



Monitoring Nutrient Uptake of Chard (*Beta vulgaris* var. *cicla* L.) Exposed to Exogenously Applied Nitric Oxide under Drought Stress

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ABSTRACT: Nutrient uptake of chard under well-watered and drought conditions with different NO applications were investigated in this research. NO solutions were prepared with four doses (0, 50, 100, 150 and 200 µM) of sodium nitroprusside as a nitric oxide source and exogenously applied on only seeds (s) or together with seed and foliar (sf) of chard under different levels of drought according to 100% (control), 67% and 33% of the water required to reach field capacity. Nutrient uptake drastically decreased under drought stress. However, progressive effects of NO applications on nutrient uptake have been observed under both well-watered conditions and under drought. The increase in N and P uptake of plant were significant especially for doses of 100 and 150 µM for both s and sf applications. The highest Fe, Cu and Zn content were obtained from 150 µM sf NO application, while the highest Mn and B were obtained from 150 µM s and 200 µM sf NO application in 67% level. However, in 33% level the highest Cu and B content were obtained from 150 µM sf NO application, while the highest Mn and Zn obtained from 100 µM sf and the highest Fe obtained from the 200 µM s NO application.

Keywords: Water deficit, Plant nutrients, Nitric oxide

Kuraklık Stresi Altında Dışarıdan Nitrik Oksit Uygulanan Pazıda (*Beta vulgaris* var. *cicla* L.) Besin Alımının İzlenmesi

ÖZ: Bu çalışmada farklı NO uygulamaları altında tam sulanmış ve kurak koşullarda yetiştirilmiş pazı bitkisinin besin maddesi alımı incelenmiştir. Uygulamalar, bitki kök bölgesinde tarla kapasitesine ulaşmak için gerekli suyun %100 (kontrol), %67 ve %33'ünün sağlandığı kuraklık seviyelerinde, nitrik oksit donörü olarak dört doz (0, 50, 100, 150 ve 200 µM) sodyum nitroprussid ile NO çözeltilerinin kullanıldığı çözeltilerle sadece tohum (s) veya tohum ve yapraklara (sf) olmak üzere dışarıdan uygulanmıştır. Kuraklık stresi altında besin alımı önemli ölçüde azalmıştır. Bununla birlikte, NO uygulamalarının besin alımı üzerindeki geliştirici etkileri hem tam sulanan koşullar hem de kurak koşullar altında gözlemlenmiştir. Bitkinin N ve P alımındaki artış, özellikle hem s hem de sf uygulamaları için 100 ve 150 µM'lik dozlarda önemli olmuştur. % 67 sulama seviyesinde en yüksek Fe, Cu ve Zn içeriği 150 µM sf NO uygulamasında olurken, en yüksek Mn ve B ise 150 µM s ve 200 µM sf NO uygulamasından elde edilmiştir. Ancak %33 seviyesinde en yüksek Cu ve B içeriği 150 µM sf NO uygulamasından elde edilirken, en yüksek Mn ve Zn 100 µM sf'den, en yüksek Fe ise 200 µM s NO uygulamasından elde edilmiştir.

Anahtar Kelimeler: Su kıstı, Bitki besinleri, Nitrik oksit

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INTRODUCTION

Abiotic stresses, such as high salinity, low or high temperature, deficient or excessive water, and heavy metals reduce the average yield for most of the plants by more than 50% (Bray et al., 2000). These stress factors are posing a severe hazard to the ecosystem and agriculture, accounting for great economic loss (Wania et al., 2016). Among the various abiotic stress conditions, drought stress is the most devastating factor. Plants respond to drought stress at multiple levels such as metabolic processes of the plant (Liu et al., 2016), plant growth (McWilliams, 2003; Ekinci et al., 2015; Sahin et al., 2015), photosynthetic characteristics (Yang et al., 2000; Farooq et al., 2009; Wu et al., 2014) and WUE (Xue et al., 2006; Qiu et al., 2008; Liu et al., 2016). Plant nutrient uptake and their transport gradually decrease under drought conditions (Heidari and Karami, 2014). Studies have shown that drought can decrease nutrient uptake from soil, the decrease in concentration of nitrogen (N) and phosphorus (P) in plant tissue under drought stress were stated earlier (Waraich et al., 2011; He and Dijkstra, 2014). Understanding the physiology of stress responses and then transfer this to develop a molecular understanding on mechanisms of drought tolerance in plants is crucial. Using different solutions to examine the mechanism of foliar spraying is not a new technique. Recently exogenous application of nitric oxide (NO) used to improve stress tolerance in plants thanks to its critical role in plant starting from germination to, ripening of fruit and senescence of organs at all the stages through growth and development (Arasimowicz and Floryszak-Wieczorek, 2007; Siddiqui et al., 2011).

