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Screening of Fenugreek (*Trigonella foenum graecum* L.) Germplasm Lines for Diosgenin Potential

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Authors' contributions

This work was carried out in collaboration between all authors. Author HD performed the experiment, analysed the results and performed statistical analysis. First draft was prepared by authors HD, DS and SA gave it to final shape.

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Short Research Article

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ABSTRACT

Fenugreek seeds were evaluated for diosgenin content and productivity which can be useful for pharmaceutical industries and farmers. Statistical significance was tested by ANOVA (n=5) followed by comparisons of means by DMRT ($p \leq 0.05$). Fenugreek seeds were grown in the experimental fields of Vegetable Research Center, Pantnagar, Uttarakhand, India for cropping year 2014. Two local varieties Pant Ragini (PR), Pusa Early Bunching (PEB) and eight accessions PM(C)1, PM 2, PM 3, PM 4, PM 5, PM 6, PM 7 and PM 8 were selected to evaluate high diosgenin content and productivity. Results indicate that fenugreek is a good source of diosgenin with high productivity, ranging from 0.40 to 0.60 g/100 g and 7.78 to 14.95 Kg ha^{-1}, respectively. Significant differences ($p \leq 0.05$) were observed among the accessions and local varieties with respect to diosgenin content and productivity. The higher diosgenin content was recorded in local variety, PEB (0.60%) and PR (0.59%), followed by accession PM 7 (0.59%) and PM2 (0.50%) respectively. The highest diosgenin productivity was recorded in PR (14.95 Kg ha^{-1}) followed by PEB (14.54 Kg ha^{-1}), PM 7 (14.46 Kg

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ha^{-1}) and PM (C)1 (13.90 Kg ha^{-1}). The maximum seed yield was recorded in accession PM(C)1 (2.98 T ha^{-1}) followed by PR (2.52 T ha^{-1}). Fenugreek seeds, used as functional foods or pharmacology industry due to bioactive substance diosgenin, contribute nutraceutical and pharmacological attributes. Fenugreek may be a feasible alternative for diosgenin production because of lower production costs in a short growing period, ease of cultivation and consistent seed yield. Variation in genotype as well as plant species bioactive compound composition has been contradictory for their availability and commercial use. This study may be useful in selecting superior fenugreek genotypes for targeted pharmaceutical purposes and also for a breeding program, further facilitate the development of more reliable genotype for industrial use.

Keywords: Diosgenin; productivity; trigonella; spectrophotometer.

1. INTRODUCTION

Phytochemicals present in food and spices are gaining popularity over synthetic drugs as they act via multiple molecule targets that synergize to prevent or treat chronic illness. Phytochemicals are considered safe with better bioavailability. Diosgenin (Fig. 1a) is a spirostanol saponin structurally similar to cholesterol and other steroids [1]. Diosgenin has great interest to the pharmaceutical industry because it is often used as a raw precursor for the production of steroidal drugs and hormones such as testosterone, glucocorticoids and progesterone [2,3] and may attribute pharmacological properties such as hypoglycemic activity, hypocholesterolemic and to treat obesity [1], chemopreventive agent [4] and dyslipidemias [5].

The annual requirement of diosgenin for pharmaceutical industry in India is 60 tonns in year 1999 and the annual requirement of diosgenin in the world is 3,000 tons and that in India 150-200 tons, that will be increase in upcoming years [6,7]. In India, the main raw material as a source of diosgenin for the production of pharmaceutical drugs is *Solanum lacinatum*, *Dioscorea deltoidea* and *D. prazeri*. The total production of diosgenin in India is only 25-30 tons, the rest being met by imports [7,8]. In India, diosgenin is mostly produced from *Dioscorea deltoid* approximately 15-20 tonnes annually by a process that is costly and difficult requiring many years before the tuber grown to a size with significant content of diosgenin [6]. Diosgenin a naturally occurring steroid saponin is commercially procured from tubers of certain wild species of Maxican yam (*Dioscorea* species). However, the process is costly and time consuming. It require several years before a yam tubers grown to a size where they possess a significant concentration of diosgenin to be used as a source of raw material. In India, the main raw material for the production of steroidal drug is *Solanum lacinatum*, *Dioscorea deltoidea* and *D. prazeri* [8].

