

THE INFLUENCE OF ASCORBIC ACID ON GERMINATION AND SEEDLINGS GROWTH OF *TRIGONELLA FOENUM-GRÆCUM* L.

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Abstract: The effect of ascorbic acid treatment on the seed germination and seedling growth processes in the early ontogenetic stages in the *Trigonella foenum-graecum* L. was investigated. Four different concentrations of ascorbic acid were used (50 mg/l, 100 mg/l, 150 mg/l and 200 mg/l). The following indicators were considered: the final germination percentage, mean germination time, germination rate index, coefficient of velocity of germination, the length of the root, the length of the hypocotyl, the length of the seedling, the seedling fresh weight and the seedling vigor index. The main effects were: statistically insignificant changes in the indicators associated with germination; obvious increase of the root and seedling length, seedling fresh weight accumulation and enhance of seedling vigor index at 100 mg/l. The immersion of the seeds in ascorbic acid solution may improve germination and growth of *Trigonella foenum-graecum* seedlings in the early ontogenetic stages.

Keywords: ascorbic acid, seeds pretreatment, germination indices, growth process.

Introduction

Ascorbic acid, water-soluble natural vitamin, is a compound of essential importance in plant life, being involved in many biochemical and physiological processes. Ascorbic acid has an antioxidant role; together with other compounds it has a protective role against environmental stressors; it is a cofactor for many enzymes; is involved in cell division and elongation, in the regeneration of antioxidants; has a role in the process of photosynthesis and respiration (Smirnoff, 1996; Conklin, 2001; De Pinto and De Gara 2004; El-Lethy, 2011; Shah et al., 2019); influences floral induction, has a role in the synthesis of hormones, regulates senescence (Bart et al., 2006); ascorbic acid system works dynamically in seeds, production and use strategies may vary depending on seed development and functional stages (De Tullio and Arrigoni, 2003).

Research by various authors has shown that the application of exogenous ascorbic acid (seeds pretreatment or foliar application) has a role in improving the tolerance of plants to various environmental factors with a stressful effect such as salinity (Khan et al., 2006; Abdullah et al., 2021), drought (Hamama and Murniati, 2010; Razaji et al., 2014), drought and light intensity (Kamal and Faisal, 2021); pathogens (Khan et al., 2011); improves germination, seedling / plant growth under normal and stressful conditions (Azooz et al., 2013; Kumari et al., 2017; Shah et al., 2019); stimulates the synthesis of some primary and secondary metabolites (El Lethy et al., 2011; Mohamed et al., 2015).

Trigonella foenum-graecum L. (fenugreek) (Fabaceae) is an annual cultivated herbaceous species with a pronounced and persistent aromatic odor, known as a medicinal plant since antiquity (Pârvu, 2006). It is widespread in Europe, South Asia, North Africa

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and other parts of the globe. The seeds are used for medicinal purposes (traditional human and veterinary medicine), as spices, as a raw material in industry and the aerial part can be used as animal feed.

The seeds have a complex chemical composition (galactomannans, proteins, lipids, glycolipids, phospholipids, polyphenolic compounds, trigonelline, saponins, vitamin A, B vitamins, vitamin C, vitamin PP, volatile compounds, etc.) and important therapeutic properties: general tonic action on the body, stimulating action on metabolism, nervous system and neuromuscular (Pârvu, 2006); antioxidant, anti-diabetic, hypolipidemic, anti-microbial, anthelmintic, anti-cancer actions (Vígħ et al., 2017; Berroukche et al., 2018; Jagapriya, 2019).

Research on the species *Trigonella foenum-graecum* showed positive effects of ascorbic acid treatment during the processes of germination and growth of seedlings, on the enzymatic activity (Behairy et al., 2012), on plant growth and content of assimilating pigments under conditions of saline stress (Abdullah et al., 2021), as well as stimulating action on the synthesis of some secondary metabolites (Mohamed et al., 2015).

Continuing the general effort to know the biology of this species and to improve its functional parameters, the present paper aims to investigate the influence of ascorbic acid treatment on the process of seed germination and growth of newly formed seedlings, under conditions of experimental cultivation in the laboratory.

