Heat Stress Management in Bean (*Phaseolus vulgaris* L.) through Nitric Oxide and Trehalose Interventions

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Abstract:

Ambient temperatures are predicted to rise in the future owing to several reasons associated with global climate changes. These temperature increases can result in heat stress-a severe threat to crop production in most countries. High temperatures during the reproductive growth stage result in a reduction in pod and seed set in bean due to enhanced abscission of flower buds, flowers, and pods.

Two filed experiments were carried out to study the effects of Trehalose and Nitric oxide (NO) via leaf sprays to investigate how to reduce the heat stress on bean plants and to improve growth and yield of bean under late sowing conditions.

The obtained results indicated that vegetative growth, chemical composition of leaves, setting, yield and its components as well as seed quality parameters responded positively to different of trehalose and nitric oxide treatments. Foliar application with either nitric oxide or trehalose proved superiority as compared with the control in most studied parameters. The best values of vegetative growth, chemical composition, setting, seed yield and germination were achieved when plants sprayed with nitric oxide at 80 ppm in both growing seasons. Thus, this treatment could be recommended to improve common bean plants performance under similar conditions of this study.

For obtaining high seed yield and quality of bean plants under late cultivation condition (high temperature) it could be recommended that, plants should be sprayed with nitric oxide at 80 ppm three times during the growing season at 3, 5 and 7 weeks after sowing

Keywords: bean; high temperature; heat stress; nitric oxide; trehalose

Introduction:

Originating in the New World and widely dispersed because of its broad adaptation, the common bean (*P. vulgaris* L.) has become an important grain and vegetable legume on a global scale. Common bean is particularly important to the developing world in providing a source of protein, calories, and trace nutrients to individuals who cannot afford more expensive sources of nutrition. As a vegetable, the immature pods have high moisture content, with raw pods containing about 1.9% protein and 7% carbohydrate, and significant quantities of vitamin C, carotenoids, and vitamin K, which dry beans lack. Uncooked dry bean contains approximately 22% protein, several micronutrients (Ca, Fe, Mg, P, and K), complex carbohydrates (62%), soluble fiber (15%) and is a significant source of folate (**USDA 2015**).

Common bean performs best in moderate growing temperatures (>10 °C and <30 °C) with about 400 mm of precipitation during the growing season. Common bean is found throughout temperate growing regions where the season permits 60–120 days of frost-free growth as well as in the tropical highlands with growing temperatures <30 °C.

Ambient temperatures are predicted to rise in the future owing to several reasons associated with global climate changes. These temperature increases can result in heat stress-a severe threat to crop production in most countries. High temperature is one of the biggest abiotic stress challenges for agriculture. High temperature induces oxidative stress, lipid peroxidation, membrane injury, protein degradation, enzyme inactivation, pigment bleaching, and DNA strands disruption in plants (**Suzuki and Mittler 2006**). Many crop species can be damaged by high temperatures (**Hall, 1992**). Green bean (*Phaseolus vulgaris*) is a heat-sensitive crop and pod yield is decreased at high temperatures (**Nakano et al., 1997**). High temperatures during the reproductive growth stage result in a reduction in pod and seed set in bean (*Phaseolus vulgaris*) due to enhanced abscission of flower buds, flowers, and pods (**Monterroso and Wien, 1990**).

Although genetic approaches may be beneficial in the production of heat-tolerant plants, it is likely that the newly produced plants are low yielding compared to near-isogenic heat sensitive plants. Thus, considerable attention has been devoted to the induction of heat tolerance in existing high-yielding cultivars. Among the various methods to achieve this goal, foliar application of, or pre-sowing seed treatment with, low concentrations of inorganic salts, osmoprotectants, signaling molecules and oxidants as well as preconditioning of plants are common approaches. Nitric oxide and Trehalose purportedly play important roles under heat stress to confer heat tolerance (**Hasanuzzaman *et al.*, 2013**; **Asthir, 2015**).

