competitive in yielding ability with the best bread wheat varieties (Scarascia-Mugnozza *et al.*, 1991). For proper expression of genetic yield potential, new durum genotypes should have a proper combination of disease resistance, plant height, fertile tiller, spike length and kernel weight. These characteristics must be genetically manipulated to let the maximum yield stability under adverse conditions.

The principal limiting factor in the applicability of mutation breeding of wheat is the efficiency in which rare desirable mutant individuals are isolated by the selection procedures. Success of the selection depends on the size of starting population and the selection intensity. Efficient selection of mutants begins in the M_2 generation using the bulk selection techniques (Mac Key, 1984) and the selection is maintained in the M_3 generation. The selection of micro-mutations is recommended in the M_3 generation rather than the M_2 generation (Sarkar, 1986).

Variation of quantitative characters for a given generation depends on the genotype, the characters investigated and the mutagen used (Rao and Siddiq, 1977). Studies of quantitative characters in treated populations resulted a decrease in the mean values and an increase in the variability of both M_2 and M_3 generations (Scossiroli, 1965; Gaul, 1966; Gill *et al.*, 1974). Sarkar (1986) indicated that estimated variation of the quantitative characters were higher for the M_3 generation than those of the M_2 generation.

The aim of the study was to:

i. Determine induced variability of yield and the other quantitative characters such as plant height, number of fertile tiller, spike length, after selection in single plant grain yield and
ii. evaluate the applicability of micro-mutations in durum wheat breeding.

Materials and Methods

The research was conducted in 1997 (spring) and 1997-1998 (autumn) growing seasons. The test populations were M_2 and M_3 generations of durum wheat variety called "Sofu" (*Triticum durum* Desf.) resulting from Ethyl Methane Sulphonate (EMS) (0.1, 0.2, 0.3, 0.4 % for 8 hours at 24 °C without presoaking) or gamma ray treatments (Çiftçi *et al.*, 1988). Dry seeds, equilibrated at 11 % water content, were irradiated at Nuclear Research and Training Center, Ankara, TURKEY, with 50 and 100 Gy (Gy = Gray (1 Gray= 10 krad)) gamma rays at Cobalt 60 (^{60}Co) source (Anonymous, 1977). Sofu variety is a facultative type, tall, weak strawed with low yield potential and susceptible to lodging. However, Sofu variety has high protein content.

The study was designed in the randomized complete block with 3 replications. Each micro-mutant population was grown separately from each other. The M_1 plants grown after mutagenic treatments were propagated based on the spike progeny method. The plots were 1m length and 20 cm apart. The M_2 generation seeds obtained from each spike were sown to rows in spring. The base populations were constructed by selecting the normal appearing 50 or 100 single plants, analyzed for quantitative characters (Gaul *et al.*, 1969). Individual plants with 20 and 40 % yielding capacities were selected from each population.

Single plants selected by micro-mutation procedures were grown as the M_3 generation and the M_3 generation was sown in autumn. A plant progeny test was performed by analyzing quantitative characters. Based on the findings, in all populations, second step selection in the M_3 generation was sustained at a rate of 40 to 50 %.

Plant height, fertile tiller, spike length and single plant grain yield were studied in the M_2 and M_3 generations. Characters studied were determined as:

Plant Height: Main stem of plants was the distances in cm from ground level to the terminal of last spikelets.

Number of Fertile Tiller: Fertile spikes in plants were counted.

Spike Length: The length of main spikes was measured in cm.

Single Plant Grain Yield: Plants selected from populations were harvested and grains were weighed to get the single plant grain yield.

Population means (X), ranges, phenotypic standard deviation (S_f) and Coefficient of Variation (C.V. %) were calculated from mean values of replications. The data were subjected to Analysis of Variance (ANOVA) using the Statistical Software Package (MSTAT). Heritabilities (H) were estimated according to genotypic and phenotypic variances (Çağirgan, 1989). The estimation parameters for the analysis of variance table were given in Table 1.

