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INDUCED VARIABILITY IN HOMOZYGOUS AND HETEROZYGOUS GENOTYPES OF TOBACCO

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ABSTRACT

Dry seeds of homozygous pure breeding lines of chewing tobacco (*Nicotiana tabacum* L.), Sona, bandi and their F_1 hybrid were treated with chemical mutagen EMS at three concentrations (0.1, 0.3 and 0.5%) for 12 h. In M_1 generation, based on seed germination and seedling growth, homozygous material was found to be more sensitive than heterozygous material. Complete seed mortality was observed following treatment with 0.5% EMS. In M_2 generation, range widened for all characters in different populations. Mean values increased in F_2 over midparent but was significantly lowered in M_2 and F_2 M_2 for all the characters except total cured leaf yield. There was no significant difference in mean leaf thickness which marginally decreased in F_2 and increased in M_2 and F_2 M_2. Significant increase in nicotine content was observed in F_2 and M_2 but in F_2 M_2 it decreased significantly. A significant increase in variation was evident in F_2 and M_2 for all characters but in F_2 M_2 it was so for leaf breadth and first grade leaf yield. The magnitude of the induced variation was in order of $M_2 < F_2 < F_2$ M_2. Variations from hybridization and mutation were generally not cumulative.

Key words : Tobacco, *Nicotiana tabacum*, mutation, induced variability.

Variability in a crop plant can be increased either by hybridization among diverse genotypes or through induced mutation. To harness more variability, mutation has been superimposed on hybridization in several crops [1-5] but no such systematic attempt has so far been made in tobacco (*Nicotiana tabacum* L.). Hence, an attempt was made to study the chemosensitivity and to assess the variability generated by hybridization, mutagenesis and combined hybridization and mutagenesis in homozygous and heterozygous genotypes of tobacco.

MATERIALS AND METHODS

Dry seeds of two chewing tobacco (*N. tabacum* L.) homozygous cultivars, viz., Sona and Bandi, alongwith their F_1 hybrid (Sona × Bandi) were treated separately

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with the chemical mutagen ethyl methane sulphonate (EMS) at 0.1, 0.3 and 0.5% concentrations for 12 h duration in 1991-92 at room temperature. Seeds soaked in distilled water for the same duration were taken as control. Treated seeds were washed immediately with running tap water and dried under shade. A part of the seeds (100 per treatment) was placed on moist filter paper in petri dishes in three replicates to observe germination under laboratory conditions. The remaining seeds were sown separately on nursery beds measuring 1 m^2 with three replications. Ten seedlings from each treatment were pulled out after seven weeks of sowing to measure root and shoot length, leaf length and breadth and stem girth. The M_1 and F_1 M_1 plants were compared with their untreated controls. The effects of mutagen on germination and seedling growth were expressed as percentage of control.

Ten normal looking plants from each treatment in M_1 were selected randomly to raise M_2 generation. From these, 50 plants per replication were raised in a randomised block design with three replications for each treatment in M_2 generation. Normal cultural practices were followed to raise the crop. Observations on six quantitative characters (Table 2, 5) were recorded on five randomly choosen plants in each treatment and in each replication. Leaf thickness and nicotine content were estimated as per Sastry and Murty [6] and Cundiff and Markunas [7], respectively.

Range, mean, variance and coefficient of variation (CV%) were calculated. The mean values between the treatments and their respective controls were tested for their significance by CD values. F test was applied to compare the variances of F_2s with the mid-parent values, of M_2s with the respective control and of F_2M_2 with F_2s. Mean leaf thickness and nicotine content were tested by CD values.

RESULTS AND DISCUSSION

The mutagenic sensitivity of homozygous and heterozygous materials for germination and seedling growth parameters were studied in M_1 generation. In M_2 generation, nature and extent of induced variations were studied and the results are discussed below.

