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Research Article

Ameliorative Effects of Exogenously Applied Nitric Oxide on Seed Germination and Seedling Growth of Purslane Under Drought and Salinity

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Abstract

We investigated the effects of exogenously applied nitric oxide (NO) on seed germination and seedling properties of purslane (*Portulaca oleracea* L.) under different levels of drought and salinity. The seeds were soaked in different SNP (sodium nitroprusside as a NO donor) doses (0, 50, 100, 150 and 200 µM) during 12 h. Drought and salinity stress were generated by additions of PEG 6000 or sodium chloride to medium. Water potentials were: 0, -0.20, -0.75, and -1.53 MPa for both stresses. The seeds were allowed to germinate at 25oC, germination percentage, germination speed, main daily germination, root and shoot length were determined at the end of the experiment. Germination and seedling properties were inversely proportional to the concentrations of NaCl and PEG, thus purslane showed a reduction in germination and seedling growth with increased NaCl or PEG concentrations, but the reduction in PEG treatment were higher than NaCl treatment. However, exogenously NO treatments alleviated the negative impact of stress conditions for germination and seedling characteristics. 50, 100 and 200 µM doses of NO were the most efficient treatments on ameliorating the deleterious effects of salinity and drought. It can be concluded that NO applications may reduce the negative effects of moderate and high salt and drought stress during germination and early growth stage.

Keywords: Abiotic Stress; Water Deficit; Salinity; Nitric Oxide; Germination; *Portulaca Oleracea*

Introduction

Abiotic stress factors affect plant growth significantly and cause significant decreases in productivity and quality in agricultural produce. Environmental stress factors such as drought, salinity, low and high temperatures are important abiotic stresses which limit agricultural production [1]. The most important stress factors that cause damage to agricultural production are known as drought and salinity in the world [1,2].

High salt concentration causes osmotic and ion stress in plants and increase the leakage of electrons from the transport of electrons in chloroplasts and mitochondria and causes excessive production of reactive oxygen species (ROS) in plants [3-5]. Salinity reduces the growth rate resulting in smaller leaves, shorter lengths and sometimes fewer leaves [1]. Drought conditions have a negative effect on photosynthetic activity, cause a change in chlorophyll content and components in the cell, decrease plant root and shoot growth, inhibit photochemical activities and decrease the activity of enzymes in Calvin circle [6-10].

NO, an important signal molecule, is a product of nitrogen metabolism known as a stress regulator in signal transduction pathways [11]. NO is stated to have multiple biological roles in developmental processes such as germination, root organogenesis, stoma closure, flowering and senescence [12]. In addition, NO was found to be involved in environmental responses such as drought, temperature, salinity, heavy metal and UV-B radiation in plants [2,13-18].

NO decreases the oxidative damage caused by salinity in seedlings and increases the growth in the seedlings and dry weight under salt and drought stress [2,19,20]. In addition, the researchers reported that the pretreatment of NO in plants under stress conditions increases the total soluble protein, effectively contributing to a better balance between carbon and nitrogen metabolism [20,21]. NO affects the downstream of ABA in the adaptation reaction to water-deficiency stress by inducing stoma closure [22]. NO increases the capacity of antioxidants, stability of cellular membranes and improves the photosynthetic capacity [23].

Purslane is providing both novel biologically active substances and essential compounds for human nutrition [24]. The purslane plant was found to be 6.3 dS m^{-1} (EC) threshold and moderately tolerant to salinity [25]. Also, it was determined that purslane is a drought-tolerant plant [24]. Purslane outstanding tolerance to chloride salinity makes this species a promising halophyte candidate for saline agriculture [26].

The current scientific literature demonstrates that NO usage is one-way to improve the plant's tolerance on stress conditions. However, the effect of NO on seed germination and seedling growth of purslane under drought and salinity stress conditions has yet not been well studied. Thus, this experiment focuses on the effect of the exogenous seed application of NO on seed germination and seedling growth properties of purslane under saline and growth conditions.

Material and Methods

Purslane (*Portulaca oleracea* L.) was used as a plant material. One percent sodium hypochlorite was used for seed disinfection about two minutes, and then seeds were washed with distilled water immediately. Seeds were soaked in different SNP (sodium nitroprusside) solutions (0, 50, 100, 150 and 200 μM) for 12 hours in a germinator with a temperature of 23 ± 2°C. Treated seeds were dried on the papers under room temperature. Fifty disinfected seeds treated with NO were germinated in 2 folds of Whatman No. 1 filter paper (sterilized) which were placed in Petri dishes (15 cm diameter). Each dish was moistened with 10 ml solutions containing polyethylene glycol (PEG) 6000 or sodium chloride (NaCl) solutions with osmotic potential 0, -0.20, -0.75 and -1.53 MPa. In the control group only, distilled water was used.