Nitric oxide (NO) is a gaseous di-atomic radical, readily diffusible through biological membranes and readily soluble in water (**Tran *et al.*, 2011**). It's a bioactive molecule that plays a key role in plant biomass and yield production by transporting its significant role in water relations in plant cells by controlling the cellular osmotic adjustment. Besides, it plays a vital role in plant resistance in response to several a biotic stress (**Shan *et al.*, 2015**). Nitric oxide is an important concentration-dependent and redox-related signaling molecule (**Fancy *et al.*, 2017**). NO regulates various physiological processes and has a vital role in conferring tolerance to plants under abiotic stress including heat stress (**Waraich *et al.*, 2012**; **Hasanuzzaman *et al.*, 2013**). Treatment of heat-stressed mungbean plants with NO as sodium nitropruside assisted in maintaining the stability of chlorophyll a fluorescence, membrane integrity, H_2O_2 content, and antioxidant enzyme activity (**Yang *et al.*, 2006**).

Trehalose (α-D-glucopyranosyl-1,1-α-D-glucopyranoside) is a non-reducing glucose disaccharide composed of two glucose units attached through their 1-carbons that is produced by a variety of organisms including plants. It considered as a cytoprotective agent under the unfavorable environmental conditions (**Fichtner and Lunn, 2021**). Its high solubility and chemical non-reactivity allow it to accumulate at higher concentrations without disrupting the normal metabolism. Also, trehalose acts as an osmoprotectant, a carbon storage and a transport compound (**Wang *et al.*, 2020**). The beneficial role of trehalose in ameliorating stress related to its ability to improve cellular redox balance, promoting photosynthesis and enhancing antioxidant systems as well as stabilizing biological structures by crystallizing into a glassy state that resists dehydration (**Lin *et al.*, 2020**). Trehalose is believed to play a protective role against different abiotic stressful cues such as temperature extremes. Exogenously applied trehalose in low amounts mitigates physiological and biochemical disorders induced by various abiotic stresses, delays leaf abscission and stimulates flowering in crops. Under high temperature stress, the protective effect of trehalose in *Myrothamnus flabellifolius* was shown to be due to its involvement in effective protein conformation (**Doehlemann *et al.* 2006**). In another study with wheat under heat stress, **Luo *et al.* (2008)** showed that pre-treated winter wheat seeds with trehalose protected the membranes from lipid peroxidation and photosystems against heat stress.

The objectives of this work are to study the effects of Trehalose and Nitric oxide via leaf sprays to investigate how to reduce the heat stress on bean plants and to improve growth and yield of bean under late sowing conditions.

Materials and Methods

Two field experiments were carried out during two summer seasons of 2018and 2019, at private farm at Salaka Village, El-Mansoura District, Dekhalia Governorate, Egypt to investigate the response of bean plants to foliar application with Trehalose and Nitric oxide via leaf sprays under late sowing date of 1 May. Soil texture of the experimental site was Clay-loam. Meteorological data (temperature ^{0}C, relative humidity % and Windspeed km/day) for May-October months in both years of Mansoura, Dekhalia Governorate, Egypt, during 2017and 2018 seasons were obtained from The Central Laboratory for Agricultural Climate (CLAC), Agricultural Research Center (ARC), Egypt and shown in Table 1.

Table 1: Average air temperature (ºC), relative humidity (%) and Windspeed km/day.

Month	2018				2019			
	Temperature ^{0}C		Humidity %	Windspeed km/day	Temperature ^{0}C		Humidity %	Windspeed km/day
	Minimum	Maximum			Minimum	Maximum		
May	19.1	33.0	41.8	239	16.3	29.6	54.4	243
June	22.2	35.7	44.0	251	19.2	33.4	56.0	243
July	22.6	38.4	50.6	200	20.5	37.5	56.0	215
August	22.4	35.4	52.8	219	20.7	36.1	60.8	191
September	20.2	33.7	58.0	191	19.4	34.6	60.0	175
October	16.7	31.3	54.4	171	18.8	32.6	59.2	191

Common bean seeds were obtained from the Vegetable Crops Seed Production and Technology Department, Horticulture Research Institute. Sowing was done on 1 st May in the two summer seasons of 2018 and 2019. The seeds were planted at 10 cm apart in a single row. Each experimental unit included 5 ridges, each of 0.75 m width and 5.0 m long, which resulting an area of 18.75 m^{2}. Other agriculture practices such as fertilization, weeding, pest and insect control were carried out as recommended for the conventional common bean planting.

