

Original Research Article

<https://doi.org/10.20546/ijcmas.2018.705.281>

Evaluation of Micronutrients in Fenugreek (*Trigonella foenum-graecum* L.): A Viable Alternative for Micronutrient Supplementation

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ABSTRACT

Dietary antioxidants (total ascorbate, α -tocopherol and total carotenoids) and micronutrient (iron, calcium, zinc, copper and manganese) content of 25 fenugreek (*Trigonella foenum graecum* L.) genotypes were evaluated under the present investigation. Higher α -tocopherol and total carotenoid content i.e., 43.080 ± 1.542 mg α -tocopherol/100 g dry wt. and 4.890 ± 0.047 g carotenoids/100 g dry wt. was observed in RMt-305. The genotype RMt-143 showed highest iron, calcium and manganese content i.e., 184.11 ± 1.639 , 781.20 ± 4.790 and 25.65 ± 0.695 μ g/g dry wt., respectively. Total ascorbate content within fenugreek genotypes under investigation showed a positive correlation with α -tocopherol and total carotenoid content (i.e., 0.114 and 0.061, respectively). It also showed a significant ($p \leq 0.05$) positive correlation with manganese (0.298) and iron content (0.062) among the genotypes. α -tocopherol content within fenugreek genotypes also showed a significant ($p \leq 0.01$) positive correlation (0.328) with total carotenoid content another important lipid-soluble antioxidant. The iron content within the fenugreek genotypes showed a significant ($p \leq 0.01$) positive correlation with other micronutrients viz., calcium, zinc and manganese (i.e., 0.408, 0.502 and 0.299, respectively). A positive correlation was also observed between iron and total ascorbate, α -tocopherol content (i.e., 0.062 and 0.213, respectively) within the genotypes. Similarly other mineral elements viz., calcium, zinc, copper and manganese, respectively also showed a positive correlation with each other in general. The findings of the present investigation clearly show significant variation in dietary antioxidants and micronutrients among the fenugreek genotypes under investigation. Thus the genotypes with higher dietary antioxidants and micronutrient content viz., RMt-305, Pant ragini, RMt-143, Pusa early bunching, IC-066843, GM-2 and HM-355 could be utilized as a valuable source of dietary micronutrients and may serve as potential functional food to combat micronutrient malnutrition along with other favourable health promoting affect.

Keywords

Ascorbate, α -Tocopherol,
Carotenoid, Dietary
antioxidants,
Micronutrients

Article Info

Accepted:
18 April 2018
Available Online:
10 May 2018

Introduction

Deficiencies of micronutrients (minerals and vitamins) have become a major health issue globally. These deficiency disorders leads to poor quality of life, reduced economic productivity and precious human lives lost throughout the world. More than 2 billion people in the world today most of them in developing countries, are estimated to be deficient in key vitamins and minerals, particularly vitamin A, iodine, iron and zinc (Tulchinsky, 2010; Müller and Krawinkel, 2005; Bailey *et al.*, 2015). Most of them live in underdeveloped and developing countries and are typically deficient in more than one micronutrient (Hill and Mkparu, 2015). Deficiencies occur when people do not have access to micronutrient-rich foods such as fruit, vegetables, animal products and fortified foods, usually because of poor quality of diet or unavailability of dietary diversity from different food groups (Burchi *et al.*, 2011; Miller and Welch, 2013). The most vulnerable groups usually suffering from micronutrient deficiencies are pregnant women, lactating women and young children, mainly because of relatively higher requirement of micronutrients and are more susceptible to the harmful consequences of micronutrient deficiencies (Benton, 2008; Fanzo, 2015).

Iron and vitamin A deficiencies are the most prevalent forms of micronutrient malnutrition apart from zinc deficiency (Akeredolu *et al.*, 2011; Bhandari and Banjara, 2015). Iron deficiency is the most common nutritional deficiency in the world, affecting about 25% of the global population, particularly young women and children. Iron deficiency anaemia is caused by insufficient consumption of iron (or insufficient absorption of iron) and is a decrease in red blood cells (RBCs) or haemoglobin, leading to symptoms such as tiredness and fatigue; weakness, shortness of breath and reduced exercise tolerance (Longo

and Camaschella, 2015). Iron deficiency without anaemia has also been shown to reduce endurance capacity, increase energy expenditure and impair adaptation to endurance exercise in females experiencing tissue depletion (Burden *et al.*, 2015). Calcium also have significant effect on growth and development insufficient dietary calcium have negative impact on health and increase the risk of diseases such as osteoporosis (Kin *et al.*, 2007; Morris *et al.*, 2008). Zinc acts as a cofactor of various enzymes involved in macronutrient metabolism and cell replication (Soetan *et al.*, 2010; Arinola, 2008). The primary roles of zinc appear to be in cell replication and gene expression and in nucleic acid and amino acid metabolism. Vitamins A and E metabolism and bioavailability are dependent on zinc status (Szabo *et al.*, 1999).

Copper functions in the utilization of iron in an early stage of haemopoiesis. Copper deficiency results in an increase in iron in the liver, whereas an excess of copper results in a decrease in iron content of the liver, thus reflecting the role of copper in iron utilization. Copper is an essential micro-nutrient necessary for the haematologic and neurologic systems (Tan *et al.*, 2006). It is necessary for the growth and formation of bone, formation of myelin sheaths in the nervous systems, helps in the incorporation of iron in haemoglobin, assists in the absorption of iron from the gastrointestinal tract and in the transfer of iron from tissues to the plasma (Soetan *et al.*, 2010). Manganese has a variety of metabolic functions including enzyme activation, neurological function, skeletal system development, energy metabolism, reproductive function, and immunological function. It also serves as an antioxidant that protects cells from damage due to free radicals (Santamaria, 2008). Manganese also plays an essential role in regulation of cellular energy, bone and connective tissue growth and blood clotting (Erikson and Aschner, 2003).

Carotenoids are known to be very efficient physical and chemical quenchers of singlet oxygen ($^1\text{O}_2$), as well as potent scavengers of other reactive oxygen species (ROS) (Fiedor *et al.*, 2005). They are known to play a protective role in a number of ROS-mediated disorders, such as, i.e., cardiovascular diseases, several types of cancer or neurological, as well as photosensitive or eye-related disorders (Fiedor and Burda, 2014). Dietary carotenoids are thought to provide health benefits in decreasing the risk of disease, particularly certain cancers and eye disease. Carotenoid appears to have potential role as antioxidants in foods and humans. Recent experimental evidences supports the role of carotenoids in enhancement immunity i.e., proliferation of T and B lymphocyte, stimulate effector T cell functions, and increased production of certain interleukins (Eldahshan *et al.*, 2013). α -tocopherol (vitamin E) is the major lipid-soluble antioxidant that maintains cell integrity by preventing lipid peroxidation in cellular membranes (Cervantes and Ulatowski, 2017; Burton *et al.*, 1982). Vitamin E is associated with membrane stabilization and improves membrane repair (Raederstorff *et al.*, 2015; Howard *et al.*, 2011).