The germination study was carried out with 4 replications and 50 seeds were used in each replication [27]. Seeds were allowed to germinate at 25°C. Germination percentage (%) and germination speed [28], mean daily germination [29], root length (mm) and shoot length (mm) were measured at the end of the study (14 day). The germination percentage values were transformed to arc sin before ANOVA. The data were analyzed by means of Duncan multiple comparison test [30].

Results

Effects of NO treatments on germination speed, germination percentage and daily mean germination of purslane seeds under drought and salinity conditions are given in table 1. The effect of salinity, drought and NO applications on seed germination speed was found to be statistically significant ($p < 0,001$). With increasing salinity and drought severity, the germination speed values increased, and germination occurred later in the highest stress conditions than the control. According to the averages, the latest germination was in the 0 μM NO application, while the earliest germination occurred in 200 μM NO application in salinity conditions. On the other hand, in drought conditions the latest germination was in the 0 μM NO application, while the earliest germination occurred in 100 μM NO (Table 1). The 50 and 100 μM NO applications resulted in faster germination at -0.20 MPa and -0.75 MPa salt stresses, respectively. In drought conditions, the 150 μM NO applications provided the earliest germination at -0,20 MPa and -0,75 MPa stress, while 100 μM NO application showed faster germination at -1,53 MPa drought stress (Table 1).

The effect of salinity, drought and NO applications on seed germination percentage was found to be statistically significant ($p < 0,001$). With increasing salinity and drought, the germination percentage decreased in the highest stress conditions. According to the averages, the highest germination percentage was in the 150 μM NO application, while the lowest germination percentage occurred in 0 μM NO application in salinity stress conditions. On the other hand, in drought conditions the highest germination percentage was in the 100 μM NO application, while the lowest germination occurred in 200 μM NO (Table 1). The highest germination percentage was obtained from 150 μM NO application at -0.20 MPa and -0.75 MPa salt stresses and 50 μM NO application at -1,53 MPa salt stress condition. On the other hand, the highest germination percentage was obtained from 100 μM NO application at -0.20 MPa, 50 μM NO application at -0.75 MPa salt stresses and 50 μM NO and 100 μM NO applications at -1,53 MPa drought stress condition (Table 1).

The effects of applications on mean daily germination of purslane seed were shown in table 1. Increased levels of salt and drought reduced mean daily germination. The highest mean daily germination was obtained 150 μM NO application for salinity and 100 μM NO application for drought conditions (Table 1). In this study, the highest mean daily germination occurred in150 μM NO application at -0.20 MPa and -0.75 MPa salt stress levels, while the highest mean daily germination was obtained from 100 μM NO ap-