Five Foliar treatments were tested; 1-control (sprayed with distilled water); 2- Nitric oxide at 40 ppm; 3- Nitric oxide at 80 ppm; 4- Trehalose at 40 ppm and 5- Trehalose at 80 ppm. All foliar treatments were applied thrice (3 times) at 3, 5 and 7 weeks after sowing. Treatments were arranged in Randomized Complete Block Design (RCBD).

Data Recorded

1-Vegetative growth characters

At flowering stage, ten random plants from each treatment were taken to measure plant height (cm), number of leaves/ plant, fresh weight/ plant (g), dry weight/ plant (g) and leaf area/plant (cm^{2}) (**Koller, 1972**).

2- Chemical analysis of leaf

At flowering stage, top fourth leaf from five random plants were picked up and subjected for determining total chlorophyll. A digital chlorophyll meter, Minolta SPAD-502 (Minolta Company, Japan) was used. The same leaves were dried, grinded and prepared for measuring nitrogen, phosphorus and potassium according to methods described by **Cottenie *et al.*, 1982**. Proline was determined according to the method of **Bates *et al.* (1973).**

3-Seed yield and its components

During the entire flowering and seed development period ten random plants from each treatment were labeled to estimate the setting percentage. At harvest stage (after ripening and pods drying), samples of fifteen random plants from each treatment were collected and used for recording seed yield parameters i.e number of seeds per pod, number of pods per plant, seeds yield per plot (kg) and total seed yield per hectare (ton).

4-Seed quality

Germination percentage and mean germination time (days) were carried out according to ISTA rules (**ISTA, 2015**). Weight of 100 seeds (seed index) (g) and seedling length of (cm) were also determined. Seedling vigor index (SVI) was calculated according to the equation of **Abdul-Baki and Anderson (1973)**.

Statistical analysis:

All the collected data were tabulated and statistically analyzed by Statistical Analysis of variance using MSTAT-C version 4, 1987 software and the treatments means were compared using the LSD test according to **Gomez and Gomez 1984.**

Bartlett's test was done according to **Bartlett (1937)** to test the homogeneity of error variance. The test was not significant for all studied traits, so, data of the two seasons were combined.

Results and Discussions:

1-Vegetative growth characters

Data presented in Table 2 indicated that all vegetative growth characters in terms of plant height, number of leaves per plant as well plant fresh and dry weights and plant leaf area were significantly affected by different foliar application treatments. Both nitric oxide and trehalose at different concertation improved vegetative growth characters markedly in compared to control treatment. The best results were obtained when plants were treated with nitric oxide at 80 ppm, followed by trehalose at 80 ppm then nitric oxide at 40 ppm then trehalose at 40 ppm and finally the lowest values of all characters obtained from control plants.

Heat stress primarily influences the rate of plant development, which increases to a certain point and diminishes afterward (**Wahid *et al.*, 2007**). Vegetative plant parts show various morphological symptoms in response to heat stress, such as scorching and sun burning of leaves, twigs, branches and stems, senescence of leaves followed by abscission, inhibition of shoot and root growth, and discoloration of fruits, which can severely reduce yield (**Bita and Gerats, 2013**). Heat stress also causes leaf wilting, leaf curling, leaf yellowing, and reduced plant height and biomass (**Siddiqui *et al.*, 2015**). Exposure of plants to severe high temperature often reduces shoot growth, root growth, root number, and root diameter (Xu et al., 2000). Heat stress severely affects vegetative growth in legumes such as bean (<30 °C); peanut (29 and 33 ^{0}C); pea (28–30 ^{0}C) and chickpea (22–25 ^{0}C). Heat stress results in water loss from cells, reduced cell size and growth, and hence reduced leaf area and biomass (**Davies *et al.*, 1999; Liu *et al.*, 2003**).