Fenugreek seed (Fig. 1b) have significant contribution to human for treatment of variety of health related problem. Fenugreek seed contain many nutrients and other active components such as protein, vitamin C, galactomannan, trigonelline, diosgenin and minerals which are beneficial for human health [9-12] and may attributes pharmacological properties such as hypoglycemic [13], hypocholestromic [14], gastroprotective [15], chemoprotective [16] and exhibit higher antioxidant potential, reduce the intracellular ROS level since the root cause of diabetes and cardiovascular disease is known to be oxidative stress caused by ROS [17,18]. Fenugreek (Fig. 1c) may be a visible alternative for the production of diosgenin because of its shorter growing cycle, lower production cost and constitutive yield and quality [19]. Fenugreek may be used as an alternate source of diosgenin production. The diosgenin content in fenugreek varies from 0.1–0.9% [20].

India is the largest producer of fenugreek in the world. Rajasthan is the major fenugreek producer state. It is also grown on a commercial scale in Gujarat, Uttarakhand, Uttar Pradesh and Madhya Pradesh [21]. In 2012-13 total production of fenugreek was reported to be 113 thousand MT [22]. Higher seed yield per hectare will be obtained through superior varieties and better management practices and contributes to increase in the crop worldwide. So Scientific phytochemical screening to obtain superior varieties containing the rich amount of industrial important bioactive compound may acts as a bridge to minimize the gap between its current productivity and potential uses. Therefore, present study was designed for screening fenugreek genotypes with high diosgenin content

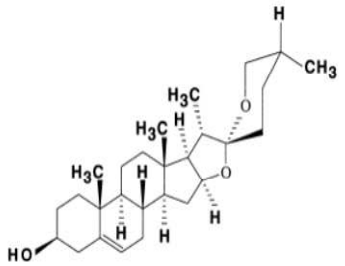

Fig. 1a. Diosgenin structure **Fig. 1b. Fenugreek seeds** **Fig. 1c. Fenugreek plant**

and productivity which can be useful for its broad utilization and commercial exploitations.

2. MATERIALS AND METHODS

2.1 Chemicals

Diosgenin and antimony pentachloride were purchased from Sigma Chemical Co. Hexane was purchased from Merck & Co and all others unlabelled chemicals and reagents were analytical grade purchased from Sd Fine Chemicals Limited, India.

2.2 Plant Sample

Ten genotypes including two checks (Pant Ragini, Pusa Early Bunching) of fenugreek seeds used in this study was initially obtained from a wide range of climatic zone and environment and selected to grow in the experimental fields of Vegetable Research Center (VRC) Pantnagar, Uttarakhand, India. The study was conducted in cropping year 2014. For analysis the fenugreek seeds randomly obtained from all the genotypes grown in the field after seed maturity. Harvested seeds were dried at 50±5℃ in a hot air oven to a constant weight then ground finally to fine flour and kept in an airtight container till analysis.

2.3 Estimation of Diosgenin

Diosgenin is extracted by hydrolysis with ethanol and sulphuric acid [23,24] and estimated in spectrophotometer [25]. For extraction of diosgenin 500 mg of dried seed powder was hydrolyzed with 30 ml of 1 M H_2SO_4 in 100% ethanol for 30 min at 100℃. After hydrolysis the solution was diluted with 20 ml of distilled water. Diosgenin was extracted thrice with 20 ml of hexane as solvent. The hexane extract (60 ml) was washed with 20 ml of 0.1 M NaOH to remove free fatty acids. 10 ml of distilled water was added to remove hydrophilic contaminants and the hexane extract was used for the estimation of diosgenin. Hexane extract (1 ml) was taken in a test tube and kept in a waterbath at 75℃. 5 ml of concentrated $HClO_4$ followed by 0.1 ml $SbCl_5$ solution (24% in 70% $HClO_4$) were added to each tubes, the tubes were incubated for 30 min at room temperature and the absorbance measured at 486 nm against blank. The diosgenin content (mg/g dry.wt) was calculated using a standard curve (10-100 µg) prepared by using diosgenin as standard.

2.4 Statistical Analysis

Results of this study represent values were expressed as means of determinations made in five replicates. Statistical significance was tested by ANOVA followed by comparisons of means by Duncan's multiple range test ($P<0.05$) calculated using SPSS 20 IBM. Stepwise regression analyses were used to determine the correlation among variables.