Responses of different plants such as cowpea (Anyia and Herzog, 2004), maize (Aslam et al., 2013), cucumber (Abd El-Mageed and Semida, 2015), squash (Ors et al., 2016), and tomato (Pazzagli et al., 2016) to drought stress have been studied earlier. Chard's response to drought stress found significant regarding to plant physiological parameters. Severe drought conditions are not sustained by plants, though mild stress slightly decreased plant fresh weight (Ekinci et al. 2020). Our objective in this study was to investigate the ameliorative effect of exogenous application of NO on the drought tolerance of the chard plant, regarding to nutrient uptake. To expand our understanding of chard responses to drought, we examined the effects of drought on the nutrient-uptake specifically focusing on N and P.

MATERIAL AND METHOD

We conducted a pot experiment in the greenhouse conditions with chard (*Beta vulgaris* var. *cicla* L.). The controlled greenhouse had an average

temperature of 20 (\pm 2) °C and humidity were around 35 (\pm 5) %. Substrate (2:1:1 v/v, soil: cattle manure fertilizer: sand) were placed in 2 L plastic pots and then the seeds were sown in.

NO solutions were prepared with four different doses (0, 50, 100, 150 and 200 μ M of sodium nitroprusside as a nitric oxide donor) and exogenously applied on only seeds (s) or together with seed and foliar (sf) of chard. Seed disinfection, foliar application of the seed and plant were applied as in Ekinci et al. (2020).

Drought treatments applied as according to 100% (control, D0), 67% (D1) and 33% (D2) of water to reach the field capacity at each irrigation event. Water retained at field capacity was determined as in Ekinci et al. (2020).

Each pot was initially planted with 6 seed then thinned to 3 seedlings per pot in the 4-leaf stage. Drought treatments were implemented at 6 to 8 leaf stage. The pots were arranged completely randomized and replicated with 5 pots. Soil moisture was measured by wet sensor (type WET-2, Delta-T Device Ltd, Cambridge, England) to maintain drought treatments. The irrigation was performed every three days until the harvest.

The samples of leaf have been taken in the harvest period and dried at oven at 68 °C for 48 h and passed 1 mm sieve size. The Kjeldahl method and a Vapodest 10 Rapid Kjeldahl Distillation Unit (Gerhardt, Königswinter, Germany) were used to determine total nitrogen (N) (Bremner, 1996). Macro and micro-elements were determined after wet digestion (HNO₃-H₂O₂ acid mixture (2:3 v/v)) of dried and ground sub-samples in microwave digestion (Bergof Speedwave Microwave Digestion Equipment MWS-2), by using an Inductively Couple Plasma spectrophotometer (Perkin-Elmer, Optima 2100 DV, ICP/OES, Shelton, CT 06484-4794, USA) (Mertens, 2005a; 2005b).

The experimental design was randomized complete block design with three replications. The differences among the means of applications were compared using the Duncan multiple tests (SPSS, 2010).

RESULTS AND DISCUSSION

The mineral content of chard leaves was affected significantly by drought applications. Under well-watered conditions only Na and Zn were significantly altered by NO applications, however the results of interacted drought and NO applications were statistically significant for all mineral contents. The Mg, Ca, Fe, Cu, Mn, Zn and B content of chard leaves decreased while Na content increased with the highest drought stress (Table 1).

Table 1. Effects of seed (s) or seed and foliar (sf) applications with different NO doses on leaf mineral content of chard under drought treatments (D0: control 100 %; D1: 67 % and D2: 33 % of the water required to reach field capacity).

