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Effect of 24-epibrassinolide pretreatments on mineral content of fenugreek (*Trigonella foenum-graecum* L.) sprouts and leaves

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Abstract
The current study was carried out in the Division of Basic Sciences and Humanities, SKUAST-K, Shalimar, Kashmir J&K, during the year 2017-18 to investigate the effect of 24- epibrassinolide (EBL) pretreatments on calcium(Ca), magnesium (Mg), potassium (K) and iron (Fe) content of fenugreek sprouts as well as in leaves of plants raised from EBL pretreated seeds. The 100% viable fenugreek seeds were pre-treated for 8 hours at room temperature with 0.1µM, 1.0 µM, 2.5 µM, 5.0 µM and 10.0 µM aqueous solution of 24-epibrassinolide (EBL). Results indicated that almost each EBL concentration used in the current study improved either one or all the minerals in both germinating fenugreek seeds as well as in leaves of plants raised from EBL pretreated seeds as compared to control. In germinated seeds the 2.5 µM EBL pretreatment was found to be optimum for enhancing Ca (94 ppm) as well as Fe (300 ppm), while as 0.1 EBL increased maximally Mg (90 ppm) and K content (8700 ppm) in them. Similarly, in case of leaves collected from plants raised from EBL pretreated seed, 2.5 µM pretreatment was found to increase optimally all of the other minerals in the leaf samples except Mg. The 2.5 µM EBL pretreatment increased Ca up to 4500 ppm, Fe up to 630 ppm, K up to 1400 ppm in leaf samples, while as 0.1 EBL pretreatment increased maximally Mg content of leaf samples up to 2150 ppm. The overall results of this study clearly indicate that fenugreek seeds pretreated with EBL have beneficial effects in enhancing many fold mineral content of plants raised from them. These observations can act as a starting point for conducting similar kind of research in other crops in order to draw some logical conclusion with respect to overall improvement of nutritional status of crops.

Keywords: 24- epibrassinolide, pretreatments, fenugreek, calcium, magnesium, iron, potassium

Introduction
Plant hormones and plant growth regulators (PGRs) have been defined as one of the main factors which influence plant growth and their primary and secondary metabolites pool (Pradeep *et al.*, 2017) [23]. Among plant hormones, recently 6th group of hormones called as brassinosteroids (BRs) have been included in them. Brassinosteroids (BRs) are common plant-produced compounds that can function as growth regulators (Maria *et al.*, 2017) [18]. However, among different growth hormones, effect of brassinosteroids in plant systems has been less investigated. In general, BRs have been reported to protect plants from various abiotic/biotic stresses such as drought stress (Behnamnia *et al.*, 2009) [3], temperature stress (Ogweno *et al.*, 2008) [20], pathogen infection (Nakashita *et al.*, 2003) [19], heavy metals (Ali *et al.*, 2008; Fariduddin *et al.*, 2009) [1], and salt stress (Hayat *et al.*, 2010) [9]. 24-epibrassinolide (EBL) is one of the most active forms of BRs available commercially with favorable safety profile (Hui *et al.*, 2015) [11] and plays prominent roles in various physiological processes, including growth, differentiation, and photosynthesis (Swamy and Rao, 2009) [27]. Till date very scant literature related to effect of 24-epibrassinolide pretreatments on mineral content of fenugreek is available. Among plants, fenugreek have been viewed as miraculous herb due to its nutrients and unprecedented health promoting activities (Murtaza *et al*, 2013) [12]. Fenugreek (*Trigonella foenum-graecum*) is an annual plant with leaves consisting of three small obovate to oblong leaflets. It is grown as a semiarid crop throughout the world. Its seeds and leaves are highly nutritious especially rich in minerals, vitamins and phytonutrients (Wani and Kumar, 2018) [2]. Fenugreek sprouts have been reported to be better source of nutrients like proteins, carbohydrates, fats, vitamins, minerals as well as secondary metabolite composition (including phenols, flavonoids, steroids and alkaloids) as compared to seeds (Laila & Murtaza, 2014) [21].

Therefore, the present investigation was carried out to assess the effect of 24-epibrassinolide pretreatments on mineral content of germinating fenugreek seeds as well as in fenugreek leaves collected from plants raised from EBL pretreated seeds.

Material and Method

The current investigation was carried out at Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Srinagar, Kashmir, J&K, India. The fenugreek seeds were collected from Seed Production Unit of Division of Vegetable Sciences, SKUAST-K, and soaked for 8 hours in aqueous solutions containing different concentrations (0.1µM, 1.0µM, 2.5µM, 5.0 µM and 10.0 µM) of 24-epibrassinolide. The pretreated seeds were air dried and germinated under dark conditions in petri-plates lined with 10 layers of moist blotting paper. The petri-plates were placed for three days in seed germinator and temperature and humidity maintained at 25 ± 2 ^{0}C and 70% respectively. In parallel the seeds pretreated with different concentrations of EBL were sown in pots and raised under uniform conditions in poly-house. The fenugreek leaves were picked at edible stage of growth and subjected to further investigations.