3. RESULTS AND DISCUSSION

Diosgenin is an important steroidal metabolite found in fenugreek seed used for starting material for synthesis of steroidal drugs and play important role in control of cholesterol metabolism, antitumor activity and to treat diabetes. The diosgenin content was found to vary significantly among accessions and ranged from 0.40 to 0.60% (Table.1). The highest diosgenin content was recorded in local variety Pusa Early Bunching (0.60%) followed by Pant Ragini (0.59%), accession PM 7 (0.59%) and PM 2 (0.50%). Similar results were also reported by [26] where diosgenin levels from mature seeds ranged from 0.28 to 0.92% among 10 accessions of fenugreek. Also, 0.55, 0.42, and 0.75% diosgenin content have been reported in three fenugreek seed samples [30]. A variation in the

seed diosgenin content between 0.33 and 1.9% from different bio-geographical areas in India was observed by [27].

The hierarchy was:

PM 3≈PM 4≈ PM 6≈ PM 5≈ PM(C)1≤PM 2 ≈ PM 8≤ PM 7≈Pusa Early Bunching≤ Pant Ragini

Regarding yield, it ranged from 1.87 to 2.98 T ha^{-1} (Table 1) and only accession PM(C)1 performed significantly better than local checks. The maximum seed yield was recorded in accession PM(C)1 (2.98 T ha^{-1}) followed by local variety Pant Ragini (2.52 T ha^{-1}) at par with PM 6 (2.50 T ha^{-1}), PM 8 (2.48 T ha^{-1}), PM 7 (2.46 T ha^{-1}), Pusa Early Bunching (2.41 T ha^{-1}), PM 4 (2.33 T ha^{-1}) and significantly higher from PM 5 (2.00 T ha^{-1}) at par with PM 3 (1.94 T ha^{-1}) and PM 2 (1.87 T ha^{-1}) and comparable to the reports by [9] where seed yield of different genotypes in irrigated environments found to be 2.5 to 4.0 T ha^{-1}.

The seed yield in increasing order was:

PM2 ≈ PM3≈ PM5 ≤ PM4 ≈ Pusa Early Bunching ≈ PM7 ≈ PM8 ≈PM6 ≈ Pant Ragini ≤ PM(C)1

The diosgenin productivity ranged from 7.78 to 14.95 Kg ha^{-1} (Table 1). The highest diosgenin productivity was recorded with local variety Pant Ragini (14.95 Kg ha^{-1}) followed by Pusa Early Bunching (14.54 Kg ha^{-1}), PM 7 (14.46 Kg ha^{-1}) and PM (C)1(13.90 Kg ha^{-1}). Diosgenin productivity ranged from 14.23 Kg ha^{-1} to 22.74 Kg ha^{-1}.

The diosgenin productivity in increasing order was:

PM 3≈PM 4≈ PM 6≈ PM 5≈ PM(C)1≤PM 2 ≈ PM 8≤ PM 7≈Pusa Early Bunching ≈ Pant Ragini.

Table 1. Diosgenin content, productivity and seed yield in fenugreek genotypes (Values are mean ± SD, n =5)

Fenugreek lines	Seed yield (Tha^{1})	Diosgenin (%)
PM(C)1	2.98^{a}	0.466^{c}
PM 2	1.87^{c}	0.498^{bc}
PM 3	1.94^{c}	0.400^{c}
PM 4	2.33^{b}	0.429^{c}
PM 5	2.00^{c}	0.466^{c}
PM 6	2.50^{b}	0.442^{c}
PM 7	2.46^{b}	0.587^{ab}
PM 8	2.48^{b}	0.506^{bc}
Pant Ragini	2.52^{b}	0.594^{ab}
PEB	2.41^{b}	0.604^{a}
Average	2.34	0.486
SEm	0.591	0.0319
CD 5%	0.169	0.0914

Data are mean ± SEm (n=5), PR= Pant Ragini, PEB= Pusa Early Bunching, IC= Advanced lines, $^{a-d}$The means of the same superscript are not significantly different (p≤0.05)

Seed yield have dominating influence over net biochemical production of diosgenin. Although, there is highly variable average level of diosgenin% which increases with seed yield. A weak relationship between diosgenin % and seed yield (R^2 = 0.062) was found for all accessions along with local check (Fig. 2.A). That is, slope for a best fit line to the data, was positive but had a low R^2. A linear relationship