Table 1: The Estimation Parameters for the Analysis of Variance Table

Sources of variation	Degrees of freedom	Mean square	Expected mean square
Blocks (r)	r-1		
Genotype (t)	t-1	M_1	$\tilde{O}^2e + r\,\tilde{O}^2g$
Error	(r-1) (t-1)	M_2	$\tilde{O}^2e$
General	(rt-1)		

Genotypic variance = $\tilde{O}^2g = (M_1-M_2) / r$
Phenotypic variance = $\tilde{O}^2p = \tilde{O}^2g + \tilde{O}^2e$
$H = \tilde{O}^2g / \tilde{O}^2p$

The difference between the mean of individually selected and the population means is defined as selection differential. Expected genetic advance (G) was separately calculated for each treatment using Yildirims' (1980) the formula: $G = H * S_f * i$ (H= heritability, S_f= phenotypic standard deviation, i= selection intensity). The deviation of selection intensity (i) was taken from the table of Falconer (1981). The expected next population means were obtained by adding expected genetic advance to population means (Yildirim, 1980).

Results and Discussion

Means: An increase in the means of plant heights was found in the M_2 generation while the other characters had lower or similar values to control after gamma irradiation (Table 2 and 3). Irradiation decreased the means of characters in a durum wheat variety in the M_2 generation (El-Rassas, 1991).

Except for 100 Gy-population of spike length, in the M_3 generation, the mean values were lower as compared to controls. A relationship between gamma ray doses and means was observed for M_2 and M_3 generations. Mean values decreased with increasing gamma ray doses. Similarly, Gaul *et al.*, (1969) noted that increasing X-ray doses resulted in a higher yield reduction of M_3 families. Population means of quantitative characters is reduced through micro-mutations (Borojevic, 1966; Anonymous, 1977; Scossiroli, 1965).

Plant height means values of the EMS populations were generally lower than that of control in both generations. The decreases in the means of the plant height indicated the selection of short culm mutants from the Sofu variety. Thus, many of the varieties released were produced by using mutations reduced plant height (Scarascia-Mugnozza *et al.*, 1991). The number of fertile tiller, spike length and single plant grain yield of micro-mutant populations had lower means as compared to control of the M_2 generation. The EMS population means for number of fertile tiller and grain yield generally decreased with increasing mutagen doses (Table 2 and 3). In contrast, no relationship between EMS doses and spike length means was observed. Except for some mutant populations, means of the M_3 generation exceeded the control means. EMS populations with high means depending on the character investigated. Many quantitative characters are controlled by polygenic systems. Under the influence of mutant genes, quantitative characters can easily be positively or negatively altered (Gottschalk and Wolff, 1983). With a 0.3 % EMS population, plant height, spike length and grain yield had the highest means. The increase of the M_3 generation mean value is probably due to the effect of selection for grain yield (Borojevic, 1965).

Genetic Variability: The populations range increase in both directions with new plus and minus variants indicated the potential of mutation breeding (Table 2 and 3). Since, the population ranges in the M_3 generation were wider than those of controls. Increasing variability in plus variants is a result of selection of individual plants with good yielding properties.

In irradiated populations, variation of coefficient of characters varied with both mutagens and generations (Borojevic and Borojevic, 1969). Plant height values in both M_2 and M_3 generations were higher in irradiated populations than those of controls. There was a positive relationship between the gamma ray dose and the degree of variation for plant height. In contrast, the variations of coefficient values of spike length were reduced with increasing gamma ray doses in segregating generations. The variation for number of fertile tiller and single plant grain yield was higher only in the M_3 generation as compared to control. Due to the importance of number of fertile tillers, the variability obtained from micro-mutant populations is useful to select lines with high yielding capacities (Gill *et al.*, 1974; Prasad *et al.*, 1980; Çağirgan, 1989). Mutant lines with high numbers of fertile tiller and single plant yield were obtained from wheat mutant populations (Bhatia and Swaminathan, 1962).