Material and methods

The biological material was represented by *Trigonella foenum-graecum* L. seeds bought from a specialised store. The seeds were disinfected with 2% hydrogen peroxide solution (for 8 minutes) and then washed several times with distilled water. The disinfected seeds were immersed in ascorbic acid solution of different concentrations (0 mg/l, 50 mg/l, 100 mg/l, 150 mg/l and 200 mg/l) at room temperature for 4 hours. Seeds subjected to ascorbic acid treatment were transferred for germination to filter papers moistened with distilled water, placed in sterile Petri dishes. For each experimental variant, three replicates were used, each replication with 15 seeds. Petri dishes were kept in laboratory conditions, at a temperature of 20°C - 23°C, the photoperiod characteristic of April 2021 for a period of 14 days. Germinated seeds were counted every 24 hours. After 14 days from the installation of the experiment, the length of the root, the length of the hypocotyl and the length of the seedling were measured and the fresh mass of the seedlings was weighed (25 seedlings for each experimental variant).

In order to estimate the response to ascorbic acid treatment, indicators of the germination process (the final germination percentage, mean germination time, germination rate index, coefficient of velocity of germination) and indicators of the seedling growth process (the length of the root, the length of the hypocotyl, the length of the seedling, the seedling fresh weight, the seedling vigor index) were evaluated. Germination indicators were calculated according to the formulas described by Kader (2005); for the seedling vigor index the formula described by Dezfuli et al. (2008) was used.

The obtained data were subjected to statistical analysis. The one-factor Anova test followed by the Tukey test ($\alpha = 0.05$) were used in order to determine the differences between means.

Results and discussions

The final germination percentage showed statistically insignificant changes compared to the control ($p>0.05$). There has been a growing trend (by 5%) in the case of 200 mg/l, and a decrease (by 5% and respectively 17.5%) at the concentrations of 150 mg/l and 50 mg/l. At the 100 mg/l, an average value equal to that of the control variant was recorded (Fig. 1).

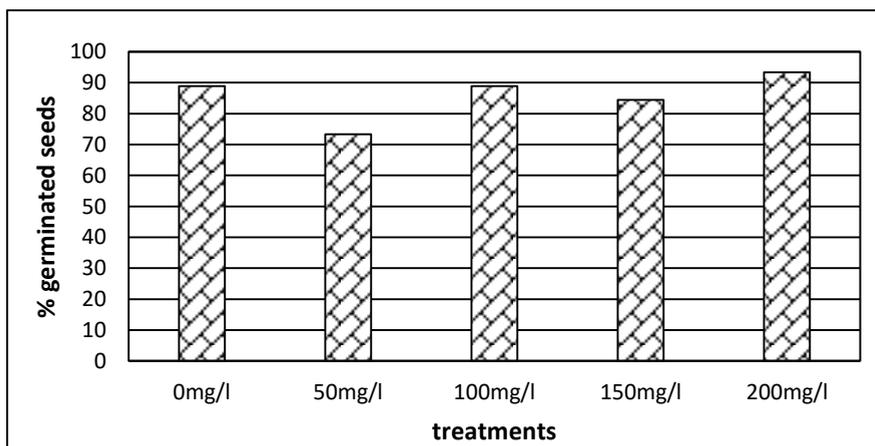


Figure 1. The final germination percentage variation

Mean germination time (MGT). Compared to the control, MGT increased to three treatment variants (at concentrations of 100 mg/l to 200 mg/l) and decreased by 2.32% to a single variant (at 50 mg/l); the changes were statistically insignificant ($p>0.05$) (Fig. 2).

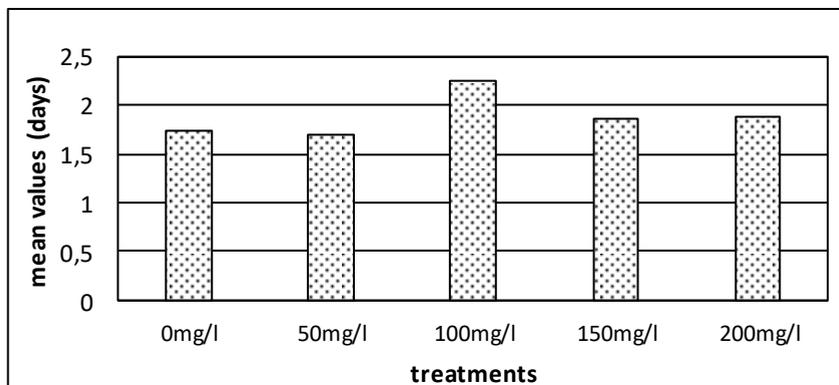


Figure 2. The mean germination time variation

The germination rate index (GRI). Compared to the control, GRI showed a slight decrease (by 6.36% to 16.41%) for all treatment variants, statistically insignificant ($p>0.05$) (Fig. 3).