The desirable effects of spraying common bean plants with nitric oxide or trehalose may be attributed to their role on resisting multiple stress. Nitric oxide is effective in improving physiological processes in plants (**Desikan *et al.*, 2004**). In addition, trehalose plays a great role in remediated physiological and biochemical parameters and plant growth (**Zulfiqar *et al.*, 2021**). Also, trehalose an energy source and has unique physicochemical properties since it efficiently stabilizes dehydrated enzymes, proteins and lipid membranes (**Fernandez *et al.*, 2010**).

Table (2): Effect of irrigation foliar application treatments on bean vegetative growth characters

Characters/ Treatments	Plant height (cm)	Number of leaves/plant	Fresh weight (g/plant)	Dry weight (g/plant)	Leaf area (cm^2/plant)
Control	36.50	20.66	44.28	8.165	285.0
Trehalose 40 ppm	38.36	21.00	55.14	10.15	304.2
Trehalose 80 ppm	40.12	21.66	62.67	13.85	350.7
Nitric oxide 40 ppm	39.28	21.33	60.78	11.95	315.9
Nitric oxide 80 ppm	41.88	21.66	68.96	16.85	372.5
LSD 5 %	1.22	1.16	3.120	1.98	38.1

2-Chemical analysis

It is clear from the data in Table 3 that the mean values of total chlorophyll, proline, nitrogen, phosphorus and potassium% in leaves of bean plants were significantly affected by different foliar application treatments. Chlorophyll, nitrogen, phosphorus and potassium% behave the same trend and increased with the application of nitric oxide and trehalose. Nitric oxide at 80 ppm exhibited the highest values followed by trehalose at 80 ppm while nitric oxide at 40 ppm came at the third rank and trehalose at 40 ppm at the fourth rank while control plants recorded the lowest values. Proline had the opposite trend as the control treatment recorded the highest value and trehalose at 80 ppm recorded the lowest value.

Photosynthesis, a vital plant process that forms sole basis for all assimilates is highly vulnerable to heat stress (**Mathur *et al.*, 2014**). High temperature affects the physicochemical properties and functional organization of thylakoid membrane thus irreversibly damaging the chloroplast protein complexes including photosystem II (PSII) (**Brestic *et al.*, 2012**). Heat stress also induce some reversible effects such as increase in photorespiration, decreased activities of Calvin–Benson cycle enzymes. Both reversible and irreversible effects of heat often lead to yield penalty as well as longer impacts on plant metabolism (**Brestic *et al.*, 2016; Mathur *et al.*, 2014**). Hence, increasing photosynthetic efficiency by enhancing heat acclimation of photosynthetic apparatus is one of the most desirable future target (**Yamori *et al.*, 2014**). It is vital to understand the processes that limit the photosynthetic productivity under heat stress and the role of NO and trehalose in ameliorating these processes.

Uchida *et al.* (2002) reported that rice seedlings treated with low levels of H_2O_2 or NO allowed the survival of more green leaf tissue and resulted in higher quantum yield for photosynthesis II than in non-treated controls under salinity and heat stress. It was also exhibited that pretreatment induced not only ROS-scavenging enzyme activities but also expression of transcripts for oxidative stress-related gene encoding sucrose-phosphate synthase, Dpyrroline-5-carboxylate synthase, and small heat shock protein 26. These findings demonstrate that H_2O_2 and NO plays an important role in tolerance of rice seedlings to both salt and heat stress by acting as signal molecules for the response. **Song *et al.* (2006)** found that application of SNP and S-nitrose-N-acetylpenicillamine, both are NO donor, dramatically alleviated heat stress-induced ion leakage increase, growth suppression, and cell viability decrease in callus of reed under heat stress and also elevated the activities of SOD, APX, CAT, and POD. These results suggest that NO can effectively protect callus from oxidative stress induced by heat stress and that NO might act as a signal in activating active oxygen scavenging enzymes under heat stress.

