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Investigation effect of ethyl methane sulfonate (EMS) on some of morphophysiological and phytochemical traits of fenugreek (*Trigonella foenum-graecum* L.)

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Abstract

Fenugreek (*Trigonella foenum-graecum* L.) is a member of the Fabaceae family which is an annual, dicotyledonous and self-pollinated plant. Since genetic diversity is the basis of plant breeding programs, it is important to create genetic mutations in order to increase diversity. To induce mutation, this experiment was conducted by applying four levels (0.1, 0.2, 0.3, and 0.4 %) of ethyl methane sulfonate (EMS) on fenugreek seeds. The treated seeds were planted in the research greenhouse on basis of randomized complete block design with three replications. The results showed that different EMS concentrations had significant effect on all the studied characteristics. The lowest values of the studied traits were observed in the control. Although the highest values of most morpho-physiological traits were obtained in 0.2 % of EMS, but the highest amount of phytochemical compounds were related higher concentrations of EMS. The content of trigonelline, diosgenin, nicotinic acid, and mucilage of grain were significantly increased with increasing EMS concentration. The highest and lowest contents of grains trigonelline were obtained in 0.3 % of EMS and control treatment, respectively. The nicotinic acid content had a positive and significant correlation with trigonelline, diosgenin and mucilage content. Also, the diosgenin content of grains had a positive significant correlation with mucilage content. In

general, the application of sufficient concentrations of EMS mutants could be used as an effective tool to increase grain yield and secondary metabolites in breeding programs of fenugreek.

Introduction

Fenugreek (*Trigonella foenum-graecum* L.) is a member of the Fabaceae family which is a yearly, dicotyledonous, self-pollinated plant. There are several species of fenugreek (Petropoulos, 2002) and diverse fenugreek genotypes are present in the world, differing in growth habits, morphology, seed quality and grain yield (Chaudhary et al., 2018). Some researchers have reported the somatic basic chromosome numbers of the genus Trigonella as 2n = 14, 16, 18, 30 and 46 (Martin et al., 2011a, b). The species of *Trigonella foenum-graecum* have the same chromosome number, 2n = 16 (Ladizinsky and Vosa, 1986). Fenugreek is adapted to different climatic, temperature and soil conditions and is cultivated in more than 20 different habitats in Asia, Europe, Africa, the America and some parts of Australia (Chaudhary et al., 2018). This plant is a kind of nutritious and healthy vegetable (Naghdi Badi et al., 2018) and its grains has various pharmacological effects including serum lipid lowering, anti-diabetic, diuretic, anti-bloating, anti-diarrhea and anti-rheumatoid (Khosla et al., 1995; Prasanna, 2000). The findings of a study have shown that fenugreek seeds from different parts of Iran are a good source for the production of 4-hydroxy isoleucine, which is effective in reducing blood sugar(Haeri et al., 2009). Ghasemi et al. (2018) reported that herbal tea of fenugreek grains improved mother's milk. Fenugreek grains are as an important source of valuable medicinal metabolites such as alkaloid trigonelline, nicotinic acid, choline, diosgenin, and steroidal saponins (Minorsky, 2002). Fenugreek's main bioactive phytochemical includes trigonelline (which is a most substantial alkaloid component), diosgenin (which is a steroidal sapogenin) and mucilage (which is an apolysaccharide compound), which plays protective roles in this plant (Zandi et al., 2015). Trigonelline is a natural alkaloid mainly found in fenugreek and other edible plants with a variety of medicinal applications. Ilavenil et al.'s (2014) findings revealed the mechanism underlying the anti-adipogenic activity of trigonelline. The small size of fenugreek is an obstacle to artificial crosses (Choudhary and Singh, 2001). Few studies have been performed on the effect of mutagens on fenugreek remediation, which can be noted in Basu et al. (2008).They successfully used EMS in the development of fenugreek mutants and produced new mutants that were more early limited growth habits, higher grains yield and quality and were consistent with the short growing season (Basu et al., 2008). Various mutagenesis are used to induce desirable high frequency mutations, including ionizing radiation and chemical mutants (Ahloowalia and Maluszynsky, 2001; Ashrafi Parchin et al., 2019). Chemical mutagenesis including ethyl methane sulfonate (EMS) ethylene imine (EI) ethidium bromide, methyl nitroso urea (MNU) n-nitroso-*n*-methyl urea (NMU) and sodium azide (NaN3). Ethyl Methane Sulphonate (EMS) is a potent chemical mutagen, extensively used in genetic research (Kumar et al., 2013). In this study, the effect of ethyl methane sulfonate (EMS) on certain morphophysiological characteristics and the main active components of Iranian

fenugreek (*Trigonella foenum-graecum* L.) were examined and the best concentration of EMS was introduced to obtain the highest yield of phytochemical compounds as well as the best mutant for future studies.

Section snippets

Plant materials and seed treatments

Studying the mutation impacts of various concentrations of ethyl methane sulfonate (EMS) on a number of morphophysiological and phytochemical fenugreek's features, a greenhouse experiment was carried out on the basis of haphazard full block design with three replications. The Fenugreek seed code TF-925 was obtained from the seed bank of the institute of Medical Plants, ACECR, Iran. The dried fenugreek seeds, containing 10 % moisture, were treated with various concentrations of ethyl methane…

Morphophysiological traits

The different concentrations of EMS had significant effects on morphophysiological traits (Table 1). The maximum grain yield was associated to 0.2 % EMS treatment, which with respects to statistics was remarkably different from the other EMS treatments (Table 2).The minimum grain yield was observed in the treatment of control. The highest and lowest grain number per pod were observed in 0.3 % and 0.1 % EMS treatments, respectively. According to LSD test (Table 2), the highest grain number per…

Discussion

This study showed that plant growth and morpho-physiological traits were altered by ethyl methane sulfonate. There are various reports of the highest mutation rate using different concentrations of mutagenic agents. In some studies, the lowest changes occurred at low concentrations (Data and Dasgupta, 2002; Kumar et al., 2013; Agarwal et al., 2015) and in some cases the highest rate of mutations was reported at moderate concentrations (Vanniarajan et al., 1996). In the present results, the…

Conclusions

In general, the findings of this study revealed that significant improvements in the morphophysiological and phytochemical features of fenugreek were triggered by the use of EMS. The lowest amounts of studied traits were related to control treatment. The highest values of

most morphophysiological traits were observed in the 0.2 % of EMS. With increasing EMS concentration, the amount of phytochemical compounds increased and the highest amount of trigonelline was observed in 0.3 % of EMS and the...

CRediT authorship contribution statement

Reza Ashrafi Parchin: Project administration, Investigation. **Ali Asghar Nasrollahnezhad Ghomi:** Conceptualization, Methodology, Formal analysis, Supervision, Writing - original draft, Writing - review & editing. **Hassanali Naghdi Badi:** Conceptualization, Methodology, Formal analysis, Supervision, Writing - original draft, Writing - review & editing. **Saeid Navabpour:** Project administration, Investigation. **Ali Mehrafarin:** Project administration, Investigation. **Ali Eskandari:** Project administration, ...

Declaration of Competing Interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the content of the paper....

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