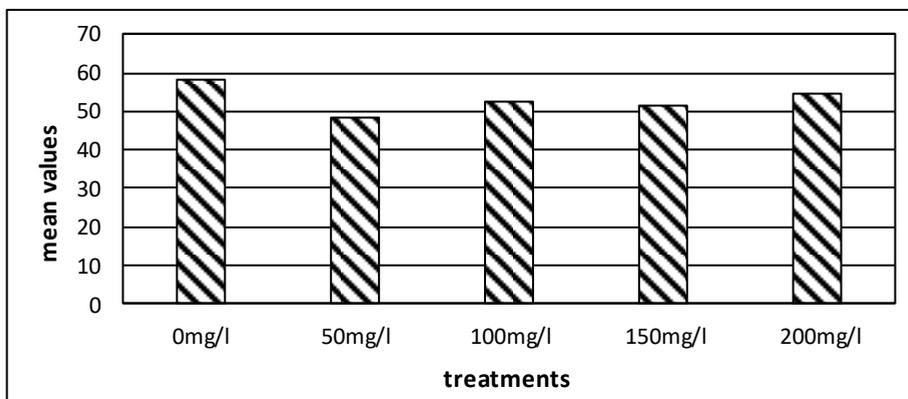


Figure 3. The germination rate index variation

The coefficient of velocity of germination. There was a decrease in the values recorded (by 6.02% - 22.83% compared with the control) in the case of concentrations of 100 mg/l, 150 mg/l and 200 mg/l. There was also a slight upward trend (by 2.95%) for the concentration of 50 mg/l. (Fig. 4). The changes found were statistically insignificant ($p>0.05$).

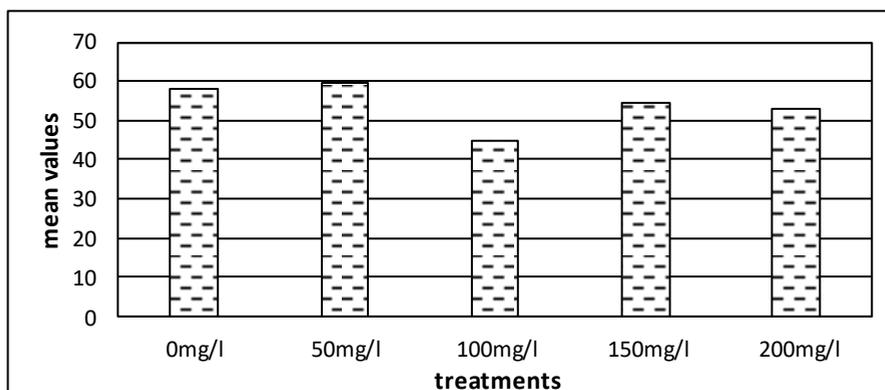


Figure 4. The coefficient of velocity of germination variation

The length of the root. Compared to the control, the root length was longer in the following treatment variants: 50 mg/l (by 0.12 %), 100 mg/l (by 24.93%) and 150 mg/l (by 4.83%). At 200 mg/l there was a decrease in root length by 12.66% compared to the control (Fig. 5). From a statistical point of view, significant differences were found ($p<0.05$) between the average values recorded for the treatment variant with a solution of ascorbic acid concentration of 100 mg/l and 200 mg/l.