Drought levels	NO (µM)	Mg mg kg ⁻¹	Na mg kg ⁻¹	Ca mg kg ⁻¹	Fe mg kg ⁻¹	Cu mg kg ⁻¹	Mn mg kg ⁻¹	Zn mg kg ⁻¹	B mg kg ⁻¹
D0	0	1972.2 ^{ns}	575.99 ^{**}	11860.0 ^{ns}	277.37 ^{ns}	55.34 ^{ns}	65.60 ^{ns}	44.54 ^{d**}	31.44 ^{ns}
	50 S	2073.3	541.88 ab	11394.7	282.56	55.24	66.31	46.94 bcd	30.38
	50 SF	2226.3	503.64 bc	11294.5	287.86	55.86	69.65	45.89 bcd	29.68
	100 S	2169.0	518.21 bc	11185.9	289.20	56.66	70.14	49.83 a	30.60
	100 SF	2207.7	486.24 c	12382.6	287.45	57.23	69.31	48.36 ab	31.33
	150 S	2252.0	496.06 c	12080.2	288.51	58.54	68.65	47.85 abc	32.39
	150 SF	2294.4	480.28 c	11485.5	290.29	54.39	72.21	47.74 abc	28.66
	200 S	2428.0	490.95 c	10979.4	292.30	55.36	70.53	45.44 cd	29.01
	200 SF	2233.5	517.49 bc	11554.2	272.58	57.13	66.50	45.94 bcd	30.63
	Mean	2206.3 A***	512.30 C***	11579.7A***	285.35 A***	56.19 A***	68.77 A***	46.95 A***	30.46 A***
D1	0	1334.4 b***	654.44 a***	7717.8 c**	185.40 b***	36.43 e***	45.13 cd***	33.60 b***	18.95 d***
	50 S	1376.1 b	657.54 a	8442.0 bc	194.17 b	39.38 d	46.77 cd	35.37 b	21.42 cd
	50 SF	1404.7 b	659.57 a	8799.0 abc	192.95 b	41.05 cd	43.57 d	34.74 b	21.50 cd
	100 S	1347.4 b	646.95 a	8957.7 ab	192.29 b	42.75 c	46.05 cd	33.38 b	24.14 bc
	100 SF	1631.2 a	520.18 b	9071.8 ab	222.58 a	45.51 b	50.62 bc	38.91 a	21.58 cd
	150 S	1613.0 a	510.00 b	9095.0 ab	219.11 a	46.57 b	58.38 a	38.92 a	26.77 ab
	150 SF	1655.4 a	499.40 b	9757.7 a	232.12 a	50.68 a	53.60 ab	40.21 a	25.87 ab
	200 S	1579.5 a	509.31 b	8573.8 bc	219.08 a	46.44 b	57.56 a	38.01 a	25.86 ab
	200 SF	1580.6 a	516.80 b	9876.5 a	221.24 a	51.48 a	51.64 abc	38.65 a	27.95 a
	Mean	1502.48 B	574.91 B	8921.3 B	208.77 B	44.48 B	50.37 B	36.86 B	23.78 B
D2	0	968.9 c***	727.14 a***	5742.4 d**	141.63 g***	27.06 e***	31.04 d**	24.82 e***	13.96 d***
	50 S	1009.5 e	687.47 b	5848.1 cd	153.96 ef	27.76 e	33.48 cd	26.58 cd	15.47 cd
	50 SF	1103.2 cd	625.82 c	5773.5 cd	165.58 d	28.21 e	35.78 abc	27.80 bc	13.77 d
	100 S	1172.6 bc	604.48 cd	6166.5 bcd	176.82 bc	29.54 d	36.76 ab	29.48 a	15.46 cd
	100 SF	1206.3 ab	601.06 cd	6528.0 abc	169.68 d	30.94 c	37.22 a	29.57 a	17.61 b
	150 S	1209.2 ab	616.07 cd	6766.4 ab	182.18 ab	33.05 b	33.29 cd	28.54 ab	19.69 a
	150 SF	1265.4 a	581.54 d	6695.8 ab	161.99 de	34.78 a	35.88 abc	27.70 bc	20.69 a
	200 S	1215.6 ab	605.79 cd	6528.1 abc	190.43 a	31.25 c	33.91 bcd	28.69 ab	17.54 b
	200 SF	1081.7 d	627.98 c	7162.4a	152.66 f	34.60 a	35.87 abc	25.36 de	17.36 bc
	Mean	1136.92 C	630.81 A	6356.8 C	166.11 C	30.80 C	34.80 C	27.62 C	16.84 C
DXNO Interaction	P>0.05	P<0.001	P>0.05	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001