There is growing experimental evidence of the possible role of α -tocopherol in oxidative stress-related diseases including neurological conditions such as Alzheimer's, Parkinson's and Down's syndrome (Schrag *et al.*, 2013; Sung *et al.*, 2004; Buhmann *et al.*, 2004; Liu *et al.*, 2007; Perrone *et al.*, 2007). Ascorbic acid (Vitamin C) is known to play a crucial role in immune system function (Maggini *et al.*, 2007; Webb and Villamor, 2007) and amino acid metabolism. It assists in lowering blood cholesterol by increasing its elimination from the body (Dave and Patil, 2017). Vitamin C regulates internal microenvironment by maintaining appropriate redox balance within the cell. Altered redox balance is associated

with various diseases i.e., obesity, cancer, neurodegenerative diseases, hypertension and autoimmune diseases thus vitamin C can be used as an adjunct in treatment of these diseases (Figueroa-Méndez and Rivas-Arancibia, 2015). Vitamin C can even overcome the effects of phytic acid, polyphenols etc., (Hurrell and Egli, 2010; Hallberg and Hulthén, 2000; Davidsson, 2003). Dietary vitamin C is also important in its ability to facilitate proper iron levels. Higher iron absorption (six folds) within individuals with high daily dietary intake of vitamin C has been observed (Pacier and Martirosyan, 2015; Hallberg, 1981). Fruits and vegetables offer a diverse mixture of nutrients that promote good health, and it is generally thought that they will be more beneficial to human health than dietary supplements (Cooper, 2004; Blomhoff *et al.*, 2006). Vegetables offers a promising source and its bioavailability can be increased by employing suitable strategies (Bachrach, 2001; Maggioli and Stagi, 2017; Miller *et al.*, 2001; Bhide *et al.*, 2013).

Fenugreek (*Trigonella foenum-graecum* Linn.) an ancient medicinal herb is used in various traditional medicinal practices throughout the world. The leaves and seeds of the plant are widely used in various food preparations and as an ingredient in medicinal preparations (Syeda *et al.*, 2008). The seed find widespread use as condiments (Meghwal and Goswami, 2012). Fenugreek is associated with various therapeutic properties including antioxidant, anti-inflammatory, hepatoprotective effects (Kumar *et al.*, 2012; Albasha and Azab, 2014), anti-diabetic and lipid lowering effect (Neelakantan *et al.*, 2014). Fenugreek seeds contain high amount of carbohydrates (45-60%), proteins (20-30%), oils (5-10%), polyphenols, alkaloids, free amino acids, minerals including calcium and iron, vitamin A, B₁, C and nicotinic acids (Norziah *et al.*, 2015).

Recent experimental evidences have identified fenugreek as a valuable multipurpose plant with well proven therapeutic effects and as raw material for pharmaceutical industry, especially steroidal hormones (Nasroallah and Kolsum, 2013). Fenugreek seeds have been used in development of therapeutic foods especially for diabetes (Vijaykumar and Deepa, 2010; Jain *et al.*, 2015) but its potential use to supplement micronutrient deficiencies has not yet been tried out. More research is needed to identify target bioactives and the levels required for a beneficial effect on long-term health and the prevention of chronic diseases. These studies need to compare cultivars with varying levels of bioactives as well as specific individual or combinations of bioactives in animal models and human clinical trials to identify the key targets for the development of truly health enhanced cultivars of fruit. The need of the hour to address global micronutrient malnutrition requires the need for many strategies. Conventional approaches including micronutrient supplementation and fortification, has certain limitations. Promotion of suitable food based approaches to enable adequate intakes of micronutrients by much of the population includes dietary diversification strategies and use of nutrient rich foods.

Dietary diversification to combat micronutrient malnutrition is only possible by the promotion of functional foods. Furthermore, health-promoting compounds from fruits and vegetables, either as pure compounds or as standardized extracts, have unmatched chemical diversity and thus can provide opportunities for exploring novel biological activities (Patil *et al.*, 2014). As a result of an increasing demand for chemical diversity, programs screening compounds from fruits and vegetables are growing rapidly throughout the world. The main objective of the present investigation was to evaluate the

genotypic difference in different fenugreek genotypes w.r.t different micronutrients and identification of potential genotypes, which could be used to overcome micronutrient deficiencies in human beings.

Materials and Methods

Chemicals and reagents

The compounds β -carotene, 2, 2'-bipyridyl, α -tocopherol, N-ethylmaleimide were purchased from Himedia (India). All other chemicals and reagents used were of analytical grade.

Plant material

The present study was undertaken to investigate fenugreek genotypes w.r.t micronutrients i.e., Iron, zinc, manganese, copper, calcium, carotenoids, ascorbic acid and α -tocopherol in seeds of different genotypes. The seeds of the fenugreek genotypes (25) under varietal trials of All India Coordinated Research Project (AICRP) on seed Spices were used in the present investigation. Fenugreek genotypes viz., AM-316, UM-271, UM-222, UM-258, UM-265, UM-273, UM-274, UM-279, UM-325, IC-143843, IC-143850, IC-143816, IC-066843, UM-126, RMt-303, RMt-305, Pusa early bunching, RMt-1, GM-2, RMt-2, RMt-361, RMt-143, Pant ragini, HM-355 and UM-366 were collected from Vegetable Research Centre (VRC), GBPUA&T, Pantnagar, district Udham Singh Nagar (Uttarakhand, India).

Total ascorbate (Vitamin C)

Total ascorbate content within fenugreek genotypes was estimated by the method of Law *et al.*, (1983). To 500 mg powdered seed samples, 10 % (w/v) trichloroacetic acid was added and vortexed mixed. The resulting extract was kept in ice for 5 min. 10 μ l NaOH was added and the extract centrifuged in a

microfuge for 2 min. The supernatant was used for the estimation of total ascorbate content. To 200 µl of supernatant, 900 µl 200 mM NaH₂PO₄ buffer (pH 7.4), and 200 µl of 1.5 mM-dithiothreitol was added and mixed thoroughly. The reaction mixture was left at room temperature for 30 min and 200 µl of 0.5 % (w/v) N-ethylmaleimide was added to remove excess DTT. Colour was developed in the reaction mixture by adding 1000 µl of 10 % (w/v) trichloroacetic acid, 800 µl of 42 % (v/v) O-phosphoric acid, 800 µl of 65 mM 2, 2'-bipyridyl in 70 % (v/v) ethanol and 400 µl of 3 % (w/v) FeCl₃. The reaction mixture vortexed and were incubated at 42°C for 60 min. Absorbance of the resulting solution was measured at 525 nm. Ascorbic acid (10-100 µg) was used as the standard. Total ascorbate content within fenugreek genotypes was expressed as µg ascorbic acid equivalents g⁻¹ fresh wt.

