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## Physiological Role of Mannitol on *Vicia Faba* Plants Undergo Sandy Soil Conditions

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**Abstract**

Background: During two consecutive winter seasons of 2019/2020 and 2020/2021, two experiments in the field were done at the Research and Production Station, National Research Centre, El-Nubaria Province, El-Behira Governorate, Egypt. This experiment aimed to evaluate the physiological effect of mannitol at 0, 10, 20 and 30 mM on growth, productivity, and nutritional value of faba bean plants (Misr 2 and Sakha 3).

Results: Results show that faba bean cv. Misr 2 was more adaptable to grow under sandy soil conditions than faba bean cv. Sakha 3, since it characterized by higher vegetative growth parameters, photosynthetic pigments, seed yield, yield components, and nutritive value. Mannitol treatments at 20 and 30 mM significantly increased plant fresh and dry weight, total photosynthetic pigments, seed number and weight/plant, seed yield(Kg/feddan),reducing sugar and starch accompanied by significant decreases in total phenolic content, tannins and vicine content. It is worthy to mention that mannitol treatments at 0, 10, 20 and 30 mM significantly increased seed yield (Kg/feddan) by 23.73, 31.88 and 40.15% respectively. Regarding interaction between cultivars and mannitol treatments, it was noted that 30 mM mannitol increased seed yield (Kg/feddan) by 56.37% in Misr 2 and by 22.21% in Sakha 3 relative to corresponding controls. It is obvious that response of faba bean cv. Misr 2 to mannitol treatments was more pronounced than that of faba bean cv. Sakha3.

Conclusion: It could be concluded that Misr 2 cultivar was more adaptable to grow under sandy soil conditions and show more effectively response to mannitol treatments than Sakha 3 cultivar. Mannitol treatments caused significant increases in most investigated parameters of both cultivars and its promotive effect increased by increasing mannitol levels. Mannitol at 30mM was the most pronounced treatment.

***Keywords:*** *Vicia faba, Reducing sugar, Mannitol, Sandy soil*

## 1. Introduction

Faba bean (*Vicia faba*) is one of the most important winter legume crops. It is a protein-rich food for both humans and animals [1]. Faba bean seeds are good sources of protein (~ 25% in dried seeds), cellulose, starch, and minerals [2]. At the same time, its cultivation increased the amount of soil nitrogen compounds [3].The yield of legumes can be increased by two ways by vertical expansion through the using best agricultural practices, including the use of compatible solutes as mannitol and/or by horizontal expansion through land reclamation. Sandy soil in a new cultivating area is mostly subjected to a variety of abiotic stress circumstances, as saline soil, saline water, water deficit, temperature fluctuations, high irradiances and nutrient deprivation.

In general, plants have evolved complex and dynamic systems incorporating a wide range of biochemical and physiological processes to overcome the effects of abiotic stresses [4].The accumulation of osmoprotectant such as polyols (or sugar alcohols), protects the plant cells against harmful osmotic and metabolic imbalances caused by various stresses [5, 6].

Polyols are non-reducing carbohydrates with high solubility, thus making them ideal for use as translocatable carbohydrates that transported in the phloem [7]. Polyol translocation in the phloem has a number of benefits, including increased carbon efficiency [8, 9], and better protection against

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hydroxyl radicals [10,11]. Furthermore, accumulation of polyols enhances tolerance of abiotic stress tolerance [12, 13, 14, 15, 16]. According to metabolome study, polyols are synthesized as a minor component in several plant species, as tomato and bean [17, 18]. Mannitol (also known as mannite or manna sugar) is a white, crystalline solid substance with the chemical formula $C_6H_8$ $(OH)_6$ that belong to polyols (sugar alcohols) [19], and serves as osmotic adjustment agent [20]. D-mannitol is the most prevalent polyol in nature. It is produced by yeasts, bacteria, algae, fungi, and many plants [21]. In many plant species, mannitol is produced as a primary photosynthetic product [22, 23, 24, 25]. It accounts for almost half of total translocated photoassimilates [26]. Special attention was paid to physiological role of mannitol, its consumption by plants, its biosynthesis pathway, its effects on plants as an osmolyte, and its role as antioxidant to scavenge free radical [10;11, 13, 27). *In vitro* [10] and *in vivo* [10 27], mannitol has also been proven to be an oxygen radical quencher. In addition, the accumulation of efficient osmolytes such as mannitol improved plant tolerance to salinity or water deficiency [29, 30, 31, 32, 33, 34].

