Theor Appl Genet (1984) 67:203–205

Myo-inositol induced growth in ethyl methanesulphonate treated tobacco

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Received May 20, 1983
Communicated by D. von Wettstein

Summary. Tobacco seeds treated with ethyl methanesulphonate produces mutations as well as physiological growth debility. The addition of myo-inositol to seeds undergoing mutagenic treatment stimulated growth and increased survival of subsequent plants with negligible effect on the mutation frequency.

Key words: Ethyl methanesulphonate – Mutation – Myo-inositol – *Nicotiana tabacum,* tobacco

Introduction

Ethyl methanesulphonate (EMS), a potent chemical mutagen, produces such deleterious physiological effects on growth and survival in tobacco that it often limits the isolation of useful mutants (Sato 1976; Appa Rao and Ramavarma 1978; Rao and Moses 1982). This mutagenic agent has produced similar effects in other plants and attempts to diminish them have not been successful (Savin et al. 1968; Khvostova 1970; Privalov 1972; Wong 1975). Pollard et al. (1961) demonstrated the growth promoting potential of myo-inositol (MI) and since then it has been routinely included in plant cell and organ cultures, including those of tobacco (Nitsch 1977). In the light of these reports of stimulated growth, experiments were conducted using MI to study its' restorative effects on EMS treated tobacco. The results of these tests are presented.

Material and methods

Seeds of flue-cured tobacco variety 'EC 15103' were presoaked in distilled water and treated with different dosages of EMS and MI (Table 1). These dosages of EMS and MI were found effective from earlier studies (Appa Rao and Ramavarma 1978; Rao and Moses 1982; Nitsch 1977). A temperature of 20 °C and pH of 7.00 were kept uniformly constant for all treatments and controls. Treated seeds were washed for 1 h in running tap water and sown in 1.0 m^2 seed beds along with controls. Measurements of growth were recorded on 30-day old seedlings raised following prescribed cultural practices. Observations on survival and mutation frequency were recorded for plants of different treatments and for the controls, from field-grown plants.

Results and discussion

Germinability was not affected by treatments either with EMS or EMS supplemented with MI. Measurements of growth, survival of seedlings and mutation frequency are presented in Table 1. It can be observed that treatment with EMS induced growth reduction, especially at higher concentrations. The addition of MI stimulated growth in both dosages of EMS treatment (Fig. 1). The addition of MI to plain water also increased growth.

Among the characters studied for growth effects, the height of seedlings showed a uniform response to EMS treatments and those with an addition of MI. Normal growth was achieved in both dosages of EMS after the addition of MI. In the 0.5% EMS treatment, the addition of MI stimulated growth to an adequate height suitable for transplanting into the field.

Observations made on survival in the field also gave similar encouraging results in the treatments with additions of MI to EMS as well as to plain water.

Sterility in the form of flower bud/flower drop, pollen abortion, underdeveloped empty capsules and seeds were observed in EMS treated plants. Macromutations affecting internode length, color of mature leaves, number of curable leaves and chlorophyll mutations were also evident in all EMS treated plants. It is

Table 1. Data on growth, survival, sterility and mutation percentage observed in different treatments

Serial no.	Treatment			Measurement of growth (cm)			Survival (%)	Sterility (%)	Mutations (%)	
	Chemical	Concentration (%)	Duration (h)	Height of seedling	Length of leaf	Breadth of leaf			Chlorophyll	Others
1	EMS	0.25	24	11.07 (±0.49)	10.12 (±0.35)	3.20 (±0.16)	83.75	7.46	2.99	5.97
2	EMS+ MI	0.25 0.54	24	13.66 (±0.91)	13.87 (±0.73)	5.90 (±0.36)	93.75	1.33	1.33	6.67
3	EMS	0.50	12	1.35 (±0.32)	5.27 (±0.23)	2.87 (±0.23)	47.50	29.63	7.41	20.37
4	EMS+ MI	0.50 0.54	12	11.77 (±1.26)	13.20 (±0.67)	5.29 (±0.28)	85.00	2.94	4.41	8.82
5	Water+ MI	0.54	24	15.60 (±1.11)	11.40 (±0.53)	4.74 (±0.24)	97.50	0	0	0
6	Water		24	12.09 (±0.55)	11.22 (±0.38)	5.22 (±2.60)	86.25	0	0	0

Fig. 1. Nursery beds (60 day old) showing contrasting growth differences between (**a**) EMS treated and (**b**) treatment with the addition of myo-inositol

important to note that in treatments with the addition of MI to EMS, sterility was less pronounced but the mutation frequency was not lowered correspondingly when compared to treatments with EMS alone.

The statistical analysis for differences among means of different treatments is given in Table 2. Differences are highly significant for all growth characters between EMS treatments and those with the addition of MI. Significant differences were not found among some characters between treatments of EMS with addition of MI as well as between control and treatments with addition of MI.

Myo-inositol occupies a key position in the growth and metabolism of plants as an important membrane constituent, a reserve substance, a cofactor in galactose metabolism and a starting substance in synthesis of other cyclitols (Loewus and Dickinson 1982). It is routinely included in nutrient media used for the culture of plant cells and organs and results of stimulated growth have been observed by Pollard et al. 1961; Braun and Wood 1962; Steinhart et al. 1962; Loomis and Torrey 1964; Shanz et al. 1967; Goforth and Torrey 1977; Nitsch 1977; Israel and Wilson 1978; Harran and Dickinson 1978.

Table 2. Comparative statistical evaluation of characters from different treatments

Treatments compared		Characters compared[b]		
A[a]	B	Height of seedling	Length of leaf	Breadth of leaf
1	2	5.83	14.29	47.64
1	3	62.58	58.99	9.67
2	4	1.64	1.37	5.98
3	4	25.99	50.59	37.79
2	5	1.19	3.91	8.35
6	5	2.19	0.34	2.84
1	6	2.36	4.85	30.38
4	6	0.28	4.81	0.76
4	5	1.69	3.13	5.02
3	6	37.73	41.24	24.59
2	6	1.23	2.77	1.23

[a] Serial number of the treatments are same as in Table 1
[b] d.f: 40; t values at P 0.05 and 0.001 are 2.021 and 3.551, respectively

Earlier studies on growth responses from combined treatments with growth regulators (IAA and GA) and mutagen (EMS) were of a lower magnitude and in some treatments the response was negative (Privalov 1972). Bandurski (1978, 1979) showed that myo-inositol is also a component of certain conjugates containing indole-3-acetic acid. There is some evidence that these conjugates, together with MI, play an important metabolic function as sources of IAA during germination, in IAA transport, as protective agents against peroxidase attack on free acid and as components of a hormonal homeostatic system responsive to environmental controls (Bandurski 1980). The recupurative effect of growth following the addition of Myo-inositol observed in the present study can be attributed to the above mentioned metabolic functions of myo-inositol, particularly in the utilization of inherent growth substances.

Acknowledgements. The author is grateful to Dr. N.C. Gopalachari, Director, Tobacco Research for facilities provided and Mr. A.S.R. Murthy for the photographs.

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