Total α-tocopherol (vitamin E)

α -Tocopherol (vitamin E) content within fenugreek genotypes was estimated by the method described by Backer *et al.*, (1980). 500 mg powdered seed samples were homogenized with 10 ml mixture of petroleum ether and ethanol (2:1.6; v/v) the resulting extract was centrifuged at 10,000 rpm for 20 min and the supernatant used for estimation of α -tocopherol.

To 1 ml of supernatant, 200 µl of 2 % 2, 2'-bipyridyl (dissolved in ethanol) was added. The reaction mixture was mixed thoroughly and kept in the dark for 5 min. The red colour developed within the reaction mixture was diluted with 4 ml of distilled water and mixed well. The resulting colour (red) in the aqueous phase was measured at 520 nm. α -tocopherol (10-50 µg) was used as the standard. The total α -tocopherol content within fenugreek genotypes was expressed as µg α-tocopherol equivalents g⁻¹ fresh wt.

Total carotenoids

Total carotenoid content in the fenugreek genotypes was estimated by the method of Jensen, (1978). Carotenoids were extracted on the basis of their solubility in acetone. 20 ml acetone was added to 1 gram powdered seed sample and extracts were kept overnight at room temperature. The extracts were filtered through whatman no. 1 filter paper. The residue was re-dissolved (twice) in acetone and filtered again so as to facilitate complete extraction of carotenoids. The combined filtrates were partitioned (thrice) with equal volume of peroxide-free ether. The pooled ether fraction was evaporated to dryness, under reduced pressure in a rotary flask evaporator at 35°C. The residue was dissolved in ethanol and treated with 60 % aqueous KOH. The resulting mixture was boiled for 10 min and partitioned (thrice) with ether. The combined ether fraction was evaporated to dryness and the residue re-dissolved in ethanol. The absorbance of ethanolic solution was measured at 450 nm. β-carotene (10-200 µg) was used as a standard Total carotenoid content was expressed as µg β-carotene equivalents g⁻¹ fresh wt.

Micronutrient estimation

Digestion of seed sample

1 gram powdered dried seed samples from different fenugreek genotypes were weighed and then transferred to digestion tubes. About 10 ml of conc. HNO₃ was added to each tube and kept overnight. The digestion tubes were, thereafter, kept on a hot plate for 10 min. The samples were cooled and 10 ml mixture of nitric acid- sulfuric acid- perchloric acid (10: 1: 4 v/v/v) was added in each sample. The samples were then kept on a hot plate for complete digestion. After cooling, 5 ml of 6 N HCl was added and volume of the digests made upto 50 ml with distilled water. The

digests were filtered with a whatmann no. 1 filter paper and transferred to storage vials. These digested samples were analyzed for zinc, iron, copper, calcium and manganese.

Determination of iron, calcium, zinc, copper and manganese in plant samples

The micronutrients i.e., iron, calcium, zinc, copper and manganese within fenugreek genotypes were determined with the help of double beam atomic absorption spectrophotometer (ECIL, Hyderabad). The instrument settings for different elements were as follows:

Statistical analysis

Analysis of variance (ANOVA) and Duncan LSD post hoc test were carried out on the experimental values (n=3) obtained in the present investigation. Correlation analysis (bivariate) was also carried out to determine the relationship between dietary antioxidants (i.e., total ascorbate, α -tocopherol and total carotenoids) and different mineral elements (i.e., iron, calcium, zinc, copper and manganese) present within different fenugreek (*Trigonella foenum-graecum* L.) genotypes under investigation. Statistical analysis and graphing were carried out by the software IBM SPSS Statistics 20 (IBM Corporation) and Sigma Plot for Windows 11.0 (Systat Software, Inc.), respectively. A statistical difference at $p \leq 0.05$ between genotypes under different estimations was considered to be significant.

Results and Discussion

Total ascorbate content

Total ascorbate content within fenugreek genotypes under investigation varied from 4.915 ± 0.077 to 51.292 ± 0.231 mg ascorbic acid equivalents/100 g dry wt. in IC-066843

and HM-355, respectively (Figure 1: A). No significant difference ($P \leq 0.05$) in total ascorbate content was observed between UM-366, IC-143850 and RMt-303 (i.e., 37.611 ± 0.363 , 37.379 ± 0.096 and 35.988 ± 1.783 mg ascorbic acid equivalents/100 g dry wt., respectively). Similarly no significant difference ($P \leq 0.05$) in total ascorbate content was observed between UM-265 and UM-222 (i.e., 29.379 ± 0.811 and 29.031 ± 0.231 mg ascorbic acid equivalents/100 g dry wt., respectively), IC-143816 and UM-274 (i.e., 27.640 ± 2.009 and 27.524 ± 1.379 mg ascorbic acid equivalents/100 g dry wt., respectively), RMt-143 and IC-143843 (i.e., 26.017 ± 0.386 and 25.669 ± 0.425 mg ascorbic acid equivalents/100 g dry wt., respectively), RMt-361 and UM-273 (i.e., 24.510 ± 1.198 and 24.046 ± 0.811 mg ascorbic acid equivalents/100 g dry wt., respectively), RMt-1 and UM-258 (i.e., 14.075 ± 0.038 and 13.959 ± 0.927 mg ascorbic acid equivalents/100 g dry wt., respectively). The 25 fenugreek genotypes can be grouped into 18 distinct groups on the basis of total ascorbate content. The genotypes within the groups differed significantly ($P \leq 0.05$) w.r.t total ascorbate content.

