ΦYTON
REVISTA INTERNACIONAL DE BOTÁNICA EXPERIMENTAL
INTERNATIONAL JOURNAL OF EXPERIMENTAL BOTANY
FUNDACION ROMULO RAGGIO
Gaspar Campos 861, 1638 Vicente López (BA), Argentina
www.revistaphyton.fund-romuloraggio.org.ar

Chilling effects after priming by nitric oxide applications on amelioration of leaf growth and photosynthetic pigments

Efecto del frío luego de la aplicación de óxido nítrico en plántulas de trigo sobre la reducción del crecimiento foliar y los pigmentos fotosintéticos

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Abstract. Chilling stress on cereal crops is the major form of cold stress that appears in some regions, and causes significant losses by depressing seed germination, and seedling growth and establishment. Hormones have the ability to reduce the stress effects in crops by various mechanisms. To determine the role of nitric oxide in mitigating chilling damages in wheat, two trials were conducted and seven wheat varieties were used. Seeds of wheat varieties after priming using three different concentrations (0, 10^{-4}, 10^{-5} M) of sodium nitroprusside as nitric oxide donor were grown under two growth conditions (control, 4 °C) according to a completely randomized design with three replicates. After measuring leaf length, leaf width and leaf number, seedlings were harvested and photosynthetic pigments were determined. Results revealed significant decrease in all the studied parameters under chilling conditions compared to controls during both trials. Nitric oxide donor at 10^{-4} M concentration showed significant improvement in all the studied aspects under both growth conditions by ameliorating the chilling effects. So, it is concluded that 10^{-4} M nitric oxide induced tolerance in seedlings against chilling stress. This accelerated photosynthetic activity by enhancing photosynthetic pigments production and ultimately seedling growth.

Keywords: Nitric oxide; Chilling stress; Wheat varieties; Chlorophyll.

Resumen. En algunas regiones, la forma principal del estrés por temperatura es el estrés por frío en cereales de cosecha. Dicho estrés causa pérdidas significativas reduciendo la germinación de las semillas y el establecimiento y crecimiento de las plántulas. Las hormonas tienen la capacidad de reducir los efectos del estrés en plantas de cosecha por varios mecanismos. Se condujeron dos ensayos para determinar el rol del óxido nítrico en reducir los daños del estrés por enfriamiento en trigo. Siete variedades de trigo fueron usadas con este propósito. Las semillas de las variedades de trigo, después del pretratamiento usando tres concentraciones (0, 10^{-4}, 10^{-5} M) de nitroprusiato sódico como donante de óxido nítrico, crecieron bajo dos condiciones de crecimiento (control, 4 °C) de acuerdo a un diseño completamente al azar con 3 réplicas. Después de medir longitud, ancho y número de hojas, las plántulas se cosecharon y se determinaron pigmentos fotosintéticos. Los resultados indicaron una reducción en todos los parámetros estudiados bajo condiciones de enfriamiento en comparación a los controles en ambos ensayos. A una concentración de 10^{-4} M. el donante de óxido nítrico mostró una mejora significativa en todos los aspectos estudiados en ambas condiciones de crecimiento, reduciendo los efectos del estrés por frío. Se concluye que el óxido nítrico a una concentración de 10^{-4} M indujo tolerancia al estrés por frío en las plantas. Esto incrementó la actividad fotosintética mejorando la producción de los pigmentos fotosintéticos y subsiguientemente el crecimiento de las plántulas.

Palabras clave: Óxido nítrico; Estrés por frío; Variedades de trigo; Clorofila.

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Received 12.XII.2017. Accepted 12.I.2018.

INTRODUCTION

Cold stress (low temperature) causes numerous alterations in morphological and physiological processes on plants (Suzuki & Mittler 2006; Zhao et al., 2009). Generally photosynthesis and many other physiological processes are susceptible to chilling stress resulting in a decline of plant growth and productivity. Chilling stress restrains different metabolic reactions thus preventing the expression of the total genetic potential of plants that is expressed as various phenotypic symptoms (Chinnusamy et al., 2007). It is reported that the majority of plants at the seedling stage are very much susceptible to low temperature stress. Various phenotypic symptoms at early stages of plant growth in response to low temperature stress are chlorosis, surface lesions, desiccation, necrosis, wilting, tissue break down and reduced leaf expansion (Jiang et al. 2002; Solanke & Sharma 2008). Low temperatures can disturb the key organs of photosynthesis (Ishikawa, 1996). Photosynthesis declines highly below 18 °C (Ramalho et al., 2003), whereas temperatures of approximately 4 °C considerably depress the photosynthetic performance (Silva et al., 2004). Partelli et al. (2009) reported that coffee plants showed 29% reduction in total Chlorophyll, 30% reduction in Chlorophyll *a*, and 27% reduction in Chlorophyll *b* during fluctuations of day/night temperatures from 25/20 °C to 13/8 °C.