The goal of this research was to look into the physiological effects of mannitol treatments on two faba bean cultivars undergo sandy soil conditions.

## 2. Methods

*Plant materials and experimental conditions*

During the winter seasons of 2019/2020 and 2020/2021, two experiments in the field were done at the Research and Production Station, National Research Centre, El-Nubaria Province, El-Behira Governorate, Egypt. Seeds of two faba bean cultivars (Misr 2 and Sakha 3) were received from Egypt's Ministry of Agriculture's Legumes Crops Research Department and selected for consistency size and colour. The soil of the experimental site was sandy soil. $P_2O_5$ as calcium superphosphate (15.5 percent) and $K_2O$ as potassium sulphate (48–52 percent) were applied at the level of 31 and 24 kg/fed, respectively, during seed bed preparation while nitrogen fertilizer as ammonium nitrate (33.5 percent) was applied at the rate of 75 kg N/fed. In the middle of November for both growth seasons, faba bean seeds were sown on a hill 25 cm apart on both sides of the ridge. At 21 days following sowing, the plants were thinned to leave two plants per hill. A split plot design with four replicates was used to set up the experiment. The main plots included two faba bean cultivars (Misr 2 and Sakha 3). Sub-plots were assigned to mannitol at 0, 10, 20 and 30 mM.

The experimental unit was $10.5m^2$, 3m long and 3.5 m wide and 70 cm apart between ridges. The plants were sprayed two times with freshly prepared solutions of mannitol at 0, 10, 20 and 30 mM at 30 and 45 days after sowing. Meanwhile, control plants sprayed with distilled water. Irrigation was done with a sprinkler irrigation system, which irrigated the plants every 5 days for 2 hours.

*Data recorded*

After 60 days from sowing, plant samples were taken to determine certain growth parameters (shoot height, leave and branch number/plant, fresh and dry weights/plant) and photosynthetic pigments. At harvest, number of pods and seeds/plant, weight of pods/plant, seed yield/plant (g) was recorded at a random of ten guarded plants from each plot. The whole plot was harvested to determine seed yield/feddan (kg). The yielded seeds were cleaned and crushed to determine total soluble sugar, reducing sugar, starch, total phenolic contents, tannins and vicine content.

*Chemical analysis*

Photosynthetic pigments were estimated by a method described by Moran [35]. The procedure for the extraction of total soluble sugars from dry powdered seeds was described by Ciha and Brun [36]. Total soluble sugar was quantified using a modified phenol sulphuric acid assay [37].The residual mass collected after the extraction of total soluble sugars was used for starch determination as the method described by McCready et al. [38]. Reducing sugar was determined by the method described by Miller (39). Determination of total phenolic compounds was done according to the method illustrated by Zheng and Wang [40].Tannins was estimated using the modified method of vanillin hydrochloric acid (MV-HCl) as described by Maxson and Rooney [41]. Vicine content was determined according to the method reported by Collier [42].

*Statistical analysis*

Before doing the analysis of variance, the acquired data were submitted to the homogeneity test [43] and Anderson–Darling normality test [44]. (ANOVA).Because the outputs demonstrated that the data homogeneity and normality were met, a combined ANOVA for the data from the two seasons was performed [45], using the Costat software tool, Version 6.303. (2004). Duncan's multiple range test revealed significant (P 0.05) differences among the treatments when the F–test indicated significant (P 0.05) differences among the treatments.