Germination Speed							
Stress	**Doses**	**0-NO**	**50-NO**	**100-NO**	**150-NO**	**200-NO**	**Mean**
Salt	0	1,37 d	1,31 d	1,29 d	1,18 d	1,32 d	1,29 C
	-0,20 MPa	1,35 d	1,15 d	1,30 d	1,19 d	1,17 d	1,23 C
	-0,75 MPa	1,86 c	1,78 c	1,69 c	1,84 c	1,74 c	1,78 B
	-1,53 MPa	3,81 b	3,86 b	4,29 a	3,89 b	3,82 b	3,93 A
Mean		2,10 ns	2,02	2,14	2,03	2,01	
Application x Salt		P < 0,001					
Drought	0	2,27 d	1,80 def	1,72 def	1,99 def	1,81 def	1,92 C
	-0,20 MPa	1,83 def	1,66 ef	1,56 f	1,52 f	1,64 ef	1,64 D
	-0,75 MPa	2,26 d	2,17 de	2,26 d	2,05 def	2,17 de	2,18 B
	-1,53 MPa	4,83 a	4,68 a	3,25 c	3,94 b	4,00 b	4,14 A
Mean		2,80 A	2,58 AB	2,20 C	2,37 BC	2,41 BC	
Application x Drought		P < 0,001					
Germination Percentage (%)							
Salt	0	94,00 bcd	96,00 ab	98,00 a	97,00 ab	95,00 bc	96,00 A
	-0,20 MPa	93,50 bcd	93,50 bcd	95,50 abc	96,00 ab	94,50 bc	94,60 B
	-0,75 MPa	90,50 e	91,00 de	91,50 cde	94,50 bc	91,00 de	91,70 C
	-1,53 MPa	77,00 f	78,00 f	77,00 f	77,00 f	75,00 f	76,80 D
Mean		88,75 C	89,63 BC	90,50 B	91,13 A	88,88 BC	
Application x Salt		P < 0,05					
Drought	0	94,50 cd	96,00 bc	98,50 a	95,00 bc	98,00 ab	96,40A
	-0,20 MPa	94,00 cd	95,50 bc	97,50 ab	95,00 bc	94,00 cd	95,20 A
	-0,75 MPa	93,00 cd	95,50 bc	92,00 cd	95,00 bc	90,00 d	93,10 B
	-1,53 MPa	9,00 ef	12,00 e	12,00 e	8,00 ef	6,00 f	9,40 C
Mean		72,63 C	74,75 AB	75,00 A	73,25 BC	72,00 C	
Application x Drought		P < 0,05					
Mean Daily Germination							
Salt	0	6,71 a-d	6,86 ab	6,86 ab	6,93 a	6,79 ab	6,83 A
	-0,20 MPa	6,68 b-e	6,64 b-e	6,82 ab	6,86 ab	6,75 abc	6,75 A
	-0,75 MPa	6,46 ef	6,50 def	6,54 c-f	6,68 b-e	6,43 f	6,52 B
	-1,53 MPa	5,50 g	5,43 g	5,50 g	5,50 g	5,36 g	5,46 C
Mean		6,34 B	6,36 B	6,43 AB	6,49 A	6,33 B	
Application x Salt		P < 0,05					
Drought	0	6,64 cd	6,82 abc	7,04 a	6,79 abc	6,43 d	6,74 A
	-0,20 MPa	6,71 bc	6,82 abc	6,96 ab	6,78 abc	6,71 bc	6,80 A
	-0,75 MPa	6,64 cd	6,82 abc	6,57 cd	6,78 abc	7,00 a	6,76 A
	-1,53 MPa	0,61 ef	0,86 e	0,86 e	0,61 ef	0,43 f	0,67 B
Mean		5,15 B	5,33 A	5,36 A	5,24 AB	5,14 C	
Application x Drought		P < 0,001					

Table 1: The effects of NO applications on germination properties of purslane under salinity and drought conditions.

ns: non-significant, different lower case letters indicate differences related to NO and stress applications, and different capital letters indicate differences related to means.

plication at -0.20 MPa, with 200 µM NO application at -0.75 MPa drought stress levels (Table 1).

The effects of applications on seedling root and shoot length under stress conditions were given in table 2. The severe drought and salt stress have caused a decrease in seedling root and shoot length. The root and shoot lengths could not measure at the highest level of drought stress factor (-1.53 MPa level). The effect was found statistically important ($p < 0,001$) for both stress factors.

Root Length (mm)							
Stress	**Doses**	**0-NO**	**50-NO**	**100-NO**	**150-NO**	**200-NO**	**Mean**
Salt	0	2,95 b	3,14 a	2,95 b	3,03 ab	2,70 c	2,95 A
	-0,20 MPa	2,36 de	2,67 c	2,24 e	2,50 d	1,93 f	2,34 B
	-0,75 MPa	1,24 h	1,91 f	1,30 h	1,60 g	1,57 g	1,52 C
	-1,53 MPa	0,22 ı	0,28 ı	0,14 ı	0,13 ı	0,18 ı	0,19 D
Mean		1,69 C	2,00 A	1,66 CD	1,81 B	1,59 D	
Application x Salt		P < 0,001					
Drought	0	3,68 abc	3,96 ab	3,48 bcd	3,42 cd	4,10 a	3,73 A
	-0,20 MPa	3,63 abc	3,94 ab	3,27 cd	3,21 cd	4,01 a	3,61 A
	-0,75 MPa	2,22 g	2,71 ef	2,34 fg	2,51 fg	3,08 de	2,57 B
	-1,53 MPa	-	-	-	-	-	-
Mean		3,18 B	3,53 A	3,03 B	3,05 B	3,73 A	
Application x Drought		P < 0,05					
Shoot Length (mm)							
Salt	0	2,64 ab	2,72 a	2,71 ab	2,68 ab	2,55 bc	2,66 A
	-0,20 MPa	2,61 ab	2,65 ab	2,45 c	2,56 abc	2,44 c	2,54 B
	-0,75 MPa	2,00 de	1,92 e	1,86 e	1,99 e	2,15 d	1,98 C
	-1,53 MPa	0,69 f	0,78 f	0,62 f	0,68 f	0,64 f	0,68 D
Mean		1,99 AB	2,02 A	1,91 AB	1,97 AB	1,94 AB	
Application x Salt		P < 0,01					
Drought	0	2,26 b	2,42 ab	2,55 a	2,64 a	2,42 ab	2,46 A
	-0,20 MPa	2,03 c	1,67 ef	1,90 cd	1,99 c	1,85 cde	1,89 B
	-0,75 MPa	1,23 h	1,63 efg	1,41 gh	1,56 fg	1,70 def	1,51 C
	-1,53 MPa	-	-	-	-	-	
Mean		1,84 C	1,91 BC	1,95 ABC	2,06 A	1,99 AB	
Application x Drought		P < 0,001					