Germination and seedling establishment are usually the most sensitive stages of plant development. The majority of studies on stress tolerance have generally included the responses of plants at these phases of developmental plant morphology in various crop species (Song et al., 2008; Tlig et al., 2008; Badridze et al., 2009). By understanding the responses of plants to stress, crops can be made more stress tolerant (Zhao et al., 2008). The role of NO in producing tolerance to abiotic stress in plants has established much consideration in the last few years. Studies suggest that NO has the potential to induce tolerance to different environmental stresses in plants. It is effectively involved in numerous morphological, biochemical and physiological processes of plants (Libourel et al., 2006; Zheng et al., 2009).

MATERIALS AND METHODS

Two consecutive trials were conducted during 2012 and 2013 in the research laboratory, Department of Botany University of Azad Jammu & Kashmir, Muzaffarabad. Seeds of seven wheat (*Triticum aestivum* L.) varieties, *viz.* Lasani (V1), Faisalabad-2008 (V2), AAS-2011 (V3), Punjab-2011 (V4), Uqab-2002 (V5), Chakwal-50 (V6) and NARC-2011 (V7) were selected for determining their tolerance to cold stress with and/ without priming. Their seeds were grown in Petri dishes lined with double layer of filter paper after priming at different concentrations (0, 10^{-4} and 10^{-5} molar) of nitric oxide donor sodium nitroprusside (snp). Half of the Petri dishes containing primed seeds were kept as control and the other half were subjected to 4 °C during 6 hours according to a Completely Randomized Design (Shibaski et al., 2009; Ansari & Sharif-Zadeh, 2012). After 17 days, leaf growth parameters (leaf length, leaf width and leaf number) were determined. Thereafter, seedlings were harvested and chlorophyll *a*, chlorophyll *b*, carotenoids and anthocyanins were determined according to Gamon and Surfus (1999).

Statistical analysis. A completely randomized Design (CRD) with 3 replicates was utilized. All the obtained data was subjected to analysis of variance using MSTAT-C software. Duncan's Multiple Range Test (DMRT) ($P \leq 0.05$) was used to compare the difference amongst treatment means (Steel et al., 1997).

RESULTS

Leaf length (LL). Leaf length is an important morphological aspect of plants that regulates plant transpirational and photosynthetic activities. Analysis of variance for leaf length revealed non-significant variations ($P > 0.05$) between the two trials data. However all factors (i.e., wheat varieties, SNP priming and growth conditions) varied significantly in trials independently. Interactions of wheat varieties and SNP concentrations in chilled and unchilled growth conditions (GC) revealed that LL varied highly significantly during both trails (Table 1). Maximum LL was shown from 10^{-4} M SNP primed Punjab-2011 under unchilled GC, followed by Faisalabad-2008 with the same priming under the same GC. Minimum LL was revealed by 0 M SNP treated Lasani under chilled growth conditions which significantly varied from all other samples.

Leaf width (LW). Leaf width showed very highly significant differences on all treatments during both trials. However, when the data from the two study years were compared, no statistical differences were detected. Comparison of SNP priming and wheat varieties under stressed and normal conditions revealed very highly significant differences for LW (Table 1). Maximum LW was observed on all the varieties treated with 10^{-4} M SNP except Lasani, Faisalabad-2008 and Narc-2011 under unchilled conditions, and Lasani and Narc-2011 with 10^{-5} M SNP priming. Minimum LW was revealed for unprimed Lasani, Aas-2011 and Uqab-2002 under chilled GC. It significantly varied to maximum LW under unchilled GC.

Leaf number (LN). Leaf number is an important aspect to determine photosynthetic activity of plants. All varieties revealed similar LN under the different priming conditions under chilling stress (Table 1). Only Punjab-2011 showed little variation in LN under unchilled GC during both trials.

Table 1. Variation in morphophysiological parameters on either primed or not wheat genotypes exposed to chilling.
Tabla 1. Variación en parámetros morfofisiológicos en genotipos de trigo pretratadas o no expuestas a estrés por bajas temperaturas.