## 3. Results

*Vegetative growth parameters*

Results show that faba bean cv. Misr 2 was characterized by higher values of vegetative growth parameters under investigation except shoot height relative to faba bean cv. Sakha3 (Table 1). Mannitol treatments significantly increased plant fresh and dry weight by increasing mannitol level. Since, 30mM mannitol significantly increased plant fresh weight by 84.58% and plant dry weight by 65.09%. Regarding interaction between cultivars and mannitol treatments, it was noted that all applied mannitol treatments increased the vegetative growth parameters of both cultivars. Mannitol at 30mm significantly increased plant dry weight of cv. Misr 2 by 103.77% and in cv. Sakha 3 by 30.89% relative to cross ponding controls. It is clear that response of cv. Misr 2 to mannitol treatments was more pronounced than cv. Sakha 3.

Table 1: Effect of mannitol on vegetative growth parameters of two faba bean cultivars undergo sandy soil conditions (Data are average of two seasons)

| Treatments | Shoot height (Cm) | Leaves number/plant | Branches number/plant | Plant fresh weight(g) | Plant dry weight/plant |
|---|---|---|---|---|---|
| Cultivars | | | | | |
| Misr 2 | 54.17 | 30.04 | 3.08 | 133.05 | 14.70 |
| Sakha 3 | 56.38 | 29.75 | 3.07 | 104.91 | 11.57 |
| LSD at 5% | 1.87 | NS | NS | 6.88 | NS |
| Mannitol treatments | | | | | |
| Control | 47.75 | 24.83 | 2.67 | 82.26 | 9.08 |
| Mannitol (10Mm) | 57.25 | 27.75 | 3.00 | 113.32 | 13.55 |
| Mannitol (20mM) | 57.08 | 32.92 | 3.33 | 128.50 | 14.92 |
| Mannitol (30mM) | 59.00 | 34.08 | 3.35 | 151.84 | 14.99 |
| LSD at 5% | 3.50 | 3.02 | 0.66 | 17.05 | 2.08 |
| Interaction between cultivars and mannitol treatments | | | | | |
| Misr 2 cultivar | | | | | |
| Control | 46.00 | 22.00 | 2.33 | 80.28 | 8.49 |
| Mannitol (10Mm) | 57.33 | 25.01 | 2.67 | 131.74 | 15.90 |
| Mannitol (20mM) | 55.67 | 35.50 | 3.67 | 146.71 | 17.12 |
| Mannitol (30mM) | 56.66 | 37.17 | 3.68 | 173.47 | 17.30 |
| Sakha 3 cultivar | | | | | |
| Control | 49.50 | 27.67 | 2.67 | 84.25 | 9.68 |
| Mannitol (10Mm) | 57.17 | 30.00 | 3.00 | 94.89 | 11.20 |
| Mannitol (20mM) | 58.50 | 30.33 | 3.30 | 110.28 | 12.72 |
| Mannitol (30mM) | 60.33 | 31.00 | 3.33 | 130.20 | 12.67 |
| LSD at 5% | NS | 4.26 | 0.94 | 24.11 | 2.54 |

*Photosynthetic pigments*

Results show that, there is no significant difference in photosynthetic pigments between two cultivars (Misr 2 and Sakha 3) except carotenoids (Table 2). Mannitol treatments at 20 and 30 mM caused significant increases of chlorophyll A, chlorophyll B and total photosynthetic pigments, since; they significantly increased total photosynthetic pigments by 11.69% and 13.64% respectively. Regarding interaction between cultivars and mannitol treatments, it was noted that mannitol treatments at 20 and 30 mM caused significant increases of chlorophyll A, chlorophyll B and total photosynthetic pigments, relative to corresponding controls. 20 and 30 mM mannitol significantly increased total photosynthetic pigments by 15.97% and 18.05% in Misr 2 as well as 7.92% and 10.36% in Sakha 3 relative to corresponding controls. It is obvious that the response of Misr 2 to mannitol treatments was more pronounced than Sakha 3.