α -Tocopherol content

α -Tocopherol content within fenugreek genotypes under investigation varied from 0.948 ± 0.021 to 43.080 ± 1.542 mg α -tocopherol/100 g dry wt. in UM-274 and RMt-305, respectively (Figure 1: B). No significant difference ($P \leq 0.05$) in α -tocopherol content was observed between Pusa early bunching and IC-143816 (i.e., 14.653 ± 1.184 and 14.653 ± 1.184 mg α -tocopherol /100 g dry wt., respectively). Similarly no significant difference ($P \leq 0.05$) in α -tocopherol content was observed between UM-265, GM-2 and IC-143850 (i.e., 9.070 ± 0.338 , 9.070 ± 0.083 and 9.070 ± 0.584 mg α -tocopherol/100 g dry

wt., respectively), UM-258 and UM-222 (i.e., 7.547 ± 0.169 and 7.547 ± 0.507 mg α -tocopherol/100 g dry wt., respectively), UM-273 and UM-271 (i.e., 6.024 ± 0.078 and 6.024 ± 0.338 mg α -tocopherol/100 g dry wt., respectively), Pant ragini and HM-355 (i.e., 3.486 ± 0.169 and 3.486 ± 0.169 mg α -tocopherol/100 g dry wt., respectively), UM-279 and UM-366 (i.e., 2.471 ± 0.507 and 2.471 ± 0.169 mg α -tocopherol/100 g dry wt., respectively), RMt-143 and UM-274 (i.e., 1.455 ± 0.169 and 0.948 ± 0.021 mg α -tocopherol/100 g dry wt., respectively). The 25 fenugreek genotypes can be grouped into 15 distinct groups on the basis of α -tocopherol content. The genotypes within the groups differed significantly ($P\leq 0.05$) w.r.t α -tocopherol content.

Total carotenoid content

Total carotenoid content within fenugreek genotypes under investigation varied from 1.450 ± 0.003 to 8.538 ± 0.018 g carotenoids/100 g dry wt. in UM-271 and UM-222, respectively (Figure 1: C). No significant difference ($P\leq 0.05$) in total carotenoid content was observed between UM-258 and UM-279 (i.e., 3.835 ± 0.032 and 3.824 ± 0.021 g carotenoids /100 g dry wt., respectively). Similarly no significant difference ($P\leq 0.05$) in total carotenoid content was observed between IC-143816 and Pant ragini (i.e., 3.505 ± 0.069 and 3.417 ± 0.010 g carotenoids /100 g dry wt., respectively), UM-265 and RMt-361 (i.e., 3.241 ± 0.252 and 3.175 ± 0.032 g carotenoids /100 g dry wt., respectively), IC-143843 and Pusa early bunching (i.e., 2.956 ± 0.040 and 2.934 ± 0.010 g carotenoids /100 g dry wt., respectively), AM-316 and UM-126 (i.e., 2.109 ± 0.027 and 2.098 ± 0.047 g carotenoids /100 g dry wt., respectively), RMt-2 and UM-274 (i.e., 1.967 ± 0.010 and 1.934 ± 0.007 g carotenoids /100 g dry wt., respectively). The 25 fenugreek genotypes can be grouped into 19 distinct groups on the basis of total

carotenoid content. The genotypes within the groups differed significantly ($P\leq 0.05$) w.r.t total carotenoid content.

Iron content

The iron content in fenugreek genotypes under investigation varied from 76.56 ± 2.421 to 184.11 ± 1.639 $\mu\text{g/g}$ dry wt. in RMt-361 and RMt-143, respectively (Table 2). No significant difference ($P\leq 0.05$) in iron content was observed between IC-066843 and RMt-305 (i.e., 177.93 ± 1.815 and 176.52 ± 2.413 $\mu\text{g/g}$ dry wt., respectively). Similarly no significant difference ($P\leq 0.05$) in iron content was observed between UM-325, GM-2 and RMt-2 (i.e., 143.34 ± 1.983 , 143.34 ± 1.148 and 142.08 ± 0.681 $\mu\text{g/g}$ dry wt., respectively), Pant ragini and UM-222 (i.e., 109.95 ± 2.014 and 109.95 ± 1.079 $\mu\text{g/g}$ dry wt., respectively), UM-265 and RMt-303 (i.e., 100.05 ± 1.792 and 100.05 ± 1.977 $\mu\text{g/g}$ dry wt., respectively), Pusa early bunching and IC-143816 (i.e., 98.82 ± 1.066 and 97.62 ± 1.333 $\mu\text{g/g}$ dry wt., respectively). The 25 fenugreek genotypes can be grouped into 19 distinct groups on the basis of observed iron content. The genotypes within the groups differed significantly ($P\leq 0.05$) w.r.t iron content.

Calcium content

The calcium content in fenugreek genotypes under investigation varied from 91.80 ± 1.324 to 781.20 ± 4.790 $\mu\text{g/g}$ dry wt. in UM-279 and RMt-143, respectively (Table 2). No significant difference ($P\leq 0.05$) in calcium content was observed between IC-143816, IC-143850, RMt-361 and UM-271 (i.e., 173.61 ± 1.032 , 173.07 ± 2.013 , 171.45 ± 3.661 and 169.86 ± 1.439 $\mu\text{g/g}$ dry wt., respectively). Similarly no significant difference ($P\leq 0.05$) in iron content was observed between UM-265 and UM-126 (i.e., 163.41 ± 1.542 and 160.20 ± 1.802 $\mu\text{g/g}$ dry wt., respectively). The 25 fenugreek genotypes can be grouped into

21 distinct groups on the basis of observed calcium content. The genotypes within the groups differed significantly ($P \leq 0.05$) w.r.t calcium content.

Zinc content

The zinc content in fenugreek genotypes under investigation varied from 15.18 ± 1.148 to 35.52 ± 1.229 $\mu\text{g/g}$ dry wt. in UM-222 and IC-066843, respectively (Table 2). No significant difference ($P \leq 0.05$) in zinc content was observed between UM-258, RMt-143 and HM-355 (i.e., 26.28 ± 1.226 , 26.28 ± 1.024 and 26.28 ± 1.330 $\mu\text{g/g}$ dry wt., respectively). Similarly no significant difference ($P \leq 0.05$) in zinc content was observed between Pant ragini and UM-366 (i.e., 23.94 ± 0.807 and 23.58 ± 1.518 $\mu\text{g/g}$ dry wt., respectively), RMt-2 and IC-143816 (i.e., 19.32 ± 0.902 and 19.23 ± 0.458 $\mu\text{g/g}$ dry wt., respectively), RMt-361, UM-274 and UM-126 (i.e., 18.06 ± 0.573 , 17.88 ± 0.505 and 17.79 ± 0.746 $\mu\text{g/g}$ dry wt., respectively), RMt-303 and RMt-305 (i.e., 17.34 ± 1.232 and 16.89 ± 0.383 $\mu\text{g/g}$ dry wt., respectively), RMt-1 and UM-222 (i.e., 15.36 ± 0.487 and 15.18 ± 1.148 $\mu\text{g/g}$ dry wt., respectively). The 25 fenugreek genotypes can be grouped into 17 distinct groups on the basis of observed zinc content. The genotypes within the groups differed significantly ($P \leq 0.05$) w.r.t zinc content.