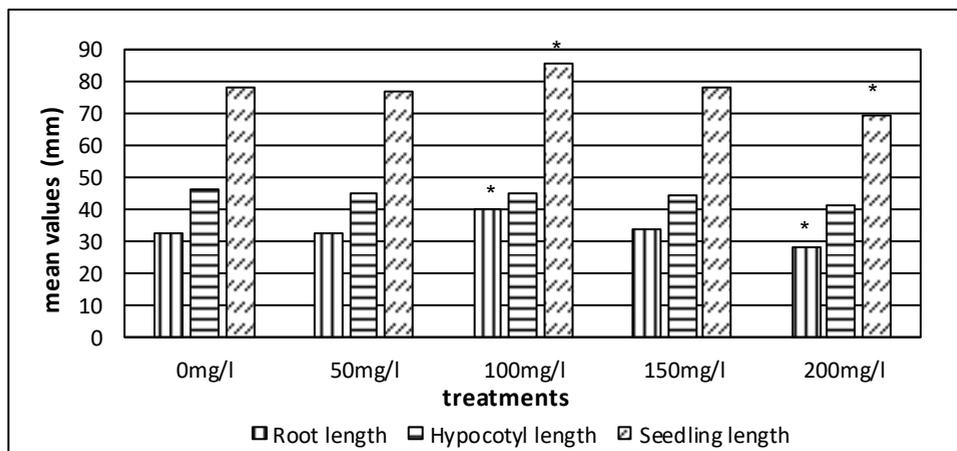


Figure 5. The length of the root, of the hypocotyl and the seedling variation (* - represent significant differences at $p < 0.05$)

The length of the hypocotyl showed a statistically insignificant decrease in recorded values by 1.48% (at 100 mg/l) to 10.7% (at 200 mg/l) (Fig. 5).

The length of the seedling, compared to the control, registered a slight increase of the values (by 9.4%) in the case of 100 mg/l and a slight decrease by 0.16% (150 mg/l) to 11.51% (200 mg/l) (Fig. 5). In statistically terms, significant differences were found ($p < 0.05$) between 100 mg/l and 200 mg/l ascorbic acid application.

The seedling fresh weight showed a trend similar to that described for the length of the seedling (Fig. 6). Statistically, significant differences were found ($p < 0.05$) between the mean values recorded for the treatment variant with ascorbic acid solution of concentration 100 mg/l and that with concentration solution 200 mg/l.

The seedling vigor index registered the highest value at the concentration of 100 mg/l (by 9.40% compared with the control). For the other treatment variants there was a slight decrease of this parameter value compared to the control (by 5.07% to 8.81%) (Fig. 7).

The results obtained in the present study indicate that ascorbic acid, in the concentrations used in the experimental model proposed by us, is a potential stimulating factor for germination and seedling growth. The influence of ascorbic acid on the studied physiological processes varied depending on the indicator studied and the concentrations tested.

Weak positive effects on germination were highlighted by the indicators studied: the final germination percentage (at 200 mg/l); the mean germination time and the coefficient of velocity of germination (at 50 mg/l).

In the literature it is mentioned that the treatment of seeds before sowing with biologically active substances has an important role in initiating biochemical changes that take place in seeds before germination (hydrolysis of inhibitors, imbibition, activation of enzymes) (Shah et al., 2019).

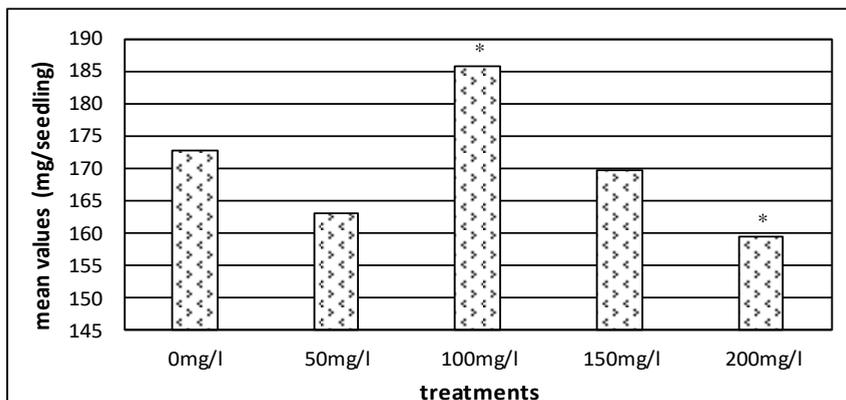


Figure 6. The seedling fresh weight variation (* - represent significant differences at $p < 0.05$)