Micro-mutant populations of EMS generally had low variation of coefficient values in the M_2 generation. Increasing of variation accompanied with a reduction of the mean values indicated that major part of the induced genetic variability is negative (Borojevic, 1991).

The variation coefficients of mutant populations were higher compared to controls in the M_3 generation. Borojevic (1991) connected the high variations in M_3 generation to the increasing of the genetic components. Estimated variations also were greater in the M_3 generation than the M_2 generation. Similarly, Sarkar (1986) reported that variations in wheat varieties increased in the M_3 generation after mutagenic treatments (EMS, NMU, X-Ray).

Table 2: The Values of Plant Height and Number of Fertile Tiller in the M_2 and M_3 Generations

	Plant height (cm)					Number of fertile tiller				
Popula.	X ± S_x	Range	C.V. (%)	F test	H	X ± S_x	Range	C.V. (%)	F test	H
					M_2 generation					
Control	107.2 ± 1.59	94 - 121	5.9	**	0.09	4.3 ± 0.22	3 - 6	20.5	NS	0.11
Gamma										
50 Gy	109.5 ± 0.67	85 - 124	6.1	**	0.87	4.4 ± 0.07	3 - 6	15.4	**	0.90
100 Gy	108.5 ± 3.31	91 - 139	11.0	NS	0.14	4.1 ± 0.17	3 - 5	14.9	NS	0.15
Control	102.5 ± 1.30	75 - 120	8.7	**	0.29	3.7 ± 0.13	2 - 7	24.2	**	0.06
EMS										
0.1 %	102.4 ± 1.01	68 - 122	9.9	**	0.33	3.8 ± 0.10	2 - 8	26.2	**	0.31
0.2 %	103.1 ± 0.85	77 - 126	8.2	**	0.34	3.7 ± 0.08	2 - 7	21.2	**	0.22
0.3 %	102.0 ± 0.72	85 - 117	7.0	**	0.25	3.5 ± 0.08	2 - 6	23.6	**	0.29
0.4 %	99.5 ± 0.84	70 - 119	8.4	**	0.30	3.5 ± 0.08	1 - 5	21.4	**	0.21
					M_3 generation					
Control	147.8 ± 1.47	112 - 166	6.9	NS	- 0.22	6.7 ± 0.24	3 - 10	24.7	NS	0.06
Gamma										
50 Gy	145.9 ± 1.52	99 - 169	9.0	NS	0.08	6.2 ± 0.27	1 - 13	37.7	NS	0.05
100 Gy	143.4 ± 2.72	111 - 168	10.4	NS	- 0.03	5.3 ± 0.54	1 - 13	55.9	NS	- 0.30
Control	146.6 ± 1.46	112 - 168	8.6	NS	0.08	5.0 ± 0.17	2 - 9	29.8	NS	- 0.15
EMS										
0.1 %	145.4 ± 1.59	100 - 168	9.5	NS	0.16	4.7 ± 0.23	2 - 11	41.6	NS	0.07
0.2 %	144.8 ± 1.75	113 - 180	10.5	NS	- 0.08	5.4 ± 0.20	2 - 11	31.6	NS	0.07
0.3 %	149.7 ± 1.20	122 - 170	6.9	NS	0.17	5.5 ± 0.22	2 - 11	35.1	**	0.38
0.4 %	144.5 ± 1.48	115 - 174	8.9	NS	- 0.03	5.2 ± 0.20	2 - 9	32.7	NS	0.09

** = 1 % level significance, NS: not significant
(X= mean, S_x= standard error, C.V. = variation of coefficient, H= heritability)

Table 3: The Values of Spike Length and Single Plant Grain Yield in the M_2 and M_3 Generations