*: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$, ns: $p > 0.05$; Values followed by the same small or capital letters are not significantly different within the columns in every drought treatments (D0, D1 and D2)

The Na content of the chard leaves increased 14% in D1 and 26% in D2 drought stress levels. Although Na content increased with drought stress, NO applications caused a significant decrease in Na content. The Ca, Fe, Cu, Mn, Zn and B content of leaves decreased almost 50% in D2 as compared to D0 (control treatment) and this reduction was around 35% in D1. These reductions might be related to drought stress levels, which are subjected to 100% of water to reach the field capacity (D0; control), and 67% (D1) and 33% (D2) of the given water in control treatment (D0). Results revealed that mineral content and the nutrient uptake by the roots of the chard leaves significantly decreased under drought stress due to the decline in soil moisture. Drought stress gradually can cause a reduction or imbalance in nutrient uptake and their transport (Heidari and Karami, 2014).

The highest Fe, Cu and Zn content were obtained from 150 μM sf NO application, while the highest Mn and B were obtained from 15 μM 0 s and

200 μM sf NO application in D1. However, in D2 the highest Cu and B content were obtained from 150sf NO application, while the highest Mn and Zn obtained from 100 μM sf and the highest Fe obtained from the 200 μM s NO application. NO are involved in many processes in plant such as primary and lateral root growth (Kopyra and Gwóźdz, 2004). Sun et al. (2017) indicated that the stimulation or inhibition of root growth is related to NO doses, and NO treatments affect nutrient fluctuation in plants. Earlier studies reported that exogenous NO treatments induced root growth under stress conditions (Zhao et al., 2007; Meng et al., 2012; Manoli et al., 2014; Trevisan et al., 2014).

Under well-watered conditions N was significantly altered by NO applications, and the results of interacted drought and NO applications were statistically significant. NO increased the percentage of N in the plant especially in some application doses regardless of seed or seed and foliar application together (Figure 1).

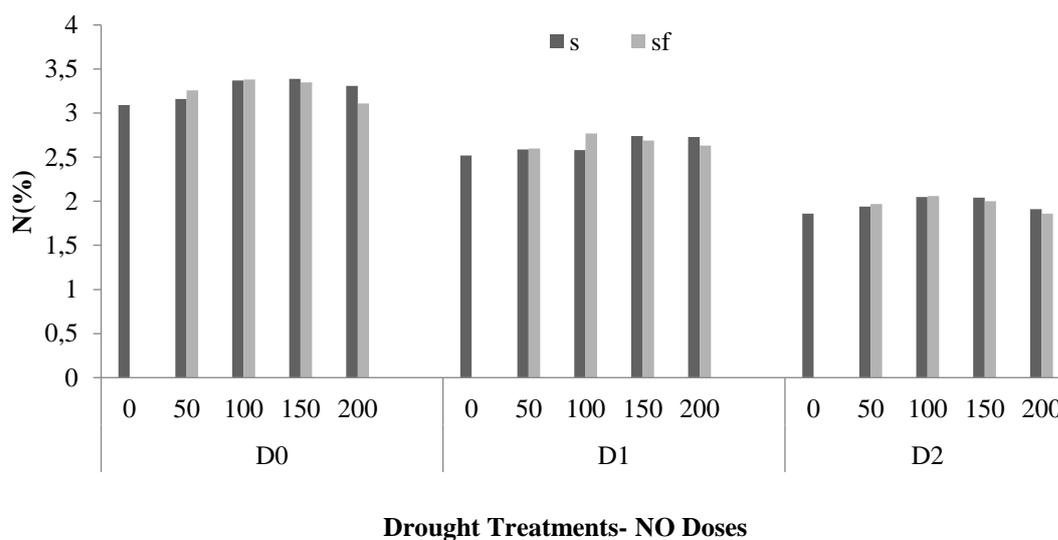


Figure 1. Effects of seed (s) or seed and foliar (sf) applications with different NO doses on leaf N content of chard under drought treatments (D0: control 100%; D1: 67% and D2: 33% of the water required to reach field capacity).