Determination of minerals content

For mineral estimation, 2g of *Trigonella foenum-graecum* sprouts as well as leaf samples were taken in a flask and 20 ml of di-acid mixture of nitric acid and perchloric acid in the ratio of 9:4 added. The flask was kept undisturbed overnight and next day placed on a hot plate at 115-118^{0}C for digestion till a watery transparent aliquot was obtained. The digested samples were then filtered and diluted with double distilled water to make the final volume up to 50 ml and used for minerals estimation. The calcium and Iron content was estimated by Versanate method described by Jackson (1973) [13] while the potassium was determined by flame photometric method Piper (1966) [22]. The magnesium was estimated with flame photometer.

Results and Discussion

Varieties of strategies are being currently employed in plant systems in order to improve nutrient status, seed germination, seedling growth and productivity of plants. Exogenous application of brassinosteroids at seed stage and through foliar application has been found to exert a wide range of biological activities of physiological importance, such as plant growth and development, reproduction, photosynthesis, flowering time and are highly effective in elicitation of several responses to various abiotic and biotic stresses (Liu *et al.*, 2013; Kanwar *et al.*, 2017) [16, 14]. Keeping in mind the diverse and important role of BR's, the present investigation was carried out with the aim to investigate the effect of EBL on mineral content of preteatmented germinated Fenugreek seeds as well leaves of plant at their edible stage of growth raised from EBL pre-treated seeds.

The vital role of mineral elements in human, animal and plant nutrition has been well recognised from past many decades, as their deficiencies or disturbances in the nutrition can cause a variety of diseases. Minerals represent one of the four groups of essential nutrients that are required by organisms to perform functions necessary for life. Among the 16 essential minerals: Ca, Mg, Fe and K represent the important group of nutrients that are needed by the animals and plants for their growth. Calcium serves as an important factor in human nutrition due to its role in bone formation and regulatory role in cellular metabolism (Ross *et al.*, 2011) [24]. In this study calcium content of EBL pre-treated germinated seeds (AM0-AM5) was found to lie in between 17 to 94 ppm (Table 1). These values are much lower than as reported previously by Hooda and Jood (2002) [10] in fenugreek sprouts. In the current study comparatively the lower content of calcium in germinated sprouts might be due to the excessive leaching out of some nutrients including calcium into soaking water during 8 hours pretreatment period or due to increased requirement for growing plant metabolism (Atlaw *et al.*, 2018) [2].

As shown in table1, in this study, the magnesium content of EBL treated germinated sprouts was found to be lie in between 15 to 90 ppm only. These values are much lower than magnesium content previously documented (153.10 mg/100g) in fenugreek sprouts (Atlaw *et al.*, 2018) [2]. Likewise, as reported for calcum, the overall magnesium content of germinated seeds may be low either due to its leaching out or due to increased requirement for growing plant metabolism (Atlaw *et al.*, 2018) [2].

The fenugreek seeds have been reported to contain good amount of iron (Doshi *et al.* 2012; Shakuntala and Naik, 2011) [4, 26]. Interestingly in this study, iron content of pre-treated germinated seeds was found to lie in between 110 to 300 ppm (Table1). The values of current study are much higher than the values as reported by Hooda and Jood (2002) [10] in germinated fenugreek seeds (11.20 mg/100g). Probably the reason for this drastic increase may be attributed to absorption of iron by germinating seeds from the water used for pretreatment purpose and EBL seems to enhance the absorption process as compared to control.

As shown in table 1, in this study potassium content of pre-treated germinated fenugreek seeds was found to lie in between 2000 to 8700 ppm and 2.5 µM EBL treatment was optimum and demonstrated much higher potassium content as reported by Kavitha *et al.* (2015) [15] in germinated fenugreek seeds (5500 ppm). Likewise, the reason for this drastic increase may be attributed to absorption of potassium by germinating seeds from the water used for pre-treatment purpose and in this case also EBL seems to enhance the absorption process as compared to control.