	Spike length (cm)					Single plant grain yield (g)				
Popula.	X ± S_x	Range	C.V. (%)	F test	H	X ± S_x	Range	C.V. (%)	F test	H
					M_2 generation					
Control	7.8 ± 0.09	7.0 - 8.3	4.5	NS	0.00	7.9 ± 0.61	3.8 - 13.2	31.1	**	0.42
Gamma										
50 Gy	7.8 ± 0.03	7.1 - 8.8	3.9	**	0.90	7.6 ± 0.15	4.7 - 12.3	20.1	NS	- 0.02
100 Gy	7.5 ± 0.05	7.1 - 7.7	2.5	NS	- 0.19	6.2 ± 0.49	4.1 - 9.9	28.4	*	0.35
Control	7.1 ± 0.09	5.7 - 8.3	8.9	**	0.20	5.8 ± 0.41	1.3 - 12.4	42.7	**	0.34
EMS										
0.1 %	7.0 ± 0.06	5.5 - 8.7	8.2	**	0.39	6.1 ± 0.27	1.2 - 14.5	42.6	**	0.28
0.2 %	7.6 ± 0.05	6.5 - 8.9	6.6	**	0.21	6.2 ± 0.18	2.2 - 10.5	27.3	NS	0.04
0.3 %	6.9 ± 0.06	5.1 - 8.2	8.1	**	0.26	5.4 ± 0.22	1.1 - 12.5	37.3	**	0.33
0.4 %	7.0 ± 0.06	5.0 - 8.1	8.6	**	0.42	4.8 ± 0.22	1.3 - 10.5	40.5	**	0.21
					M_3 generation					
Control	7.7 ± 0.15	5.7 - 9.9	13.6	NS	0.03	14.6 ± 1.09	5.5 - 19.7	29.8	NS	0.03
Gamma										
50 Gy	7.5 ± 0.14	4.5 - 10.0	16.4	NS	- 0.03	13.5 ± 0.97	4.8 - 23.7	35.9	NS	0.10
100 Gy	8.1 ± 0.18	6.3 - 10.1	12.2	NS	0.12	11.4 ± 1.16	5.3 - 16.2	32.1	NS	- 0.10
Control	7.1 ± 0.11	5.5 - 9.5	12.9	NS	0.20	10.9 ± 0.70	4.9 - 20.5	32.0	NS	0.02
EMS										
0.1 %	7.4 ± 0.11	5.5 - 9.8	12.7	NS	- 0.18	10.0 ± 0.66	5.6 - 16.4	32.8	NS	- 0.11
0.2 %	7.4 ± 0.11	5.5 - 10.0	12.9	NS	- 0.15	11.5 ± 0.68	3.2 - 17.4	29.8	NS	- 0.12
0.3 %	7.2 ± 0.11	5.0 - 9.5	13.4	NS	0.09	12.5 ± 1.07	4.0 - 26.9	43.1	**	0.33
0.4 %	7.7 ± 0.13	5.6 - 10.4	14.8	NS	0.04	12.2 ± 0.89	2.5 - 21.3	36.5	*	0.25

* = 5 % level, ** = 1 % level significance, NS: not significant
(X= mean, S_x= standard error, C.V. = variation of coefficient, H= heritability)

High variations for some of the quantitative characters were observed at low doses of EMS (e.g. 0.1 and 0.2 %). Similarly, Sarkar (1986) reported that lower doses of N-metil N-nitroz Uretan (NMU) mutagen resulted greater variances for plant height and productive tiller number in bread wheat. There was no relationship between EMS doses and degree of variation for plant height, number of fertile tiller and single plant grain yield. However, the variation values of spike length increased with increasing EMS doses for the M_3 generation. The highest variation (14.8 %) along with a high mean value (7.7 cm) was obtained from 0.4 % EMS population (Table 3). Some micro-mutant populations with high mean values and variations showed that the lines have positive mutations (Gaul, 1966; Yildirim, 1980; Çağirgan, 1989). The means of quantitative characters varied and the variations increased with mutagen treatments (El-Rassas, 1991; Borojevic, 1991).