Copper content

The copper content in fenugreek genotypes under investigation varied from 0.05 ± 0.006 to 5.82 ± 0.037 $\mu\text{g/g}$ dry wt. in UM-222 and IC-143850, respectively (Table 2). No significant difference ($P \leq 0.05$) in zinc content was observed between IC-143850, GM-2 and UM-265 (i.e., 5.28 ± 0.037 , 5.82 ± 0.053 and 5.82 ± 0.034 $\mu\text{g/g}$ dry wt., respectively). Similarly no significant difference ($P \leq 0.05$) in copper content was observed between UM-325 and RMt-143 (i.e., 4.53 ± 0.042 and

4.53 ± 0.028 $\mu\text{g/g}$ dry wt., respectively), UM-274, IC-066843 and HM-355 (i.e., 3.27 ± 0.027 , 3.27 ± 0.041 and 3.27 ± 0.034 $\mu\text{g/g}$ dry wt., respectively), UM-273, UM-271, IC-143843 and IC-143816 (i.e., 2.61 ± 0.027 , 2.61 ± 0.029 , 2.61 ± 0.019 and 2.61 ± 0.017 $\mu\text{g/g}$ dry wt., respectively), RMt-303 and Pusa early bunching (i.e., 1.35 ± 0.02 and 1.35 ± 0.016 $\mu\text{g/g}$ dry wt., respectively), UM-366, UM-358, RMt-2, RMt-305 and UM-222 (i.e., 0.06 ± 0.001 , 0.06 ± 0.001 , 0.06 ± 0.001 , 0.06 ± 0.007 and 0.054 ± 0.006 $\mu\text{g/g}$ dry wt., respectively). The 25 fenugreek genotypes can be grouped into 12 distinct groups on the basis of observed zinc content. The genotypes within the groups differed significantly ($P \leq 0.05$) w.r.t zinc content.

Manganese content

The manganese content in fenugreek genotypes under investigation varied from 0.66 ± 0.028 to 25.65 ± 0.695 $\mu\text{g/g}$ dry wt. in UM-274 and RMt-143, respectively (Table 2). No significant difference ($P \leq 0.05$) in manganese content was observed between RMt-143 and HM-355 (i.e., 25.65 ± 0.695 and 25.65 ± 0.217 $\mu\text{g/g}$ dry wt., respectively). Similarly no significant difference ($P \leq 0.05$) in manganese content was observed between UM-366 and Pant ragini (i.e., 23.13 ± 0.281 and 23.13 ± 0.728 $\mu\text{g/g}$ dry wt., respectively), RMt-2 and Pusa early bunching (i.e., 15.66 ± 0.764 and 15.66 ± 0.548 $\mu\text{g/g}$ dry wt., respectively), RMt-361 and GM-2 (i.e., 10.65 ± 0.363 and 10.65 ± 0.573 $\mu\text{g/g}$ dry wt., respectively), UM-126, RMt-305, RMt-303, RMt-1, IC-143843 and AM-316 (i.e., 5.67 ± 0.390 , 5.67 ± 0.573 , 5.67 ± 0.666 , 5.67 ± 0.571 , 5.67 ± 0.394 and 5.67 ± 0.017 $\mu\text{g/g}$ dry wt., respectively), IC-143816 and IC-066843 (i.e., 3.18 ± 0.026 and 3.18 ± 0.693 $\mu\text{g/g}$ dry wt., respectively), UM-279, UM-273, UM-271, UM-265, UM-222, IC-143850 and UM-274 (i.e., 0.66 ± 0.021 , 0.66 ± 0.016 , 0.66 ± 0.598 , 0.66 ± 0.010 , 0.66 ± 0.001 ,

0.66±0.001 and 0.66±0.028 µg/g dry wt., respectively). The 25 fenugreek genotypes can be grouped into 9 distinct groups on the basis of observed manganese content. The genotypes within the groups differed significantly ($P \leq 0.05$) w.r.t manganese content.

Total ascorbate content

Plant based foods are an important source of ascorbic acid (vitamin C). Ascorbic acid is important in metabolism of various metabolites including folic acid, tyrosine, tryptophan etc. It also have definite role in metabolism of cholesterol (Dave and Patil, 2017) i.e., assist in lowering of blood cholesterol levels.

Ascorbic acid helps in absorption of iron thereby regulating iron levels in the body. The ascorbate content within fenugreek genotypes showed a positive correlation with α -tocopherol and carotenoid content (i.e., 0.114 and 0.061, respectively). It also showed a significant ($p \leq 0.05$) positive correlation with manganese content (0.298) and iron content (0.062) among the genotypes (Figure 2: A).

Vitamin C is a multifunctional vitamin, especially its potential role as an antioxidant and its health promoting effects in prevention of cancer and blood pressure. Its crucial role in providing overall immunity is well documented (Maggini *et al.*, 2007; Webb and Villamor, 2007). The fenugreek genotypes under investigation showed a significant amount of ascorbate within the genotypes, which may be associated with availability of other essential micronutrients especially iron, manganese, α -tocopherol and carotenoids as observed by the positive correlation of these micronutrients with total ascorbate content within the genotypes. The results clearly shows that fenugreek genotypes with high ascorbate content i.e., HM-355, UM-126,

RMt-305 and Pant ragini can be used as a potential functional food and may provide desired health benefits especially against iron deficiency anaemia, maintenance of cholesterol levels and overall immunity. Presence of significant amount of vitamin C in fenugreek seeds has also been reported by Leela and Shafeekh (2008) and Srinivasan (2006). Thus ascorbate along with other antioxidants i.e., α -tocopherol and carotenoids may be responsible for imparting functional food status to fenugreek.

α -tocopherol content

α -Tocopherol (vitamin E) is a major lipid-soluble antioxidant which plays an important role in maintaining cellular integrity by preventing membrane lipid peroxidation (Cervantes and Ulatowski 2017). It has an essential role in preventing cellular damage caused by various oxidative stress associated diseases viz., Alzheimer, Parkinson's disease etc. Recent studies have shown a positive correlation between vitamin E supplementation and prevention of diseases like atherosclerosis, cataract, cardiovascular diseases, neural tube defects and cancer.

The vitamin E content within fenugreek genotypes under investigation showed a significant ($p \leq 0.01$) positive correlation (0.328) with total carotenoids another important lipid-soluble antioxidant. It also showed a positive correlation with ascorbate, iron and calcium content (i.e., 0.114, 0.213 and 0.036, respectively) within the genotypes (Figure 2: B). The results clearly show that fenugreek genotypes with high vitamin E content viz., RMt-305, RMt-303, Pusa early bunching and IC-143816 are rich source of vitamin E and can be used for vitamin E supplementation and thereby may provide potential health benefits in prevention of neurological and other oxidative stress associated diseases.