During heat stress, trehalose pretreatment protects the ultrastructure of chloroplasts and some polypeptides in thylakoid membranes and also improves the photosynthetic capacity of thylakoids which indicates a protective role of trehalose or its metabolite for the thylakoid membrane. Under heat stress, proteins in the thylakoid membranes and the photosynthetic capacity were protected by trehalose pretreatment (**Luo *et al.* 2010**).

Table (3): Chlorophyll content, proline, nitrogen, phosphorus and potassium percentages of bean in response to foliar application treatments.

Characters/ Treatments	Chlorophyll	Proline (%)	N%	P%	K%
Control	14.72	11.77	3.003	0.498	1.193
Trehalose 40 ppm	17.30	10.58	3.122	0.510	1.255
Trehalose 80 ppm	20.25	10.05	3.491	0.522	1.347
Nitric oxide 40 ppm	18.12	10.36	3.302	0.514	1.313
Nitric oxide 80 ppm	20.72	9.725	3.597	0.537	1.360
LSD 5 %	0.96	0.22	0.115	0.017	0.038

3-Seed yield and its components

Data in Table 4 revealed that all seed yield and its components were significantly and positively affected by nitric oxide and trehalose foliar treatments.

Setting percentage, number of seeds per pod and number of pods per plant as major yield components improved markedly with different foliar applications. Nitric oxide at 80 ppm was the superior treatment in this regard and recorded an increment of 25.92, 61.28 and 36.07% over the control treatment for setting percentage, number of seeds per pod and number of pods per plant, respectively. Seed yield per plot and total seed yield per hectare had the same trend in their response to foliar application treatment. Nitric oxide at 80 ppm was the superior treatment in this regard as it increased seed yield per hectare by 30.34% over control treatment.

For establishment of seed, the pollen grains must interact with a receptive stigma, followed by pollen tube growth to reach the ovules for fertilization, and embryo and endosperm development (**Barnabás *et al.*, 2008**). Some of these events may be impacted by the adverse environmental conditions frequently encountered by crop plants (**Driedonks *et al.*, 2016**). High temperature may arrest fertilization by inhibiting the development of male **(Jain *et al.*, 2007**) and female gametophytes (**Snider *et al.*, 2009**). Reduced fertilization is a common problem associated with heat due to disruption of meiosis and fertilization in various species including beans.

Gross and Kigel (1994) reported that high temperatures of 27/32^{0}C at sporogenesis reduced pollen viability and yield in heat-sensitive genotypes of bean, due to failed anther dehiscence, pollen sterility, low pod and seed set. Temperature fluctuations during seed filling drastically reduce yield in legumes such as common bean (**Rainey and Griffiths, 2005**). Reductions in various yield attributes due to heat stress has been reported in common bean (**Vara Prasad *et al.*, 2002**; **Rainey and Griffiths, 2005**). Heat stress results in water loss from cells, reduced cell size and growth, and hence reduced leaf area and biomass. When growing conditions are favorable, plants continue vegetative growth without setting pods or filling fewer pods (**Davies *et al.*, 1999; Liu *et al.*, 2003**).

High yield losses have been reported in snap bean under heat stress (**Tsukaguchi *et al.*, 2003**). **Gutiérrez-Rodríguez *et al.* (2003)** studied the biomass and yield of bean plants raised in two different seasons, i.e., winter and summer, and found that the winter-sown crop had 41 and 38% higher biomass and yield, respectively than the summer-sown crop.

The increment of yield and its components achieved by foliar application of nitric oxide and trehalose is a good reflection of increased vegetative growth parameters, i.e., plant height, number of leaves, total fresh and dry weights and leaf area (Table 2) and chemical constituents of leaves as mineral contents (N, P and K), Chlorophylls (Table 3).