Variances and Heritability: Except for 100 Gy-population in the M_2 generation, the analysis of variance revealed that variability of plant height, the number of fertile tiller and spike length among lines was statistically significant at 1 % level. However, there were generally no significant differences in the M_3 generation between the lines (Table 2 and 3). Mutagen doses (100 Gy, 0.1, 0.3 and 0.4 %) for single plant grain yield in the M_2 generation resulted differences in the variability among lines. Yield variances between generations was not consistent (Table 3).

For the response of selection, heritability estimates of micro-mutant populations in the M_2 generation were generally higher compared to the controls. Hence, further selection is expected to be more effective for later generations. In contrast, estimates for single plant grain yield were lower than those of controls (Table 2). There was no relationship between mutagen doses and heritabilities of characters.

The heritability values for some mutant populations, depending on characters, were higher than those of controls in the M_3 generation. High heritabilities values in both generations indicated that the variability in the populations is fixed the selection. Due to the occurrence of negative variances that was affected by environmental variability, negative estimates for mutant populations were obtained in generations. The increases in heritability after mutagen treatments are an indication of effective selection in the population. Increases of heritability for grain yield were obtained from some progeny populations (50 Gy, 0.3 and 0.4 % EMS) with high means and variations (Table 3). The spike length showed a slight increase in heritability and a low response to selection (Ibrahim and Sharaan, 1974; Yildirim, 1980).

Effects of Selection: Means of plants selected were similar to their base populations. Population means varied with mutagen treatments (Table 4). In irradiated populations, means were lower than those of controls in the both M_2 and the M_3 generations. In contrast, yields of the EMS treated populations had high values.

Table 4: The Selection Values for Single Plant Grain Yield (g) in the M_2 and M_3 Generations

Popula	Base or progeny population n_1	Base or progeny population (X_1)	Selected population n_2	Selected population (X_2)	Selection differential $(X_2 - X_1)$	Proportion (P) (n_2 / n_1)	Selection intensity (i)	Standard deviation (S_f)
				M_2 generation				
Control	50	7.9	10	9.4	1.5	0.20	1.40	2.46
Gamma								
50 Gy	100	7.6	25	9.7	2.1	0.25	1.27	1.53
100 Gy	50	6.2	10	6.8	0.5	0.40	1.40	1.77
Control	100	5.8	20	7.5	1.7	0.20	1.40	2.49
EMS								
0.1 %	100	6.1	25	9.2	3.1	0.25	1.27	2.58
0.2 %	100	6.2	25	8.3	2.1	0.25	1.27	1.70
0.3 %	100	5.4	25	7.7	2.3	0.25	1.27	2.00
0.4 %	100	4.8	25	6.9	2.1	0.25	1.27	1.94
				M_3 generation				
Control	10	14.6	5	19.2	4.6	0.50	0.80	4.36
Gamma								
50 Gy	25	13.5	10	18.2	4.7	0.40	0.97	4.85
100 Gy	10	11.4	5	14.4	2.9	0.50	0.80	3.66
Control	20	10.9	10	14.0	3.1	0.50	0.80	3.49
EMS								
0.1 %	25	10.0	10	13.5	3.4	0.40	0.97	3.29
0.2 %	25	11.5	10	14.7	3.3	0.40	0.97	3.42
0.3 %	25	12.5	10	17.3	4.9	0.40	0.97	5.37
0.4 %	25	12.2	10	16.5	4.3	0.40	0.97	4.44

(n_1= number of plant in base or progeny population n_2=number of plant selected, X_1= mean of base or progeny population, X_2= mean of plants selected)

Table 5: Expected and Realized M_3 Means and Genetic Advance for Single Plant Grain Yield after Selection in the M_2

