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Exogenous arginine improved fenugreek sprouts growth and trigonelline production under salinity condition

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ABSTRACT

Fenugreek (*Trigonella foenum-graecum* L.) is an aromatic and spice herb whose seeds have medicinal and nutraceutical properties. Trigonelline is one of the valuable alkaloids in fenugreek seeds with therapeutic potential for diabetes and central nervous system disease. In order to improve fenugreek sprout growth and trigonelline production, an experiment examining two factors was conducted on the basis of complete randomized design (CRD) with three replications. Fifty healthy seeds were put in petri dish and subjected to different arginine (0, 10, 20, 30 and 40 µM) and/or NaCl concentrations (0, 75 and 150 mM). To identify the best time in which the highest trigonelline content was accumulated and its possible mechanisms, trigonelline, proline and nicotinic acid contents were measured at 4, 8 and 12 days after beginning the experiment. Results showed that the arginine application mitigated the salinity induced reduction in germination and sprout growth. At early stages of sprout growth (4 days after treatments), salinity induced changes in the assimilate partitioning into proline, trigonelline and nicotinic acid accumulation. However, application of arginine adjusted the salinity effects. Over time (4–8 and 12 days after treatments), arginine application together with salinity increased the trigonelline and nicotinic acid content and the highest efficiency was achieved by 20 µM arginine and 150 mM salinity. In general, the highest yield of trigonelline in fenugreek sprouts, as the most important index, was observed when they were subjected to 75 mM NaCl and 20 µM arginine and it was noticeable in 12-day-old ones. Therefore, arginine and NaCl exogenous application, depends on their concentrations, were a valuable method for improving the trigonelline content of fenugreek sprouts.

1. Introduction

Fenugreek (*Trigonella foenum-graecum* L.) is an important legume crop native to Asia and Southern Europe. The whole plant is used as a high quality vegetable and forage, as it is rich in protein, vitamins and minerals, while the seeds are used as human and animal food (Mehrafarin et al., 2010; Laila and Murtaza, 2015; Zandi et al., 2015). Fenugreek seeds are used as an herbal remedy to treat a wide range of disease such as diabetes and fever (Eidi et al., 2007). Trigonelline is the most substantial alkaloid component found in fenugreek seeds, which plays a key role in its medicinal effects (Zandi et al., 2015). Despite the nutritional importance of fenugreek seeds for both human and animals, its use in diets is restricted due to presence of anti-nutritive factors. These factors include phytate, tannins and oxalates (Udensi et al., 2005). Sprouting is one of the food processing approaches that cause reduction in anti-nutritional factors (El Maki et al., 2007; Aremu et al., 2010). This process also raises the levels of bioactive compounds, nutritive value and health qualities of the seeds, which is pharmaceutically important (Fernández-Orozco et al., 2006; Swieca et al., 2014; Swieca, 2016; Gu et al., 2017). In addition, it's clear that plant sprouts are unique sources of highly concentrated nutrients such as proteins, lipids, sugars, minerals, tocopherols (vitamin E) and many other substances (Marton et al., 2010). Therefore, production of fenugreek sprouts with high levels of trigonelline is desirable.

Elicitors are physical or chemical factors that play an important role in production of commercially important compounds. There is some evidence that the quality of sprouts could be improved by elicitors (Swieca, 2016; Qian et al., 2016), and it is an effective method for improving the nutraceutical quality of sprout, however it may negatively influence growth and nutritional quality of sprouts. Numerous studies have regarded environmental stresses as abiotic elicitors that can be deliberately used to enhanced secondary metabolite biosynthesis (Selmar and Kleinwachter, 2013; Mustafavi et al., 2016). However, this response can vary within species and it is reasonable to suggest that the stress response-related production of secondary metabolites will also vary. Taarit et al. (2010) on *Salvia officinalis* L., Haghighi et al. (2012)

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on *Plantago ovata* Forsk. and Gengmao et al. (2014) on *Carthamus tinctorius* L. showed that salinity stress increased accumulations of secondary metabolites. Swieca (2015) reported that application of abiotic elicitors (osmotic and ion-osmotic shocks) improved 2-day-old sprout pro-health potential via an increase of phenolic contents. Recently, Naik and Al-Khayri (2016) and Gorelick and Bernstein (2014) classified salinity stress into physical (or abiotic) elicitors which induced secondary metabolites accumulation. Apart from abiotic stresses, exogenous application of signaling molecules, polyamines and trace elements, not only provided significant protection against stress-induced damages in plants, but also affected secondary metabolite accumulation. Arginine is one of the most functionally diverse amino acids in living cells and a major storage and transport form for organic nitrogen in plants. In addition to serving as a constituent of proteins and an essential metabolite for many cellular and developmental processes, Arginine is a precursor for biosynthesis of polyamines, proline and nitric oxide (Winter et al., 2015). Numerous researches have showed that exogenous application of arginine significantly promoted plant growth and productivity (Nassar et al., 2003), and mediates plant environmental stress tolerance (Zeid, 2009; Nasibi et al., 2011; Zhang et al., 2013). The increased nutraceutical quality of sprouts through elicitation has opened up a new area of research, which could have important health and economic benefits. To the best of our knowledge, no study has been conducted concerning the medicinal sprouts. Therefore, the objective of the present study was to elicitation of trigonelline production in fenugreek sprouts by providing arginine exogenously and salinity stress.