Table 2. The effects of NO applications on root and shoot length of purslane on salinity and drought conditions.

Different lower case letters indicate differences related to NO and stress applications and different capital letters indicate differences related to means.

NO applications had important impact on seedling root and shoot length. For salinity condition, the highest root length was obtained from 50 µM NO application for all stress levels. On the other hand, for drought condition the highest root length was occurred in 200 µM NO for -0.20 MPa and -0,75 MPa levels. 50 µM NO application showed the highest shoot length at -0.20 MPa and -1.53 MPa salinity levels, while 200 µM NO application gave the highest shoot length at -0,75 MPa drought levels.

Discussion

Salinity and drought stress conditions hindered the investigated parameters in this experiment. Germination properties of purslane seeds were adversely affected by salinity and drought stress. Salinity and drought stresses are physiologically related, because both induce osmotic stress and most of the metabolic responses of the affected plants are similar to some extent. Both stress conditions may cause significant reductions in the rate and final percentage of germination [31]. Similarly, drought and salinity stress conditions negatively affected the shoot and root length. At zero potential, both shoot and root lengths reached their highest values. All other treatments gradually reduced the seedling growing. There are a lot of studies showing that stress conditions lead to decrease in growth properties of crops [7,9]. The first measurable effect resulting from drought and salt stress is the decline in growth. This is due to the cessation of cellular expansion. Cellular expansion process has been reported to be susceptible to salinity and drought stress conditions [32].

To investigate the interactive role of NO on the drought and salinity, we applied 50, 100, 150 and 200 μM NO, and found that NO treatments mitigated the adverse effect of stress conditions on germination and seedling growth. As a result of the study, the effect of NO applications in the purslane germination attributes is not much in stress-free conditions. Exogenous NO has a strong stimulating effect on seed germination under stress or non-stress conditions [33]. Similarly, it was observed NO treatments on seed alleviated stress conditions on germination properties in different crops by enhancing antioxidant capability [3,34]. Exogenous NO application has effective treatment to protect plants from oxidative damage under salt and drought stress by increasing various antioxidant defense enzymes activity (SOD, APX, CAT and GR) [3,35].

The purslane seeds are relatively salinity and drought tolerant, but especially -1.53 MPa severity of salinity and drought caused significant reductions on germination and root and shoot length of seed. However, 50 μM doses of NO at the most severe salt level and 50 and 100 μM doses of NO at the most severe drought level mitigated the negative impact of stress. In terms of root and shoot lengths, the negative effect of stress was less in 50 μM NO application at the highest salinity and in 50 μM and 200 μM NO applications at the highest level of drought as compared to the other applications. This situation can be explained by the fact that NO has antioxidant properties and can act as a signal in activating ROS-scavenging enzyme activities under stress [20]. In addition to the regulatory role in some physiological processes such as germination, flowering, fruit ripening and senescence, NO plays a protective role against different abiotic stresses such as drought, salinity, temperature [36,37].

In the study, it was found out that drought is more deleterious than salinity on observed parameters of purslane. Root and shoot measurement could not be performed in level of -1.52 MPa of drought. This also shows that purslane is more resistant to salinity than drought during germination and seedling. Kumamoto., et al. [25] reported that the purslane was moderately tolerant to salinity. And also, the researchers suggest that purslane could be used to rehabilitate saline soils and halophyte candidate for saline agriculture [26,38,39].

Conclusion

As a result of the study, germination properties of purslane under salinity and drought conditions slightly decreased at lower levels of stress, but drastically decreased at the highest stress levels. However, the seedling growth decreased gradually depending on increased stress conditions. It can be concluded that purslane is relatively resistant to low and moderate salt and drought stress during germination and early seedling growth. NO applications can mitigate the negative effects of moderate and high salt and drought stress.

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