	Parameters→	LL (cm)	LW (cm)	LN	Chl a (μmol/mL)	Chl b (μmol/mL)	Car (μmol/mL)	Antho (μmol/mL)
Years 2012-2013	0 M × Lasani	7.667 N	0.300 E	3.000	0.008 H	0.020 CDE	0.009 J	0.052 L
	0 M × Faisalabad-2008	8.667 IJK	0.317 DE	3.000	0.010 GH	0.019 C-F	0.013 HI	0.072 K
	0 M × Aas-2011	9.117 FG	0.350 CD	3.000	0.013 EFG	0.018 C-F	0.014 GHI	0.087 I
	0 M × Punjab-2011	9.583 E	0.350 CD	3.333	0.016 CD	0.020 CDE	0.017 DEF	0.098 H
	0 M × Uqab-2002	7.967 M	0.300 E	3.000	0.012 FG	0.008 I	0.014 F-I	0.073 K
	0 M × Chakwal-50	7.933 MN	0.400 AB	3.000	0.015 DE	0.017 D-G	0.015 E-I	0.096 H
	0 M × Narc-2011	7.720 MN	0.333 DE	3.000	0.015 DE	0.013 GH	0.013 HI	0.144 C
	10^{-4} M × Lasani	9.233 F	0.350 CD	3.000	0.011 GH	0.012 H	0.013 I	0.070 K
	10^{-4} M × Faisalabad-2008	10.650 B	0.350 CD	3.000	0.018 C	0.022 BC	0.020 CD	0.133 D
	10^{-4} M × Aas-2011	10.150 C	0.417 AB	3.000	0.016 CDE	0.018 C-F	0.018 DE	0.104 G
	10^{-4} M × Punjab-2011	11.567 A	0.433 A	3.333	0.027 A	0.031 A	0.026 A	0.192 A
	10^{-4} M × Uqab-2002	9.267 F	0.400AB	3.000	0.015 DEF	0.013 GH	0.016 E-H	0.072 K
	10^{-4} M × Chakwal-50	8.983 FGH	0.400 AB	3.000	0.018 C	0.020 CDE	0.022 BC	0.128 E
	10^{-4} M × Narc-2011	8.467 KL	0.433 A	3.000	0.022 B	0.025 B	0.022 BC	0.097 H
	10^{-5} M × Lasani	8.650 IJK	0.400 AB	3.000	0.016 CD	0.016 E-H	0.014 F-I	0.082 J
	10^{-5} M × Faisalabad-2008	9.750 DE	0.350 CD	3.000	0.014 DEF	0.022 BC	0.016 E-I	0.169 B
	10^{-5} M × Aas-2011	8.600 JK	0.300 E	3.000	0.016 CD	0.020 CDE	0.017 DEF	0.099 H
	10^{-5} M × Punjab-2011	9.933 CD	0.383 BC	3.333	0.024 B	0.030 A	0.023 AB	0.148 C
	10^{-5} M × Uqab-2002	8.900 GHI	0.400 AB	3.000	0.014 DEF	0.015 FGH	0.017 D-F	0.109 F
	10^{-5} M × Chakwal-50	8.283 L	0.400 AB	3.000	0.018 C	0.021 BCD	0.018 DE	0.132 DE
	10^{-5} M × Narc-2011	8.800 HIJ	0.400 AB	3.000	0.018 C	0.021 BCD	0.016 EFG	0.110 F
Years 2013-2014	0 M × Lasani	7.667 N	0.300 E	3.000	0.008	0.020 C-F	0.011	0.049 K
	0 M × Faisalabad-2008	8.667 IJK	0.333 D	3.000	0.010	0.018 C-G	0.035	0.071 J
	0 M × Aas-2011	9.117 FG	0.350 D	3.000	0.013	0.018 D-G	0.014	0.087 HI
	0 M × Punjab-2011	9.583 E	0.350 D	3.167	0.016	0.019 C-F	0.017	0.094 GH
	0 M × Uqab-2002	7.967 M	0.300 E	3.000	0.012	0.007 J	0.016	0.071 J
	0 M × Chakwal-50	7.933 MN	0.400 C	3.000	0.015	0.017 E-H	0.014	0.099 EFG
	0 M × Narc-2011	7.717 MN	0.300 E	3.000	0.015	0.013 HI	0.014	0.124 D
	10^{-4} M × Lasani	9.233 F	0.350 D	3.000	0.011	0.012 I	0.012 (9%)	0.067 J
	10^{-4} M × Faisalabad-2008	10.650 B	0.350 BC	3.000	0.018	0.021 BCD	0.020 (43%)	0.134 CD
	10^{-4} M × Aas--2011	10.150 C	0.417 AB	3.000	0.016	0.018 C-G	0.016 (14%)	0.102 EFG
	10^{-4} M × Punjab-2011	11.567 A	0.433 AB	3.167	0.027	0.030 A	0.024 (41%)	0.190 A
	10^{-4} M × Uqab-2002	9.267 F	0.400 C	3.000	0.029	0.013 HI	0.016 (0%)	0.067 J
	10^{-4} M × Chakwal-50	8.983 FGH	0.400 C	3.000	0.018	0.019 C-G	0.020 (43%)	0.128 D
	10^{-4} M × Narc-2011	8.467 KL	0.450A	3.000	0.022	0.024 B	0.020 (43%)	0.094 GH
	10^{-5} M × Lasani	8.667 IJK	0.400 C	3.000	0.016	0.016 F-I	0.014 (27%)	0.079 IJ
	10^{-5} M × Faisalabad-2008	9.733 DE	0.350 D	3.000	0.014	0.022 BC	0.014 (60%)	0.165 B
	10^{-5} M × Aas-2011	8.583 JK	0.300 E	3.000	0.016	0.019 C-F	0.014 (0%)	0.097 FGH
	10^{-5} M × Punjab-2011	9.933 CD	0.400 C	3.333	0.024	0.030 A	0.024 (41%)	0.144 C
	10^{-5} M × Uqab-2002	8.900 GHI	0.400 C	3.000	0.014	0.015 GHI	0.015 (6%)	0.106 EF
	10^{-5} M × Chakwal-50	8.283 L	0.400 C	3.000	0.019	0.021 B-E	0.018 (29%)	0.130 D
	10^{-5} M × Narc-2011	8.783 HIJ	0.400 C	3.000	0.018	0.020 B-E	0.016 (14%)	0.110 E