Table 2: Effect of mannitol on photosynthetic pigments of two faba bean cultivars undergo sandy soil conditions (Data are average of two seasons)

| Treatments | Chlorophyll A | Chlorophyll B | Carotenoids | Total photosynthetic pigments |
|---|---|---|---|---|
| | (mg/g fresh leaf tissues) | | | |
| Cultivars | | | | |
| Misr 2 | 1.15 | 0.32 | 0.25 | 1.72 |
| Sakha 3 | 1.08 | 0.31 | 0.20 | 1.56 |
| LSD at 5% | NS | NS | 0.04 | NS |
| Mannitol treatments | | | | |
| Control | 1.03 | 0.29 | 0.21 | 1.54 |
| Mannitol (10Mm) | 1.08 | 0.36 | 0.22 | 1.61 |
| Mannitol (20mM) | 1.15 | 0.32 | 0.24 | 1.72 |
| Mannitol (30mM) | 1.19 | 0.34 | 0.22 | 1.75 |
| LSD at 5% | 0.05 | 0.02 | NS | 0.07 |
| Interaction between cultivars and mannitol treatments | | | | |
| Misr 2 cultivar | | | | |
| Control | 1.10 | 0.30 | 0.24 | 1.64 |
| Mannitol (10Mm) | 1.11 | 0.31 | 0.24 | 1.66 |
| Mannitol (20mM) | 1.19 | 0.32 | 0.26 | 1.77 |
| Mannitol (30mM) | 1.22 | 0.34 | 0.25 | 1.81 |
| Sakha 3 cultivar | | | | |
| Control | .97 | 0.29 | 0.18 | 1.44 |
| Mannitol (10Mm) | 1.06 | 0.30 | 0.21 | 1.57 |
| Mannitol (20mM) | 1.11 | 0.33 | 0.23 | 1.67 |
| Mannitol (30mM) | 1.17 | 0.34 | 0.19 | 1.70 |
| LSD at 5% | 0.08 | 0.02 | NS | 0.10 |

*Seed yield and yield components*

Results show that cv. Misr 2 was characterizes by higher seed yield and yield components than cv. Sakha 3 under sandy soil conditions (Table 3). Mannitol treatments significantly increased pod weight/plant, number and weight of seeds /plant and seed yield (Kg/feddan). Mannitol treatments at10, 20, and 30mM significantly increased seed yield (Kg/feddan) by 23.73%, 31.89%, and 40.15% respectively relative to control. Regarding interaction between cultivars and mannitol treatments, it was noted that mannitol treatments significantly increased seed yield and yield components under investigation except number of pods/plant which showed non-significant increases. Mannitol at 30 mM significantly increased seed yield (Kg/feddan) of Misr 2 by 56.37% and Sakha 3 by 22.21 % relative to corresponding controls.

*Nutritional and antinutritional components of the yielded seeds*

Results show that cv. Misr 2 was characterized by higher content of reducing sugars, starch and vicine relative to cv. Sakha 3. While, cv. Sakha 3 was characterized by higher content of total soluble sugar, phenolic, and tannins (Table 4). Mannitol treatments significantly increased total soluble sugar, reducing sugars, starch and significantly decreased total phenolic, tannins and vicine content. Regarding interaction between cultivars and mannitol treatments, it was noted that mannitol treatments significantly increased total soluble sugars and non-significant increased in reducing sugar and starch in both cultivars. Respect to anti-nutritive content, mannitol treatments caused non-significant decreases in phenolic and vicine content and significantly reduced tannins.