M_1 GENERATION

Germination of seed was affected more severely in homozygous than in the heterozygous treated materials at all the EMS concentrations (Table 1). Gradual reduction in germination percentage was observed with increase in concentration of mutagen, reaching more than 50% lethality at 0.5% EMS in both homozygous and heterozygous populations. There was hardly any seedling emergence at 0.5% EMS in the nursery bed, thus restricting further studies to 0.1 and 0.3% EMS treatments only. Non emergence of seedling at 0.5% EMS may be due to direct sowing of the seeds in the nursery [8]. Reduction in germination with increasing mutagen

concentration [9] and more than 50% lethality [10] have also been reported in flue-cured virginia tobacco (*N. tabacum* L.). Greater reduction in germination in homozygous materials have been observed in hexaploid triticale [11] and sorghum [12] also.

Table 1. Mean (% of control) of different seed and seedling characters in EMS treated homozygous and heterozygous genotypes of tobacco

Character	EMS (%)	Sona	Bandi	average	Sona × Bandi (F_1)
Germination, %	0.0	96.5	96.0	96.2	93.0
	0.1	81.4	78.7	80.1	87.0
	0.3	71.0	72.4	71.7	76.9
	0.5	34.7	33.9	34.3	39.8
Root length, cm	0.0	3.1	3.0	3.1	2.2
	0.1	78.1	21.7	49.9	58.7
	0.3	10.1	10.0	10.1	30.9
Shoot length, cm	0.0	9.3	10.8	10.1	8.7
	0.1	90.9	63.3	77.1	99.7
	0.3	48.9	57.2	53.1	73.9
Leaf length, cm	0.0	8.6	12.5	10.6	9.3
	0.1	87.1	54.9	71.0	97.1
	0.3	57.6	39.4	48.5	71.6
Leaf breadth, cm	0.0	3.6	5.4	4.5	4.2
	0.1	88.9	62.9	75.9	96.2
	0.3	60.6	43.1	51.9	78.6
Stem girth, cm	0.0	1.4	1.5	1.5	1.3
	0.1	80.9	74.5	77.7	103.8
	0.3	22.0	64.1	43.1	88.7

SEEDLING GROWTH

A perusal of different biological parameters of seedling growth, e.g. root length, shoot length, leaf length, leaf breadth and stem girth showed reduction in their mean values with increase in mutagen concentration, except for stem girth at 0.1% EMS in the heterozygous material (Table 1). Inhibition of seedling growth is an indicator of genetic damage caused by the mutagen. It was more pronounced in the homozygous parents than in their heterozygotes. This may be due to greater resistance offered by the gene complexes present in the heterozygotes. This conclusion is supported by similar findings in other crops [11, 12]. Among the two homozygous lines, Bandi showed greater reduction in almost all seedling parameters than Sona

following treatment with EMS (Table 1). Differential varietal responses have been reported in flue-cured tobacco [13], groundnut [14] and Lathyrus [15].

M_2 GENERATION

The range for different quantitative characters related to growth and yield increased due to hybridization (F_2), due to mutagenesis in homozygous lines (M_2) and due to hybridization-cum-mutagenesis (F_2M_2). An increased range in both positive and negative directions is a clear index of the fact that polygenic variability is released, however, widening of range in all the treated population was mostly towards negative side resulting into reduced mean values for different characters (Table 2). The increase in range provides variants with extreme value for different characters that were not available in the original populations.

The mean values for six quantitative characters related with growth and yield increased in F_2 over the average parental mean which indicates genetic differences among the parental lines. In mutagen treated homozygous and heterozygous populations, there was a lowering of means (Table 2). Significant decreases in mean was observed in variety Sona for all the characters except leaf breadth and total cured leaf yield and for plant height, leaf length and leaf breadth in variety Bandi. In heterozygous mutagenic population (F_2M_2), mean values decreased significantly as compared to that in F_2 population for all the six quantitative characters except total cured leaf yield. This might be due to induction of more mutations in negative direction. Reduction in mean value due to mutation and differential varietal response to mutagenesis have also been reported in chewing tobacco [16] and in flue-cured tobacco [17, 18].