2. Material and methods

2.1. Plant materials and seed treatments

To investigate the effects of exogenous arginine and sodium chloride (NaCl) on fenugreek sprout growth and trigonelline production, one experiment examining two factors was conducted on the basis of complete randomized design (CRD) with three replications. The first factor was arginine different concentrations and the second factor was three salinity levels. Fenugreek (*Trigonella foenum-graecum*) seeds with the 985-MPI-SB code were procured from the seed bank of the medicinal plants institute, ACECR. The surface of intact seeds was sterilized with 3% sodium hypochlorite solution for 5 min with ratio 1:5 (g ml^{-1}), and then the seeds thoroughly rinsed with distilled water for 10 min. Fifty healthy seeds were put in each sterilized glass petri dish (9 cm diameter) over Whatman filter paper (No. 1) and filter paper was moistened with 15 ml of distilled water (control) and various concentrations of NaCl (0, 75 and 150 mM) and/or arginine (0, 10, 20, 30 and 40 µM). Arginine and NaCl concentrations were added to germination and growth medium according to their treatments. The seeds were incubated under the same conditions in the germination device with a 16 h light and 8 h dark cycle and 70% humidity at 25 °C. The sprouts were grown in the presence of the elicitors for a total of 12-days. To evaluate some important metabolic changes during growth, the sprouted fenugreek samples were harvested at 4, 8 and 12 days after beginning the experiment. It should be noted that a separate set of experiment was conducted for each harvest time. At each harvesting time all the sprouts were collected, and for the next harvesting time, the same experiment started from the beginning. Therefore, the sprouts at each stage were separately harvested at related time. Then each sample was divided into two parts. A part of the sample was frozen in liquid nitrogen and stored in freezer −80 °C till measurement of proline content. Another part of the sample was dried in an oven during 48 h at 70 °C for determination of trigonelline and nicotinic acid content.

2.2. Trigonelline and nicotinic acid analysis

Approximately 1 g powder of dried fenugreek sprouts was weighted, and then mixed with 1 g of magnesium oxide (MgO) and 20 ml distilled water. The mixture was incubated in a water bath at 100 °C for 20 min. After cooling the mixture was filtered through Whatman paper (No. 4) and its volume was brought to 25 ml with distilled water. Absorbance of the solutions was measured in UV–vis spectrophotometer apparatus at 268 and 264 nm for trigonelline and nicotinic acid, respectively. The trigonelline and nicotinic acid concentration of the sample was determined using a standard curve and was expressed as milligram per gram dry weight (Oraei, 2009).

2.3. Proline analysis

Approximately 0.5 g of the frozen fresh plant material was homogenized in 10 ml of 3% aqueous sulfo-salicylic acid by mortar and pestle in ice. The homogenate was centrifuged for 10 min at 15,000 rpm and the supernatant was used for proline content measurement. 2 ml of the supernatant was reacted with 2 ml ninhydrin reagent and 2 ml of glacial acetic acid in a test tube for 1 h in water bath at 100 °C, and the reaction was terminated by placing test tubes in ice bath. In the following 4 ml toluene was added to the reaction mixture and mixed vigorously with a test tube stirrer for 15–20 s. Then the reaction mixture was incubated at room temperature for up to two-phase mixtures is formed. The upper phase that consists of toluene and proline was separated from the aqueous phase and the absorbance read at 520 nm using toluene for a blank. The proline concentration was determined from a standard curve (0, 4, 8, 12, 16 and 20 mg L^{-1}) and was expressed as micromole proline per gram dry weight (Troll and Lindsley, 1954).

2.4. Statistical analysis

All the data were subjected to analysis of variance using SAS software. In cases where interaction effects were significant ($P \leq 0.05$), The SLICE option of the LSMEANS was used to test the simple effects of sprout harvesting time within each level of salinity. However, if slicing of interaction effects were significant ($P \leq 0.05$), the difference between means were compared by Duncan's Multiple Range Test.

3. Results

3.1. Germination and sprout growth

Table 1 showed that, germination was significantly affected by both salinity and arginine concentrations and also their interaction. Slice interaction for different arginine concentrations within each level of salinity showed that the arginine treatments had no significant effects on seed germination under control condition, but it was significantly changed under moderate (75 mM NaCl) and severe (150 mM NaCl) salinity conditions. According to Fig. 1, despite the fact, fenugreek seed germination was decreased with increasing the NaCl concentration from 0 to 150 mM, this change was ameliorated by increasing the arginine concentration from 0 to 40 µM.

Current study showed that simple and interaction effects of salinity and arginine on sprout dry weight (SDW) at all three harvesting times were statistically significant (Table 1). As shown in Fig. 2a, although SDW was decreased under salinity stress (without arginine application), dry weight of 4-day-old sprouts was improved by arginine increment up to 20 µM. Thereafter, further increases in arginine concentrations had unfavorable effects on SDW (Fig. 2a). According to Fig. 2b and c, 8-day-old and 12-day-old sprouts were differently affected by arginine and salinity. In non-saline condition, arginine fortified-media had negative effect on dry weight of sprouts on day 8 or 12 after treatment. Stimulatory effects of arginine on stressed-sprout growth had lasted to 8 days after treatment, wherein the maximum values of SDW were achieved by 10 and 30 µM of arginine for moderate and severe salinity, respectively (Fig. 2b). At 12 days after treatment, SDW of all three stressed and unstressed sprouts were decreased by arginine application (Fig. 2c).