Table 1: Effect of 24-epibrassinolide pre-treatments on Mineral content of germinated fenugreek seeds

Treatments	Calcium (ppm)	Magnesium (ppm)	Iron (ppm)	Potassium (ppm)
AM0: DW (control)	17.0	15.0	110.0	2000.0
AM1: 0.1 µM EBL	42.0	90.0	260.0	8700.0
AM2: 1.0 µM EBL	51.0	27.0	220.0	7000.0
AM3: 2.5 µM EBL	94.0	24.0	300.0	5000.0
AM4: 5.0 µM EBL	82.0	22.0	180.0	3100.0
AM5:10.0 µM EBL	61.0	19.0	150.0	2200.0
CD ($p \leq 0.05$)	11.0	7.0	21.0	26.0

Although milk and milk products serve as the major source of dietary calcium requirement in humans, equally green leafy vegetables can also serve as an important source to add daily dietary calcium requirements. As shown in table 2, as compared to calcium content of leaves in control (3200 ppm), the calcium content of leaves from the plants raised from EBL pre-treated seeds was much higher. These values of current study are also much higher than the values reported in fenugreek leaves by Golaszewska and Wierzbowska (2017) [8]. Among the different concentrations used, 2.5 µM EBL pre-treatment was found to cause maximum increase (4500 ppm) in calcium content of leaves that can serve as good source of this vital mineral. However, the bioavailability of calcium depends upon various factors including presence of chelators like phytic acid and oxalate in leaves and thus warrants research in this direction.

Green leafy vegetables are the best source of magnesium and acts as cofactor in more than 300 enzyme systems that regulate diverse biochemical reactions in the body, including protein synthesis, muscle and nerve function, blood glucose control, and blood pressure regulation (Glasdam and Peters, 2016). Recent reports suggests that fenugreek leaves are rich source of magnesium with 0.67 g/kg (Golaszewska and Wierzbowska, 2017; Gharneh and Davodalhosseini, 2015) [8, 6]. In the current study, the magnesium content of leaves from the plants raised from EBL pre-treated seeds was much higher as compared control leaves (Table 2). Among the different concentrations used, 2.5µM EBL pretreatment caused the maximum increase in this particular mineral and thus suggest this treatment to be the best concentration to raise magnesium content of fenugreek leaves.

Iron deficiency is a major public health problem in developing countries, it affects up to 50% of infants, children, and women of child- bearing age in poorer populations of Africa, Asia, and Latin America (Saleh *et al*, 2013) [25]. Iron (Fe) is an essential micronutrient in human nutrition and is essential constituent of haemoglobin, and may be an important factor to increase the biosynthesis of haemoglobin. As shown in figure 1, in the current study, as compared to control the iron content of leaves from the plants raised from EBL pre-treated seeds was much higher and 2.5 µM EBL was found to cause almost 4 times increase in iron content of fenugreek leaves as reported by Golaszewska and Wierzbowska (2017) [8]. The current study indicates that EBL pre-treated fenugreek leaves can act as a rich source of iron.

Equally, Potassium is a mineral that plays an important role in controlling the amount of fluid in the body and potassium-rich diet is linked to many powerful health benefits. It may reduce blood pressure and water retention, protect against stroke and help in osteoporosis and kidney stones. In this study as depicted in table 2, the potassium content of the leaves from the plants raised from EBL pre-treated seeds was found to be much higher than control. The 2.5 µM EBL pre-treatment demonstrated much higher potassium content in fenugreek leaves as compared to potassium values reported by Golaszewska and Wierzbowska (2017) [8] in fenugreek leaves. The results clearly indicate that EBL treatment can enhance this particular mineral in plants that can in turn act as good source of dietary potassium.

Table 2: Effect of 24-epibrassinolide pre-treatments on mineral content of fenugreek leaves

Treatment	Calcium (ppm)	Magnesium (ppm)	Iron (ppm)	Potassium (ppm)
AM0: DW (control)	3200	1170	320	1040
AM1: 0.1 µM EBL	3500	2150	520	1100
AM2: 1.0 µM EBL	3500	1620	510	1150
AM3: 2.5 µM EBL	4500	1850	630	1400
AM4: 5.0 µM EBL	3800	1720	550	1200
AM5:10.0 µM EBL	3500	1520	450	1200
CD ($p \leq 0.05$)	12	17	13	28

Conclusion

The results of the current study are an indication that fenugreek seeds treated with EBL can increase calcium, iron, potassium and magnesium content in germinating sprouts as compared to control. Interestingly, EBL pre-treatments demonstrated great potential to increase many fold these selected minerals in the leaves of plants raised from EBL pre-treated seeds. Among the different concentrations used in the study 2.5µM EBL was found to be best pre-treatment that caused the maximum increase in Fe, K, Mg and Ca of the leaves of plants raised from EBL pre-treated seeds. These leaves can in turn act as good source of dietary calcium, magnesium, iron and potassium. However, the bioavailability of these minerals depends upon various factors including presence of chelators like phytic acid and oxalate in leaves and thus warrants further research in this direction. These observations can act as a starting point for conducting similar kind of research in other crops in order to draw some logical conclusion with respect to improvement of nutritional status especially mineral content of crops.

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