Fig.1 (A) Total ascorbate content; (B) α -tocopherol content; (C) Total carotenoid content in in different fenugreek (*Trigonella foenum-graecum* L.) genotypes. Data shown are mean value \pm standard error (n=3). (* Note: The values with same superscript are not significantly different at ($P \leq 0.05$) according to Duncan LSD post hoc analysis)

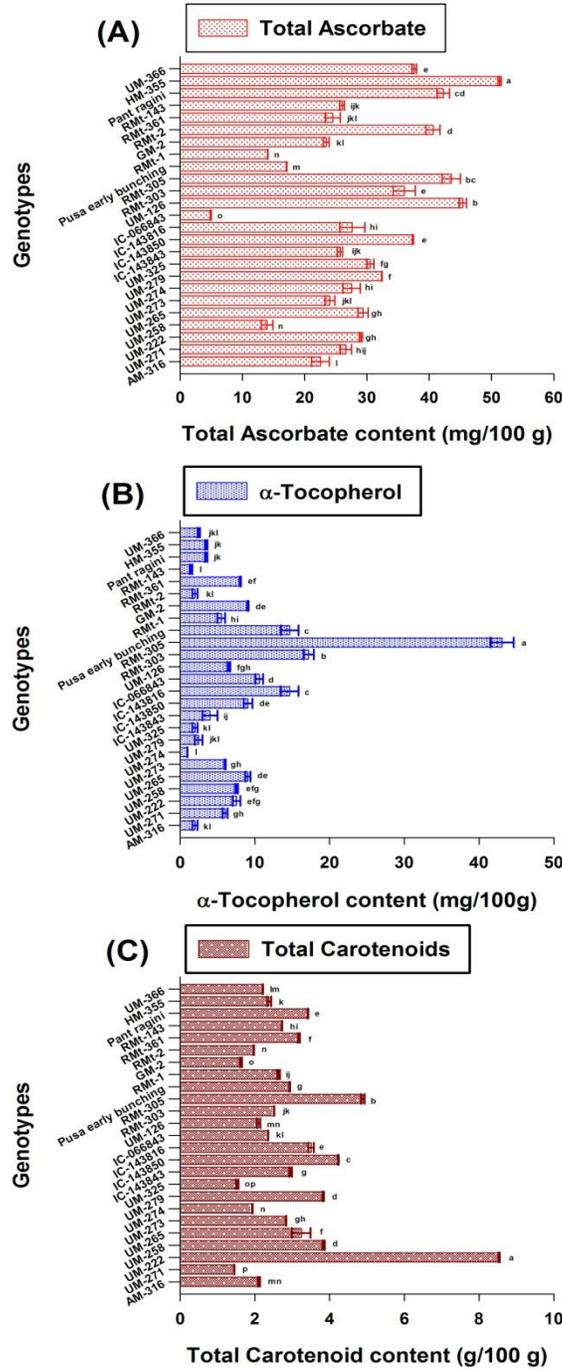


Fig.2 Correlation analysis (A): Between total ascorbate content and mineral elements (i.e., iron, calcium, zinc, copper & manganese, respectively); (B): Between α -Tocopherol content and mineral elements (i.e., iron, calcium, zinc, copper & manganese, respectively); (C): Between Total carotenoid content and mineral elements (i.e., iron, calcium, zinc, copper & manganese, respectively), within seeds of different fenugreek (*Trigonella foenum-graecum* L.) genotypes. Data shown are mean value \pm standard error (n=3)

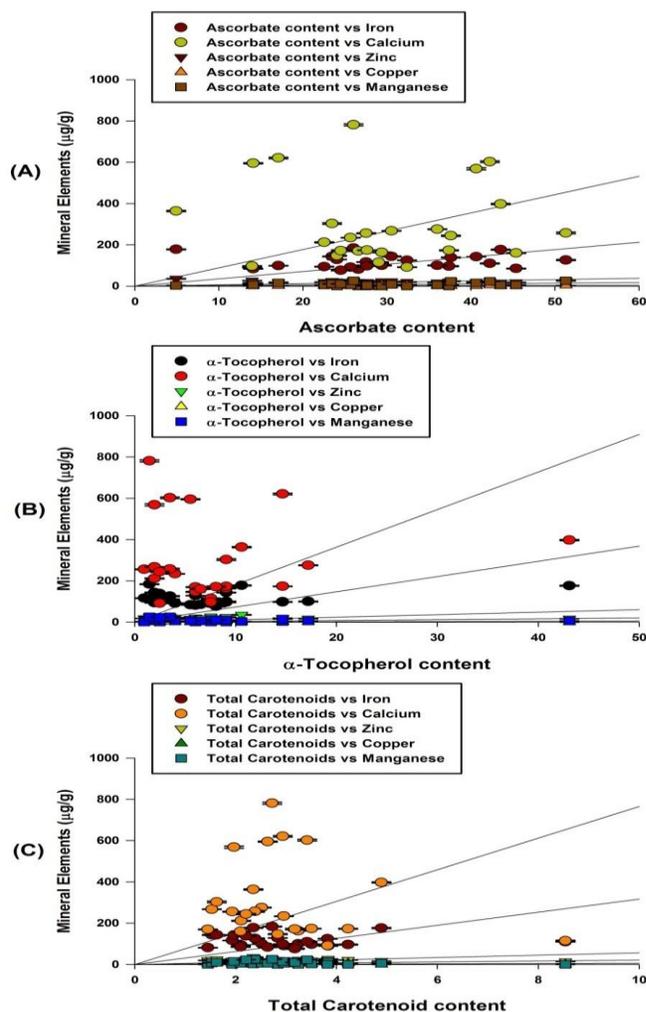


Table.1 Instrument settings for determination of different elements

Element	Wavelength (nm)	Slit width (nm)	Light source	Flame type
Iron (Fe)	248.3	0.2	Cathode Lamp	Air acetylene flame (Oxidizing)
Calcium (Ca)	422.7	0.2	-do-	-do-
Zinc (Zn)	213.9	1	-do-	-do-
Copper (Cu)	324.8	0.2	-do-	-do-
Manganese (Mn)	279.5	0.2	-do-	-do-

Table.2 Micronutrient content in different fenugreek (*Trigonella foenum-graecum* L.) genotypes. Data shown below are mean value ± standard error (n=3)