The mean values for two quantitative characters related with quality viz., leaf thickness and nicotine percentage show variable trends (Table 3). An increased leaf thickness due to mutagenization in both homozygous and heterozygous genotypes was observed though, these increases were not significant. Leaf thickness, on the other hand, decreased (9.1%) in F_2 whereas in M_2 it increased by 17.6% and in F_2M_2 by 23.7 % (Table 4). Mean nicotine content increased significantly in F_2 over the mid- parental value. Significantly increased nicotine content percentage was observed due to mutagenesis in homozygous (M_2) but in F_2M_2 nicotine content decreased significantly as compared to the F_2 (Table 3). There was almost an identical increase (30%) of nicotine percentage in F_2 and M_2 whereas in F_2M_2 the nicotine content decreased by 17.6% (Table 4).

Table 2. Range (R), mean (M), variance (V) and coefficient of variation (CV) for various characters in homo- and heterozygous genotypes in F_2, M_2 and F_2M_2

		Sona (M_2)			Bandi (M_2)			Sona × Bandi (M_2F_2)		
		EMS (%)								
Character	Parameter	0.0	0.1	0.3	0.0	0.1	0.3	0.0(F_2)	0.1	0.3
Plant height (cm)	R	37-50.5	30-55	24-54	41-53.5	35-56	30.5-55	41.5-58	40-59	37.5-58
	M	42.8	41.5**	37.3**	45.4	42.7**	42.5**	47.6	46.2*	44.4**
	V	9.6	38.8*	41.1**	10.6	36.3**	39.8**	30.7**	40.1	44.8
	CV	7.2	15.0	17.2	7.2	14.1	14.8	11.6	13.7	15.1
Leaf length (cm)	R	43-56	39.5-62	33-60.5	41-54	37.5-57	34-57	43.5-60	39.5-60	37-59
	M	49.8	46.8**	45.6**	47.6	45.7*	43.0**	50.1	48.3	47.3**
	V	16.3	31.9**	47.5**	13.9	31.9**	31.6**	31.6**	39.7	38.7
	CV	8.1	12.1	15.1	7.9	12.43	13.1	11.2	13.1	13.2
Leaf breadth (cm)	R	31-41	29-44	27-45.5	27-38	25-43	23-43.5	32.5-45	30-46	30-47.5
	M	33.6	34.2	33.1	31.7	31.2	29.7	38.6	35.9**	35.5**
	V	8.9	23.7**	34.8**	10.5	27.0**	38.6**	22.4**	38.1*	40.4**
	CV	8.9	14.3	17.8	10.2	16.7	20.9	12.3	17.2	17.9
Internode length (cm)	R	3-4.4	2.7-4.6	2.4-4.9	3.2-4.7	3.1-5.3	2.8-5.1	3.2-4.8	2.9-5.1	3-5.4
	M	3.6	3.4*	3.4*	3.7	3.7	3.6	3.7	3.5**	3.5**
	V	0.1	0.2**	0.5**	0.2	0.3**	0.4**	0.3**	0.4	0.4
	CV	8.8	13.8	19.9	10.4	15.0	16.7	15.7	17.7	17.2
Total cured leaf yield (g)	R	147-245	150-268	132-259	132-228	122-240	125-249	145-256	140-261	141-265
	M	175.8	175.2	173.2	164.6	162.6	158.4	184.7	182.1	180.7
	V	509.4	917.9**	979.5**	477.4	859.1**	960.4**	942.9**	1165.6	1267.8
	CV	12.8	17.3	18.1	13.3	18.0	19.8	16.6	18.8	19.6
First grade leaf yield (g)	R	86-130	75-151	62-141	65-126	58-135	60-148	79-138	72-144	70-145
	M	100.6	99.8	88.1**	87.7	85.8	84.6	100.7	07.7	95.2*
	V	229.7	429.1**	496.9**	162.5	476.8**	573.4**	414.5**	626.1**	649.5*
	CV	15.1	20.8	25.3	14.5	25.4	28.3	20.2	25.6	26.8

Table 3. Mean leaf thickness has nicotine content in homo- and heterozygous genotypes in F_2, M_2 and F_2M_2

Genotype	EMS %	Leaf thickness (mm)	Nicotine content (%)
Sona (M_2)	0.0	21.2	2.9
	0.1	26.5	4.4
	0.3	23.5	3.7
Bandi (M_2)	0.0	21.7	2.9
	0.1	26.2	3.6
	0.3	24.4	3.6
Sona × Bandi (F_2M_2)	0.0	19.5	3.8
	0.1	32.2	3.1
	0.3	26.0	3.2
SE mean		1.8	0.2
C.D, 0.05		NS	0.6