Table 3: Effect of mannitol on seed yield and its components of two faba bean cultivars undergo sandy soil conditions (Data are average of two seasons)

| Treatments | Pods number/plant | Pods weight/plant (g) | Seeds number/plant | Seeds weight /plant (g) | Seeds yield (Kg/fedaan) |
|---|---|---|---|---|---|
| Cultivars | | | | | |
| Misr 2 | 16.33 | 23.34 | 47.17 | 58.99 | 1439.36 |
| Sakha 3 | 14.58 | 19.02 | 36.83 | 42.22 | 1047.68 |
| LSD at 5% | 1.64 | 2.84 | 9.63 | NS | NS |
| Mannitol treatments | | | | | |
| Control | 13.83 | 17.50 | 30.17 | 37.25 | 1003.32 |
| Mannitol (10Mm) | 15.17 | 20.75 | 42.66 | 44.19 | 1241.40 |
| Mannitol (20mM) | 16.00 | 22.80 | 46.16 | 58.00 | 1323.16 |
| Mannitol (30mM) | 16.83 | 23.67 | 49.00 | 62.97 | 1406.20 |
| LSD at 5% | NS | 1.59 | 5.65 | 12.31 | 17.24 |
| Interaction between cultivars and mannitol treatments | | | | | |
| Misr 2 cultivar | | | | | |
| Control | 14.33 | 18.51 | 35.67 | 45.17 | 1054.20 |
| Mannitol (10Mm) | 16.67 | 21.99 | 45.33 | 46.61 | 1484.76 |
| Mannitol (20mM) | 17.00 | 25.58 | 51.33 | 70.26 | 1570.16 |
| Mannitol (30mM) | 17.33 | 27.28 | 56.34 | 73.92 | 1648.32 |
| Sakha 3 cultivar | | | | | |
| Control | 13.33 | 16.49 | 24.67 | 29.33 | 952.48 |
| Mannitol (10Mm) | 13.67 | 19.51 | 40.00 | 41.78 | 998.08 |
| Mannitol (20mM) | 15.00 | 20.02 | 41.01 | 45.74 | 1076.20 |
| Mannitol (30mM) | 16.33 | 20.07 | 41.67 | 52.03 | 1164.08 |
| LSD at 5% | NS | 2.24 | 7.99 | 17.41 | 24.36 |

Table 4: Effect of mannitol on some nutritional value and anti-nutritional value of the yielded seeds of two faba bean cultivars undergo sandy soil conditions (Data are average of two seasons)

| Treatments | Nutritional value | | | Anti-nutritional value | | |
|---|---|---|---|---|---|---|
| | Total soluble sugar | Reducing sugar | Starch | Total phenolic content | Tannins | Vicine content |
| | (g/100g dry weight) | | | | | (mg/100g dry weight) |
| Cultivars | | | | | | |
| Misr 2 | 1.87 | 0.94 | 34.47 | 2.18 | 1.07 | 428.74 |
| Sakha 3 | 2.10 | 0.80 | 33.18 | 2.62 | 1.24 | 400.55 |
| LSD at 5% | 0.10 | NS | NS | 0.08 | NS | 4.24 |
| Mannitol treatments | | | | | | |
| Control | 1.21 | 0.26 | 30.50 | 2.62 | 1.32 | 433.16 |
| Mannitol (10Mm) | 1.69 | 0.91 | 33.20 | 2.38 | 1.22 | 422.01 |
| Mannitol (20mM) | 2.35 | 1.14 | 35.27 | 2.34 | 1.09 | 411.77 |
| Mannitol (30mM) | 2.69 | 1.18 | 36.33 | 2.27 | 1.00 | 391.64 |
| LSD at 5% | 0.16 | 0.20 | 1.02 | 0.17 | 0.06 | 7.50 |
| Interaction between cultivars and mannitol treatments | | | | | | |
| Misr 2 cultivar | | | | | | |
| Control | 1.01 | 0.38 | 31.61 | 2.43 | 1.20 | 448.18 |
| Mannitol (10Mm) | 1.44 | 0.97 | 33.49 | 2.25 | 1.08 | 437.37 |
| Mannitol (20mM) | 2.33 | 1.18 | 35.86 | 2.07 | 1.02 | 429.32 |
| Mannitol (30mM) | 2.68 | 1.22 | 36.92 | 1.97 | 0.99 | 400.10 |
| Sakha 3 cultivar | | | | | | |
| Control | 1.41 | 0.13 | 29.38 | 2.81 | 1.44 | 418.15 |
| Mannitol (10Mm) | 1.93 | 0.85 | 32.92 | 2.51 | 1.36 | 406.65 |
| Mannitol (20mM) | 2.37 | 1.10 | 34.68 | 2.61 | 1.15 | 394.22 |
| Mannitol (30mM) | 2.70 | 1.14 | 35.75 | 2.57 | 1.01 | 383.17 |
| LSD at 5% | 0.23 | NS | NS | NS | 0.09 | NS |