S. No.	Genotypes	Iron (µg/g)	Calcium (µg/g)	Zinc (µg/g)	Copper (µg/g)	Manganese (µg/g)
1	AM-316	93.87±1.558 ^{kl}	211.71±2.499 ⁿ	19.41±0.681 ^{ijk}	1.98±0.028 ^g	5.67±0.017 ^g
2	UM-271	81.51±1.148 ^o	169.86±1.439 ^o	19.77±0.487 ^{hij}	2.61±0.029 ^f	0.66±0.598 ⁱ
3	UM-222	109.95±1.079 ^h	115.11±2.222 ^f	15.18±1.148 ^m	0.054±0.006 ^k	0.66±0.001 ⁱ
4	UM-258	90.15±1.547 ^m	96.87±1.216 ^s	26.28±1.226 ^b	0.06±0.001 ^k	13.17±0.478 ^d
5	UM-265	100.05±1.792 ⁱ	163.41±1.542 ^p	22.50±0.545 ^{def}	5.82±0.034 ^a	0.66±0.010 ⁱ
6	UM-273	128.49±0.866 ^e	147.33±2.115 ^q	21.60±0.669 ^{efg}	2.61±0.027 ^f	0.66±0.016 ⁱ
7	UM-274	116.13±0.923 ^g	255.72±1.723 ^k	17.88±0.505 ^{kl}	3.27±0.027 ^e	0.66±0.028 ⁱ
8	UM-279	124.77±1.232 ^f	91.80±1.324 ^t	20.04±1.033 ^{ghi}	5.19±0.066 ^b	0.66±0.021 ⁱ
9	UM-325	143.34±1.983 ^c	267.51±1.150 ^j	22.86±0.613 ^{cde}	4.53±0.042 ^c	8.16±0.366 ^f
10	IC-143843	91.41±2.014 ^{lm}	234.24±1.033 ^m	21.06±1.258 ^{fgh}	2.61±0.019 ^f	5.67±0.394 ^g
11	IC-143850	96.36±2.101 ^{jk}	173.07±2.013 ^o	21.96±0.675 ^{ef}	5.82±0.037 ^a	0.66±0.001 ⁱ
12	IC-143816	97.62±1.333 ^{ij}	173.61±1.032 ^o	19.23±0.458 ^{ijk}	2.61±0.017 ^f	3.18±0.026 ^h
13	IC-066843	177.93±1.815 ^b	363.60±2.958 ^g	35.52±1.229 ^a	3.27±0.041 ^e	3.18±0.693 ^h
14	UM-126	85.23±1.541 ⁿ	160.20±1.802 ^p	17.79±0.746 ^{kl}	0.69±0.07 ⁱ	5.67±0.390 ^g
15	RMt-303	100.05±1.977 ⁱ	275.58±1.529 ⁱ	17.34±1.232 ^l	1.35±0.02 ^h	5.67±0.666 ^g
16	RMt-305	176.52±2.413 ^b	397.50±2.674 ^f	16.89±0.383 ^l	0.06±0.007 ^k	5.67±0.573 ^g
17	Pusa early bunching	98.82±1.066 ^{ij}	620.70±3.647 ^b	18.24±0.987 ^{ijkl}	1.35±0.016 ^h	15.66±0.548 ^c
18	RMt-1	83.97±1.064 ^{no}	594.90±2.480 ^d	15.36±0.487 ^m	0.60±0.091 ^j	5.67±0.571 ^g
19	GM-2	143.34±1.148 ^c	303.00±3.904 ^h	24.30±0.712 ^c	5.82±0.053 ^a	10.65±0.573 ^e
20	RMt-2	142.08±0.681 ^c	568.50±5.012 ^e	19.32±0.902 ^{ijk}	0.06±0.001 ^k	15.66±0.764 ^c
21	RMt-361	76.56±2.421 ^p	171.45±3.661 ^o	18.06±0.573 ^{kl}	0.63±0.02 ^{ij}	10.65±0.363 ^e
22	RMt-143	184.11±1.639 ^a	781.20±4.790 ^a	26.28±1.024 ^b	4.53±0.028 ^c	25.65±0.695 ^a
23	Pant ragini	109.95±2.014 ^h	602.40±4.114 ^c	23.94±0.807 ^{cd}	3.9±0.028 ^d	23.13±0.728 ^b
24	HM-355	126.03±2.465 ^{ef}	257.31±3.856 ^k	26.28±1.33 ^b	3.27±0.034 ^e	25.65±0.217 ^a
25	UM-366	138.39±1.990 ^d	243.90±2.733 ^l	23.58±1.518 ^{cd}	0.06±0.001 ^k	23.13±0.281 ^b

* Note: The values with same superscript are not significantly different at (P≤0.05) according to Duncan LSD post hoc analysis

Total carotenoid content

Carotenoids serves as potent quenchers of reactive oxygen species (Fiedor *et al.*, 2005) which are associated with various oxidative stress mediated disorders viz., cardiovascular, neurological diseases as well as various types of cancers. Dietary carotenoids provides health benefits and are derived from various plant based sources i.e., fruits and vegetables. They mainly function as antioxidants. There is growing experimental evidence in the possible role of carotenoids as potential mediator of various immune responses (Eldahshan *et al.*, 2013).

Total carotenoid content within fenugreek genotypes under investigation showed a significant ($p \leq 0.01$) positive correlation (0.328) with α -tocopherol content another important group of lipid-soluble antioxidant in fenugreek (Figure 2: C). Presence of carotenoids (β -carotene) within fenugreek has also been reported by Srinivasan, 2006. The results clearly show the presence of significant amount of carotenoids within seeds of different fenugreek genotypes. The genotypes with high carotenoid content viz., UM-222, RMt-305 and IC-143850 may be utilized as potential functional foods particularly in combating vitamin A malnutrition as carotenoids are an important precursor of vitamin A.

Iron content

Iron (Fe) is an essential micronutrient in human nutrition and is the main factor responsible for occurrence of iron deficiency anaemia in humans. Plants and plant based foods are an important source of micronutrients including iron, but the bioavailability of iron from plant based foods may be influenced by various dietary factors such as inhibitors and enhancers of iron absorption. Presence of iron in fenugreek

seeds has also been reported by Doshi *et al.*, 2012 and by Shakuntala *et al.*, 2011 in seed endosperm of fenugreek.

