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EFFECT OF CHEMICAL MUTAGENS ON STEROIDAL SAPOGENINS IN *TRIGONELLA* SPECIES

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Key Word Index—*Trigonella corniculata; T. foenum-graecum*; Leguminosae; ethyl methane sulphonate (EMS); methyl methane sulphonate (MMS); sodium azide (NaN_3); diosgenin; tigogenin.

Abstract—A two- to four-fold increase in the steroidal sapogenins (diosgenin and tigogenin) was observed in the plants and seeds obtained from seeds of *T. corniculata* and *T. foenum-graecum* treated with low concentrations of chemical mutagens (EMS, MMS and NaN_3). However, a decrease in their levels was recorded at high concentrations of the mutagens.

INTRODUCTION

Trigonella species are known to contain steroidal sapogenins [1–6], especially diosgenin, and thus, they are of great significance to the pharmaceutical industry. Mutagens have been used to improve the quality and quantity of the chemicals produced by crops [7], but very little work has been done on the effect of chemical mutagens on secondary metabolites in general and steroidal sapogenins in particular. Although, the effect of radiation on diosgenin has been investigated in *Dioscorea bulbifera* [8], *D. floribunda* [9] and *Costus speciosus* [10]. An increase in the solasodine content in *Solanum viarum* was observed upon EMS treatment [11]. The combined effect of irradiation and incubation with sodium azide on diosgenin content were similarly studied in *T. foenum-graecum* [12]. In the present investigation we have studied the individual effects of ethyl methane sulphonate (EMS), methyl methane sulphonate (MMS) and sodium azide (NaN_3) on diosgenin and tigogenin levels in *T. corniculata* L. (Piring Sak) and *T. foenum-graecum* L. (Fenugreek) with the aim to regulate their biosynthesis in these plant species.

RESULTS AND DISCUSSION

In both plant species, an increase in diosgenin and tigogenin levels was observed in the plants and seeds obtained from seeds treated with low concentrations of the mutagens, whereas the levels were significantly decreased at higher concentrations (Figs 1–3).

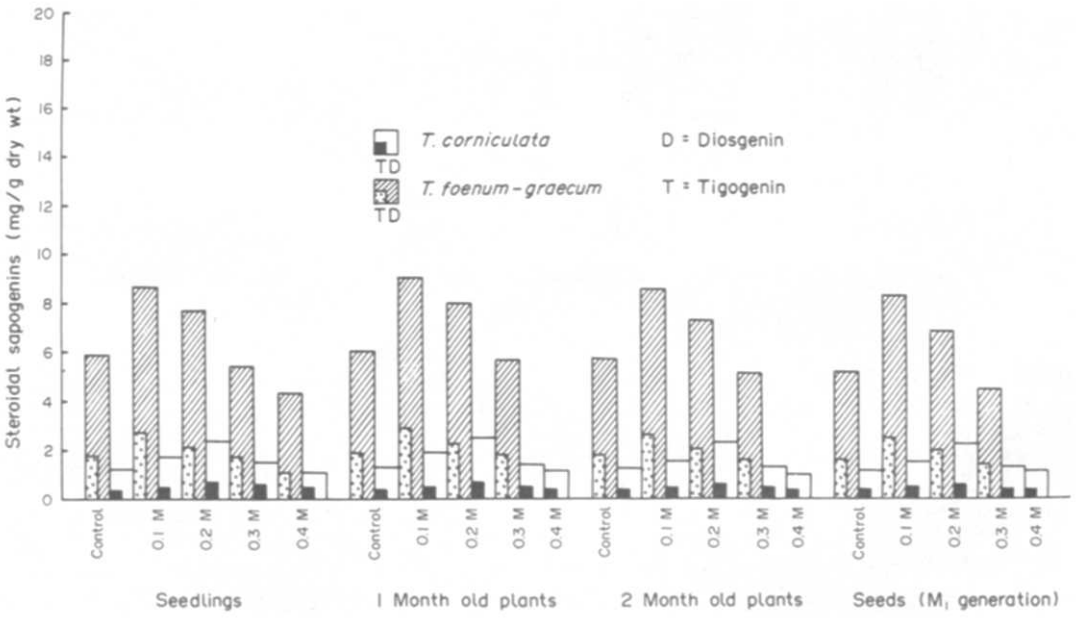

Fig. 1. Steroidal sapogenins in *Trigonella* species at various stages of growth after EMS treatment.