## 4. Discussion

The enhancement effect of foliar application of mannitol on faba bean growth parameters (Table 1), photosynthetic pigments (Table 2), seed yield an yield components (Table 3), and some chemical composition of the yielded seeds of two cultivars of faba bean (Table 4) may be attributed to physiological roles of mannitol as a carbon compound which is transported through hyphae and/or an antioxidant to quench reactive oxygen species (ROS) to prevent oxidative damage, store reducing power, and participate in redox balancing as well as its impact on plants as osmolyte [10, 13, 28).

Furthermore, Karakas et al. [12] reported that mannitol treatment improved the dry weight of transgenic tobacco plants under salt stress when compared to salt-treated untransformed tobacco. It is known that when mannitol applied, it can be taken up and used as a source of carbon and energy by plants [46, 24] affecting dry matter built up of plants. Kaya et al. [47] reported that foliar applications of mannitol enhanced maize biomass. This could be owing to its use in the leaves as a source of C [25, 48] or their role in cellular osmotic adjustment [49]. The osmotically active organic solutes as mannitol [9, 25] play a key role in osmotic adjustment, a phenomenon which has been widely reported to play an active role in maintaining cell turgor [50, 51]. Mannitol serves as a protein and membrane structure stabiliser, as well as a photosynthetic protector against salt and drought stress [52, 9, 11, 28, 53, 25, 27]. More beneficial effects of foliar-applied mannitol might be due to the fact that mannitol is a major product of photosynthesis and hence a source of energy in a variety of plant species. It accounts for up to half of the entire translocated photoassimilates [26]. Furthermore, according to Kaya et al. [47], foliar-applied mannitol was effective in increasing the leaf chlorophyll content of maize plants, which was favourably related with increased photosynthetic rate and thus higher biomass output. Stoop et al. [9] reported that mannitol acts as a scavenger of reactive oxygen species, preventing lipid peroxidation of lipids and cell damage and is beneficial in minimizing the negative effects of oxidative stress on biological membranes. Gao et al. [54] showed that mannitol have a role in cellular ion transport. Seckin et al. [49] suggest that exogenous application of mannitol could protect wheat plants from the damaging effects of salt-induced oxidative stress by enhancing the activity of antioxidant enzymes. In addition, Ramadan and Shalaby [55] mentioned that melatonin treatments (0, 2000 and 4000 mg $l^{-1}$) increased stem length, head width, head weight, total yield, vitamin C, total sugars, chlorophyll a and b, dry weight and minerals content (N, P and K) of white cabbage under salinity. Sattar et al. [16] stated that mannitol treatments increased total phenolic contents, total flavonoids content and free radical scavenging capacity in potato cultivars with increasing treatment concentrations.

## 5. Conclusions

It could be concluded that Misr 2 cultivar was more adaptable to grow under sandy soil conditions and show more effectively response to mannitol treatments than Sakha 3 cultivar. Mannitol treatments caused significant increases in most investigated parameters of both cultivars and its promotive effect increased by increasing mannitol levels. 30mM mannitol was the most pronounced treatment.

## Availability of data and materials

The data sets generated and/or analyzed during the current study are included in this published manuscript.

## Competing interests

The authors declare that they have no competing interests

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## Authors' contributions

MG Dowood designed and performed the experiment, responsible of all the physiological and biochemical analysis and also wrote and reviewed the manuscript.

ME El-Awadi designed and performed the experiment, responsible of all responsible of agriculture, collecting samples during plant growth and harvest and recording data.

IM El-Metwaly responsible of statistical analysis.

All authors read and approved the final manuscript.

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