The iron content within the fenugreek genotypes under investigation showed a significant ($p \leq 0.01$) positive correlation with other micronutrients viz., calcium, zinc and manganese (i.e., 0.408, 0.502 and 0.299, respectively). There was also a positive correlation between iron and total ascorbate, α -tocopherol content (i.e., 0.062 and 0.213, respectively) within the genotypes (Figure 2: A & B). This clearly shows that fenugreek seeds may serve as an important source of iron supplementation and may help in combating dietary iron deficiency with enhanced absorption particularly in presence of vitamin C. Thus fenugreek genotypes with high iron content viz., RMt-143, IC-066843 and RMt-305 may serve as an important dietary source of iron along with other micronutrients.

Calcium content

Calcium serves as an important factor in human nutrition due to its role in bone formation and regulatory role in cellular metabolism (Linder, 1991). Although milk and milk products serves as the major source of dietary calcium requirement within humans (Chan *et al.*, 1995), vegetables also serves as an important source to meet dietary calcium requirement. But the bioavailability of calcium depends upon various factors including presence of chelators like phytic acid and oxylate within plants (Weaver and Heaney, 1991).

The calcium content within fenugreek genotypes under investigation showed a significant ($p \leq 0.01$) positive correlation with other micronutrients viz., iron and manganese (i.e., 0.408 and 0.576, respectively). A positive correlation between calcium and α -

tocopherol (Figure 2B), zinc content (i.e., 0.036 and 0.095, respectively) was also observed among the genotypes. Dietary calcium is known to assist in iron utilization (Fleck, 1976) apart from playing essential role in nerve transmission and muscle functioning. The results of the present investigation clearly show that fenugreek seeds are a potential source of dietary calcium along with other important micronutrients and therefore can favourably affect human health in general. Fenugreek genotypes with high calcium content viz., RMT-143, Pusa early bunching and Pant ragini could be utilized as a functional food to combat micronutrient malnutrition especially of calcium and iron faced by millions in developing and underdeveloped countries.

Zinc content

Zinc (Zn) along with other micronutrients plays an important role in human nutrition. Zinc serves as a cofactor for different enzymes involved in a variety of metabolic processes including carbohydrate, lipid and protein metabolism. Generally the zinc content in foods is low and its availability depends upon several physiological and dietary factors (Camera and Amaro, 2003).

The zinc content within fenugreek genotypes under investigation showed a significant ($p \leq 0.01$) positive correlation with other micronutrients viz., iron, copper and manganese content (i.e., 0.502, 0.407 and 0.339, respectively) within the genotypes. Zinc is a multifunctional micronutrient with an important role in tissue repair and wound healing apart from its effects on insulin action. The results clearly show that fenugreek may serve as an important dietary source of zinc along with other essential micronutrients. Fenugreek genotypes with high zinc content viz., IC-066843, RMT-143 and HM-355 may well be utilized as an

important functional food especially w.r.t zinc and other micronutrient nutrition and may favourably affect human nutrition.

Copper content

Copper is an essential micronutrient required for proper functioning of haematological and neurological systems (Tan *et al.*, 2006). It is also required for iron incorporation in haemoglobin apart from assisting in iron absorption and transfer. Copper acts as a cofactor for enzymes involved in blood clotting, signal transduction etc. (Kim *et al.*, 2008). Clinical disorders associated with deficiency of copper include heart failure, anaemia and impaired reproductive system (Soetan *et al.*, 2010). Copper is also known to regulate cholesterol biosynthesis and thereby its concentration within blood (Lei *et al.*, 2017).

The copper content within fenugreek genotypes under investigation showed a significant ($p \leq 0.01$) positive correlation with zinc content (i.e., 0.407). There was also a positive correlation between copper and iron content (i.e., 0.189) within the genotypes. The results clearly show that fenugreek is an important dietary source of micronutrients including copper. The fenugreek genotypes with high copper content viz., IC-143850, GM-2, UM-265 and UM-279 may serve as an important dietary source of available copper along with other micronutrients and thus have a favourable health promoting affect.

Manganese content

Manganese as a micronutrient is required for carbohydrate and lipid metabolism, growth, reproduction and development of skeletal system. It serves as a cofactor in enzymes i.e., pyruvate carboxylase, superoxide dismutase etc. Manganese has a variety of metabolic functions including enzyme activation

especially enzymes of protein and fat metabolism. It is known to play an important role in skeletal system development apart from having neurological, reproductive and immunological functions (Soetan *et al.*, 2010). It also serves as an antioxidant that protects cells from damage due to free radicals (Santamaria, 2008)

The manganese content within fenugreek genotypes under investigation showed a significant ($p \leq 0.01$) positive correlation with iron, calcium and zinc content (i.e., 0.299, 0.576 and 0.339, respectively). Manganese content among the genotypes also showed a significant ($p \leq 0.05$) positive correlation (Figure 2: A) with ascorbate content (0.289). Presence of Mn in fenugreek has also been reported by Shakuntala *et al.*, 2011. The results clearly reveal that fenugreek genotypes vary significantly w.r.t Mn content along with other micronutrients. The genotypes with high manganese content viz., RMt-143, HM-355, UM-366 and Pant ragini may well be utilized as functional food with significant amount of micronutrients including manganese.

The present investigation clearly reveals functional food potential of fenugreek especially w.r.t micronutrients i.e., vitamin C, vitamin E, total carotenoids, iron, calcium, zinc, copper and manganese supplementation. Fenugreek genotypes viz., RMt-305, Pant ragini, RMt-143, Pusa early bunching, IC-066843, GM-2 and HM-355 with high micronutrients may be utilized as potential source of dietary micronutrients. The results also reveal that the genotypes vary significantly w.r.t different micronutrients thereby their availability for micronutrient supplementation. The micronutrients also showed a significant positive correlation among themselves thereby clearly showing that they may have significant effect on their bioavailability among themselves upon digestion and subsequent absorption, for

example a positive association between iron, total ascorbate, calcium, zinc and manganese was observed among the fenugreek genotypes under investigation. Thus the findings of the present investigation may help in identification of potential fenugreek genotypes which may be utilized in combating micronutrient malnutrition by dietary supplementation of genotypes with high micronutrient content.

Conflict of interest

The authors declare no conflict of interests regarding the publication of this paper.

Acknowledgements

The financial support provided by Council of Scientific and Industrial Research (CSIR) and the infrastructural grant provided by DST (Department of Science and Technology, Govt. of India) is duly acknowledged.

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How to cite this article:

Naveen Chandra Pant, Manisha Tewari, Rakesh Dhoundiyal, C.S. Pandey, J.P. Singh and Sanjeev Agrawal. 2018. Evaluation of Micronutrients in Fenugreek (*Trigonella foenum-graecum* L.): A Viable Alternative for Micronutrient Supplementation. *Int.J.Curr.Microbiol.App.Sci.* 7(05): 2446-2464. doi: <https://doi.org/10.20546/ijcmas.2018.705.281>