Table (4): Seed yield and its components of bean as affected by different foliar application treatments

Characters/ Treatments	Setting %	Number of seeds/pod	Number of pods/plant	Seed yield/plot (kg)	Seed yield/hectare (ton)
Control	48.25	4.330	14.00	3.924	2.093
Trehalose 40 ppm	54.75	5.000	15.75	4.442	2.369
Trehalose 80 ppm	58.70	6.330	17.75	4.946	2.638
Nitric oxide 40 ppm	56.70	6.000	16.99	4.672	2.492
Nitric oxide 80 ppm	60.76	7.000	19.05	5.115	2.728
LSD 5 %	3.18	0.89	0.85	0.012	0.091

4-Seed quality

Seed quality and its traits are major issue for the next year planting. Thus, it is very critical to have high quality seed for next year. Seed index (weight of 100 seed) and germination percentage were improved by applying nitric oxide (40 or 80 ppm) or trehalose (40 or 80 ppm) over the control treatment and NO at 80 ppm had the higher seed index and germination percentage. Mean germination time had a progressive reduction as a result of applying nitric oxide or trehalose at any concentration and NO at 80 ppm was the best treatment in this regard. Seedling length responded positively also to foliar treatment and NO at 80 ppm or trehalose at 80 ppm were the best treatments followed by NO at 80 ppm then trehalose at 80 ppm. Control treatment recoded the lowest value. Seedling vigor index increased with applying nitric oxide or trehalose compared with control. NO at 80 ppm recoded the highest values then trehalose at 80 ppm followed by NO at 40 ppm then trehalose at 40 ppm and control treatments (Table 5).

Elevated temperatures during seed filling and maturation can increase the proportion of seeds that are shriveled and abnormal at physiological maturity and result in seeds that exhibit reduced germination and seedling vigor in soybean (**Egli *et al.*, 2005**). Furthermore, in legumes such as soybean, heat stress leads to the retention of chlorophyll in mature seeds, which can reduce seed quality (**Teixeira *et al.*, 2016**). Low leaf photosynthetic rates during seed filling in heat-stressed plants are a major cause of reduced seed size (**Awasthi *et al.*, 2014**). The accumulation of various seed components (mainly starch and proteins) may be inhibited by heat stress due to inactivation of enzymatic processes involving starch (**Ahmadi and Baker, 2001**) and protein synthesis (**Triboï *et al.*, 2003**). Reduced seed weight was associated with reduced starch biosynthesis enzyme activities (ADP-glucose pyrophosphorylase and soluble starch synthase) in the endosperm during seed filling when the temperature increased above a threshold level (**Singletary *et al.*, 1994**). High temperature stress increased the percentage of shriveled seed and reduced seed size in common bean (**Vara Prasad *et al.*, 2002**).

Improvements in seed quality traits i.e seed index, germination percentage, mean germination time, seedling length and seed index vigor of common bean seeds after harvesting as a result of foliar spraying with nitric oxide and trehalose at different concentrations may be due to the role of nitric oxide in improving growth and dry matter accumulation and the benefit gained by foliar application with trehalose an essential signal metabolite in plants biosynthesis, carbon metabolism, linking growth and seed development (**Meitzel *et al.*, 2021**).

Table (5): Effect of foliar application treatments on bean seed quality

Characters Treatments	Seed index (g)	Germination %	Mean germination time (days)	Seedling length (cm)	Seed vigor index
Control	42.43	78.33	4.500	09.85	7.481
Trehalose 40 ppm	45.13	87.66	3.450	11.15	9.774
Trehalose 80 ppm	46.90	89.33	2.850	14.30	12.77
Nitric oxide 40 ppm	46.03	88.66	3.010	13.20	11.70
Nitric oxide 80 ppm	48.20	90.33	2.250	15.50	14.00
LSD 5 %	1.34	1.07	0.44	0.96	1.03

From the fore-going results, it is possible to infer that exogenous application through nitric oxide or trehalose play a critical role in alleviating the adverse effects of high temperature by regulating a myriad of physio-biochemical plant processes. Furthermore, more research work still needed in this regard.

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