Table 4. Comparative effect of EMS treatment on leaf thickness and nicotine content in relation to genetic background in F_2, M_2 and F_2M_2 populations

Genotype	Leaf thickness		Nicotine content	
	mean	deviation %	mean	deviation %
parents	21.5	-	2.9	-
F_2	19.5	- 9.1	3.8	+ 30.1
M_2	25.2	+ 17.6	3.8	+ 30.8
F_2M_2	24.1	+ 23.7	3.1	- 17.6

Variance increased significantly after hybridization over the mid-parental variance (Table 2). The magnitude of deviation in coefficient of variability in F_2 ranged from 27.3% for total cured leaf yield to 61.7% for plant height (Table 5). This increased variability resulted from the recombination and segregation of genes of the parents following hybridization. But the small magnitude of variability for yield restricts its usefulness. In homozygous varieties, mutagenic treatment significantly increased the variances over control for all the six quantitative characters (Table 2). The magnitude of deviation of coefficient of variation was highest ranging from 39.8% for total cured leaf yield to 112.4% for plant height (Table 5). The increased variability may be attributed to induction of mutations in some of the polygenes governing these characters and their subsequent segregation. Release of induced variability in a polyploid species like tobacco, however is attained in later segregating generations depending upon the buffering effect of duplicate loci. Increased variability in M_2 generation has been reported in flue-cured tobacco [17, 18]. Mutagenization in heterozygous line and their segregation in F_2M_2 population increased the variability

Table 5. Comparative effect of EMS treatment on various characters in relation to genetic background in F_2, M_2, F_2M_2

Character and population	Mean	Deviation (%) over parents	C.V.(%)	Deviation (%) over parents
Plant height				
Parents	44.1	-	7.2	-
F_2	47.6	+ 7.9	11.6	+ 61.7
M_2	41.0	- 7.1	15.3	+ 112.4
F_2M_2	45.3	- 4.9	14.4	+ 23.7
Leaf length				
Parents	48.7	-	7.9	-
F_2	50.1	+ 2.9	11.2	+ 40.8
M_2	45.3	- 7.0	13.2	+ 65.1
F_2M_2	47.8	- 4.7	13.1	+ 16.8
Leaf breadth				
Parents	32.6	-	9.6	-
F_2	38.6	+ 18.3	12.3	+ 28.2
M_2	32.1	- 1.7	17.4	+ 82.1
F_2M_2	35.7	- 7.5	17.5	+ 43.1
Internode length				
Parents	3.7	-	9.6	-
F_2	3.7	+ 1.6	15.7	+ 63.2
M_2	3.5	- 3.3	16.4	+ 70.5
F_2M_2	3.5	- 6.5	17.5	+ 11.6
Total cured leaf yield				
parents	170.2	-	13.1	-
F_2	184.7	+ 8.5	16.6	+ 27.3
M_2	167.3	- 1.7	18.3	+ 39.8
F_2M_2	181.4	- 1.8	19.2	+ 15.4
First grade leaf yield				
Parents	94.2	-	14.8	-
F_2	100.7	+ 6.9	20.2	+ 36.5
M_2	89.6	- 4.9	24.9	+ 68.5
F_2M_2	96.5	- 4.3	26.2	+ 29.6

significantly only for leaf breadth and first grade leaf yield. Character-specific increase in variance as observed in the present investigation is in conformity with the findings of Virk *et al.* [3]. The magnitude of increased variability in F_2M_2 over F_2 was low, ranging from 11.6% for internode length to 43.1% for leaf breadth (Table 5). This increase in variance of low magnitude in F_2M_2 over F_2 is in agreement with findings

of other workers [4, 5] and in contradiction with those of some others [1]. This clearly indicates that the variance produced by hybridization might be enough under the environmental condition encountered and that the superimposition of mutagenization over hybridization in not adding much to this variation. This, most probably may be due to overlapping effects of these two techniques. Such inference was also drawn by many workers while working on mutagenesis [2, 5].

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