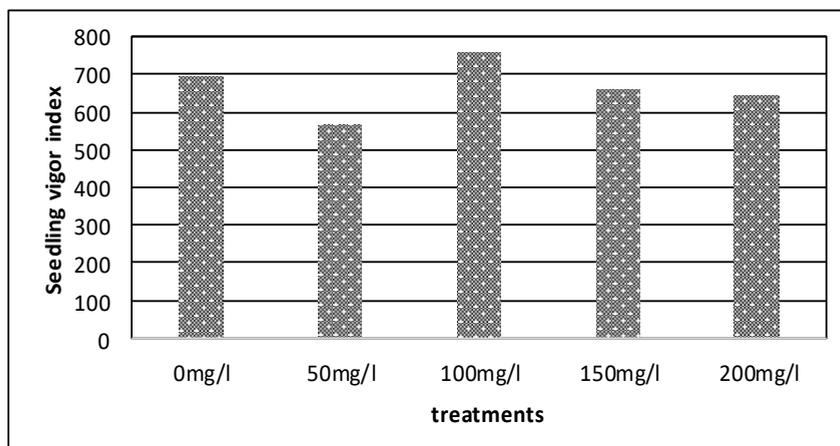


Figure 7. Seedling vigor index variation

Regarding the influence on the growth process of *Trigonella foenum-graecum* seedlings, there was an obvious stimulating effect (indicators: the length of the root, the length of the seedling and the seedling fresh weight) as well as the improvement of the seedling vigor index in the case of the concentration of 100 mg/l. The results are due in part to the role of ascorbic acid in plant life; ascorbate stimulates cell growth (Córdoba and Gonzales-Reyes, 1994). According to Kaviani (2014), ascorbic acid stimulates cell division and increases the size of cells in the region of root elongation.

Various studies indicate that ascorbic acid was not detected in the dried seeds, but during germination the content of this natural vitamin increases rapidly due to the reactivation of its biosynthesis (Xu et al., 2005; Beltrán-Orozco et al., 2020); this indicates the involvement of ascorbic acid in modulating plant growth (Beltrán-Orozco et al., 2020).

The dried seeds of *Trigonella foenum-graecum* contain ascorbic acid, the values varied from 5.36 mg/100g (Ojha et al., 2018), 7.07 mg% (Dinesh et al., 2015) to 12

mg/100g (Ojha et al., 2018). Ascorbic acid content of fenugreek seeds increases significantly during seed storage in water and germination process (Ojha et al., 2018).

The results presented in this study confirm the data from literature regarding the beneficial effect of ascorbic acid on germination and seedling growth in *Trigonella foenum-graecum* or other cultivated species.

Pretreatment of *Trigonella foenum-graecum* seeds with ascorbic acid concentrations of 50 mg/l and 100 mg/l for 24 hours positively influenced seed germination, growth in length (root, hypocotyl and seedling), accumulation of fresh mass and seedlings vigor index under normal and different saline stress conditions (Behairy et al., 2012).

Treatment of *Helianthus annuus* and *Brassica napus* seeds with ascorbic acid in concentration of 100 ppm, 200 ppm and 400 ppm stimulated seed germination and seedling growth (Dolatabadian and Modarres Sanavy, 2008).

In *Zea mays*, pretreatment of seeds with ascorbic acid of different concentrations and different exposure time positively influenced the germination and growth of seedlings. Research by Hamama and Murniati (2010) has shown that seed treatment with ascorbic acid at a concentration of 55 mM (24-hour soaking time) has improved germination rate, root and coleoptile growth, and seedling vigor index. According to Kumari et al. (2017), ascorbic acid in concentration of 100 ppm (soaking time 12 hours) showed significant positive effects on seed germination (by indicators: germination percentage and germination index) and seedling growth (by indicators: root length, coleoptile length, seedling length, seedling fresh weight and seedling vigor index) under normal conditions.

Conclusions

Treatment of *Trigonella foenum-graecum* seeds with ascorbic acid solutions had different effects depending on the concentration and indicators studied.

Immersion of seeds in ascorbic acid solution has the potential to improve germination and growth in the early ontogenetic stages in the species studied.

Future research will be focused on the identification of optimal concentrations, as well as treatment duration to improve of functional parameters in *Trigonella foenum-graecum*.

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