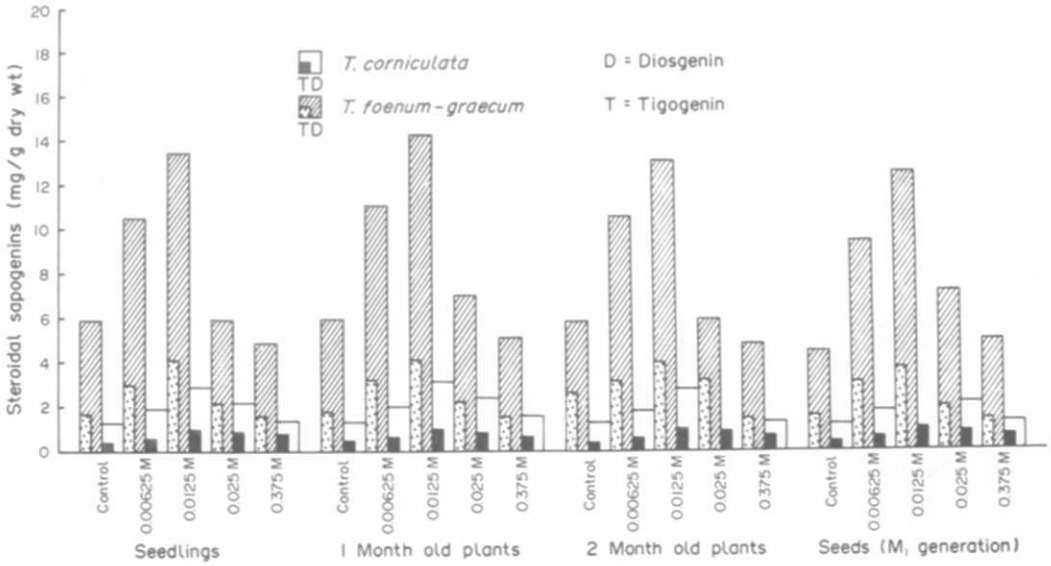

Fig. 2. Steroidal sapogenins in *Trigonella* species at various stages of growth after MMS treatment.

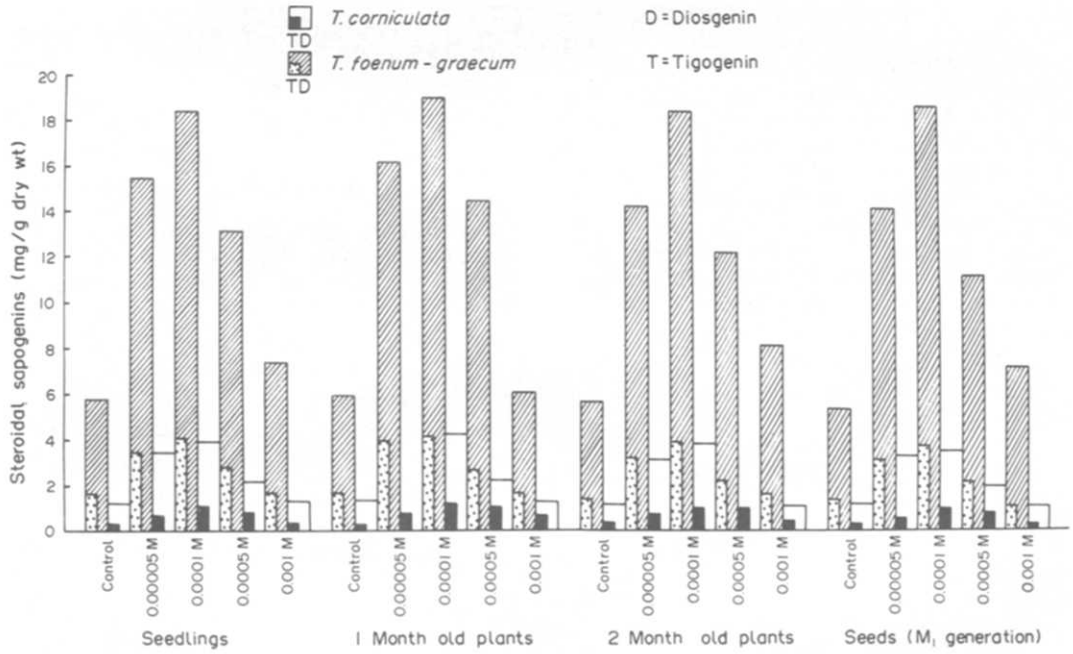

Fig. 3. Steroidal sapogenins in *Trigonella* species at various stages of growth after NaN_3 treatment.

The maximum increases in diosgenin and tigogenin levels were observed with 0.1 M EMS (2-fold), 0.025 M MMS (3-fold) and 0.0001 M NaN_3 (4-fold), in both plant species. The increases observed for each treatment were similar for seedlings, one-month-old plants, two-month-old plants and seeds. The greatest increase was brought about by NaN_3, supporting the earlier work with this mutagen [12]. An increase in the oil content in fenugreek by physical and chemical mutagenic treatments has been observed [13], and the present investigation shows that the diosgenin and tigogenin contents of fenugreek and Piring Sak can be improved by treatment with chemical mutagens.

EXPERIMENTAL

Pre-soaked seeds of *T. corniculata* and *T. foenum-graecum* were treated with different concentrations (Figs 1–3) of EMS, MMS and NaN_3 for 8, 4 and 6 hr respectively. The treated seeds were washed under running tap water for 5–10 min and the seeds germinated in petri-dishes and in pots, which were kept under normal controlled field conditions. The plants were sampled at

the seedling stage, and after one month and two months of growth. In addition the seeds were obtained from the M_1 generation. At 0.4 M EMS no plants of *T. Foenum-graecum* survived.

Each sample was dried, powdered, defatted (petrol, 40–60°) and hydrolysed with 15% ethanolic HCl (w/v). Each hydrolysate was processed further [14] using EtOAc to extract the steroidal sapogenins. Later, such samples were reconstituted in $CHCl_3$, filtered, dried again and weighed.

The steroid extracts after analytical TLC (silica gel; $CHCl_3$–hexane–Me_2CO, 23:5:2; spray-anisaldehyde reagent) were subjected to prep. TLC and the bands coinciding to diosgenin (R_f 0.59) and tigogenin (R_f 0.65) eluted and crystallized. The purity of the isolated compounds was checked by 2D-TLC (silica gel; 1D: CH_2Cl_2–MeOH–formamide, 93:6:1; 2D: cyclohexane–EtOAc–H_2O, 600:400:1). Both compounds were subjected for mp, mmp and spectral studies and the data compared with those of standards [1, 15]. For quantification of the two sapogenins, the spectrophotometric method of ref. [16] was followed after TLC on silica gel G.

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