Popula.	Base population (M_2) mean	Expected genetic advance ($G= H * S_f * i$)	Expected (M_3) mean (M_2 mean + G)	Realized (M_3) mean	Realized genetic advance (M_3 mean-M_2 mean)
Control	7.9	1.45	9.34	14.6	6.75
Gamma					
50 Gy	7.6	0.00	7.62	13.5	5.90
100 Gy	6.2	0.87	7.11	11.4	5.18
Control	5.8	1.19	7.02	10.9	5.07
EMS					
0.1 %	6.1	0.92	6.98	10.0	3.97
0.2 %	6.2	0.09	6.29	11.5	5.27
0.3 %	5.4	0.84	6.20	12.5	7.10
0.4 %	4.8	0.52	5.32	12.2	7.36

The negative heritability estimates were used as zero in the formula of genetic advance
(H= heritability, S_f= standard deviation, i= selection intensity)

Table 6: The Expected Genetic Advance and M_4 Means for Single Plant Grain Yield after Selection in the M_3

Popula.	Progeny population (M_3) mean	Expected genetic advance (G= H * S_f * i)	Expected (M_4) mean (M_3 mean + G)	Advance (%) (M_3 / M_4 x 100)
Control	14.6	0.10	14.7	0.7
Gamma				
50 Gy	13.5	0.47	14.0	3.4
100 Gy	11.4	0.00	11.4	0.0
Control	10.9	0.06	11.0	0.5
EMS				
0.1 %	10.0	0.00	10.0	0.0
0.2 %	11.5	0.00	11.5	0.0
0.3 %	12.5	1.72	14.2	12.1
0.4 %	12.2	1.08	13.2	8.2

The negative heritability estimates were used as zero in the formula of genetic advance
(H= heritability, S_f= standard deviation, i= selection intensity)

Selection differentials of micro-mutant populations were higher than those of controls in generations. Only 100 Gy populations had low values (Table 4). The means of plants selected were in agreement with the selection differential values. Selection differential isn't the estimate of genetic advance gained by selection (Yildirim, 1980). High standard deviations obtained in the M_3 generation were an indication of the positive measure of successful selection (Yildirim, 1980). Except for 0.1 % EMS population, the phenotypic standard deviation values were lower than those of controls in the M_2 generation.

Expected genetic advances calculated from the M_2 generation data were lower as compared to the controls of both treatments (Table 5). In contrast, expected advances in 50 Gy, 0.3 and 0.4 % EMS populations were higher than those of controls (Table 6). Scosssiroli (1977) also indicated that the genetic advances were obtained by using the variability of quantitative characters through selection.

Genetic advance was increased approximately 12.1 % with the selection. Borojevic (1965) noted that the genetic advance was greater in the M_3 generation than that of the M_2 generation. Therefore, selection of micro-mutations was recommended mostly in the M_3 generation (Sarkar, 1986). Micro-mutant populations (0.3 and 0.4 % EMS) with high means along with genetic advances indicated a certain effect of selection in the populations (Table 6). Gaul *et al.,* (1969) reported that yield advance could be obtained through selection in treated populations of barley. Realized advances of irradiated populations agreed with the expected advances. EMS populations (0.2, 0.3 and 0.4 %) had high-realized values as compared to controls (Table 5).

Expected means of the M_3 generation agreed with realized means in the irradiated populations. This result enables the estimation of the next population means in advance. Although expected and realized means looks parallel in the M_3 generation, they change in the M_4 generation (Yildirim, 1980). Expected means of 0.2, 0.3 and 0.4 % EMS populations calculated from M_3 generation were found higher than that of control in the M_4 generation (Table 6). Therefore, increasing of yield potential in populations can be expected in the next generations.

Conclusion

Means of quantitative characters in treated populations varied with the mutagen treatments through selection. Estimated variations of segregating generations increased depending on the character investigated and the mutagen used. The high heritability estimates in response to selection demonstrated that the part of the induced variability remains in generations. As a result of expected genetic advances in the M_3 generation that obtaining plants with higher yield by selection is possible. Micro-mutations related to yield and quantitative characters can potentially be used in plant breeding.

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