The total amount of N decreased roughly 18% in D1 and 40% in D2 drought treatments as compared to D0. The highest N content of leaves was obtained 100 μM sf in all drought levels. Sun et al. (2017) also reported that NO regulates N distribution and uptake in many plant species.

The P content decreased 34% in D1 and 48% in D2, however, it increased with NO applications under all drought levels. The highest P content was obtained from 150 μM sf application in D0 treatments (Figure 2).

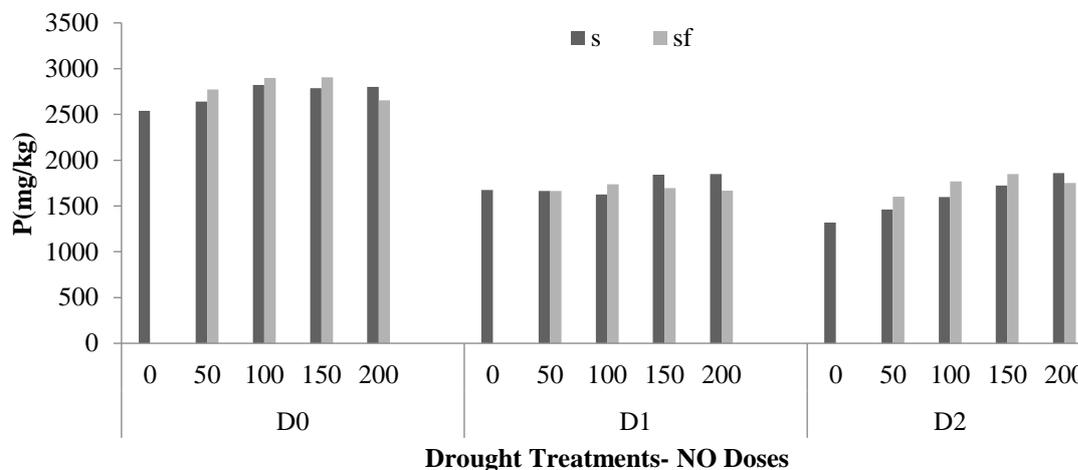


Figure 2. Effects of seed (s) or seed and foliar (sf) applications with different NO doses on leaf P content of chard under drought treatments (D0: control 100%; D1: 67% and D2: 33% of the water required to reach field capacity).

Decreased soil moisture levels may cause a reduced rate of nutrient diffusion from the soil to the root surface (Pinkerton and Simpson, 1986; Sahin et al., 2018). Previous studies have shown that drought can reduce nutrient uptake from soil especially the concentration of nitrogen (N) and phosphorus (P) in plant tissue (Waraich et al., 2011; He and Dijkstra, 2014). Moreover, Kramer and Boyer (1995) stated that a reduced root adsorbing power of plants occurs since the nutrient transport from the roots to the shoots is restricted by the reduced transpiration rates. We also have observed reduced transpiration rates under drought treatments; this can be another reason to observe the reduced mineral uptake by plants. As a result, the reduced nutrient availability under drought is one of the most significant factors for plant growth. The reduction of water availability in plants with drought stress prevents the intake of nutrients required for the plant.

CONCLUSION

This study aimed to investigate chard (*Beta vulgaris* var. *cicla* L.) nutrient uptake by applying NO solely on the seeds or together with seed and foliar applications under different levels of drought. NO applications positively induced nutrient uptake and its effect was favorable under well-watered conditions as well. The results were more significant with 100 and 150 μM doses of NO applications in all drought treatments including well-watered conditions. Our results indicated that optimized dose of NO, depending on drought stress severity, could have an important ameliorating effect in response to nutrient uptake of chard.

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Statement of Conflict of Interest

Authors have declared no conflict of interest.

Authors' Contributions

ME, SO and EY conceived and designed research. ME, SO, EY, MT, AD and US set up and conducted the experiment. ME, SO and EY wrote the manuscript. All authors read and approved the final manuscript.

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