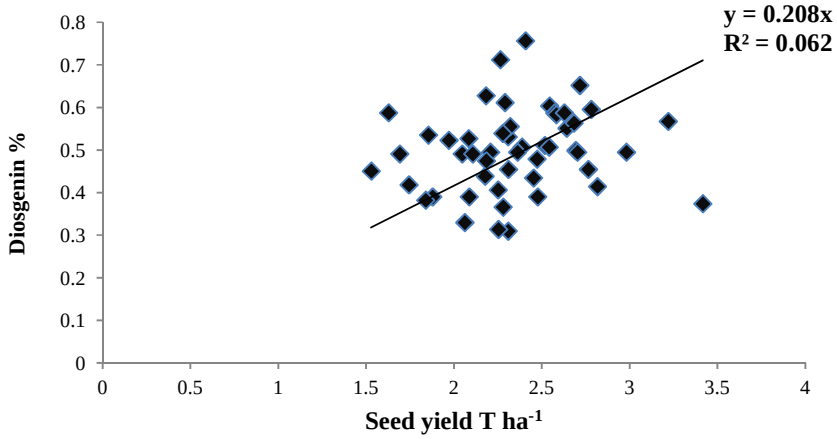

Fig. 2A

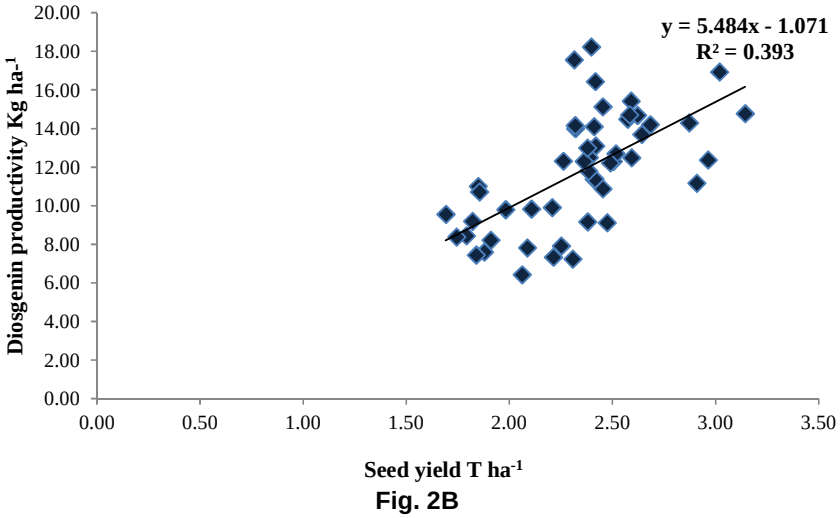

Fig. 2B

Fig. 2. Plot of seed yield versus (A) diosgenin %, (B) diosgenin productivity

was found between diosgenin productivity and seed yield (R^2 = 0.393) (Fig. 2B above) average level of diosgenin productivity increases with seed yield.

4. CONCLUSION

Fenugreek seeds traditionally used as food (spice) and medicine for treatment of many diseases mainly consumed by diabetic and hypercholestromic people. Diosgenin is one of the major bioactive constituent used for synthesis of steroidal drugs and to treat various diseases [28,29] found in the seeds of fenugreek as well as in the root tubers of wild yams (*Dioscorea villosa* L.) [30]. Fenugreek is an unassuming plant give a consistent seed yield may be a viable alternative for production of diosgenin because of lower production costs in a short growing period, ease of cultivation and its rapid growth [19,31]. The highest diosgenin content was recorded with local variety Pusa Early Bunching, Pant Ragini, accession PM 7 and PM 2. The highest diosgenin productivity was recorded with local variety Pant Ragini, Pusa Early Bunching, advanced lines PM 7 and PM (C)1. Many plants exhibit variation in proportions of bioactive constituents that can influence profitable recovery of these substances for use by functional food/medicinal plant industry and also to the consumers. Variation in genotype as well as plant species for specific bioactive compound composition can contribute to their inconsistent availability and have been criticized for the commercial use. The biochemical analysis observations from this study may be imperative for the development of an understanding about the use of fenugreek by selecting superior fenugreek genotypes for farmers and targeted pharmaceuticals purposes, food and also for a breeding program further facilitate the development of more reliable genotype for use by the industry.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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