In the second trial, 10^{-5} M SNP primed Punjab-2011 also showed similar LN under chilled GC. Other SNP concentrations showed the same minimum LN.

Chlorophyll *a*. In the present study significant (P≤0.05) effects of chilling on chlorophyll *a* of studied wheat genotypes and SNP primings was revealed during both experiments. However, comparison between the two trials data showed no significant variations. Interactions of wheat varieties with varied concentrations of SNP, and their comparison under both chilled and unchilled GC, showed that chlorophyll *a* value of WV was affected by GC as well as SNP concentrations. Both factors had the capacity to change the chlorophyll *a* of plants (Table 1). During the first trial, maximum chlorophyll

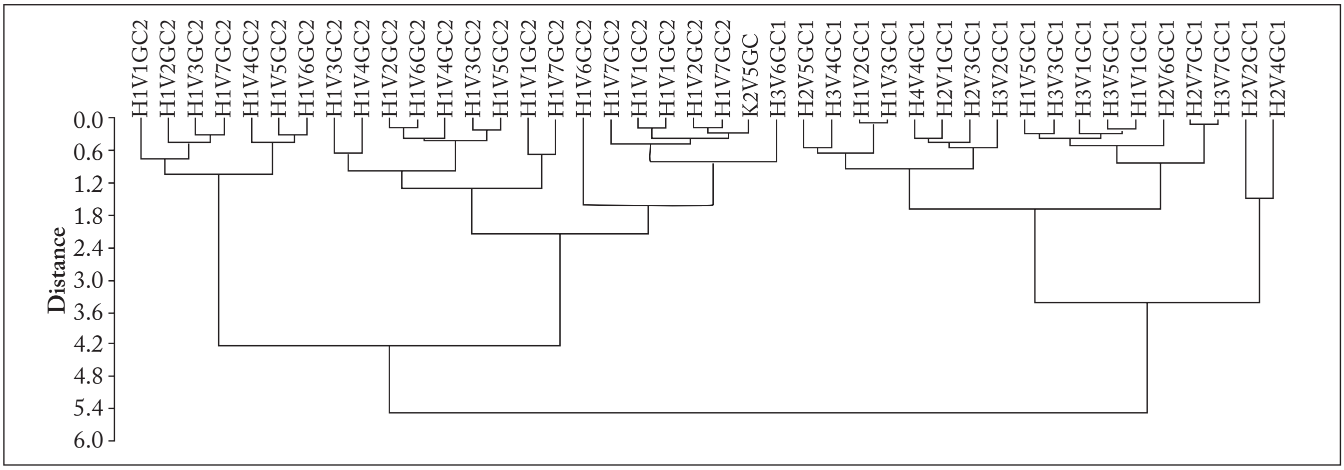

Fig. 1. Cluster analysis for all morphophysiological parameters.
Fig. 1. Análisis de cluster para todos los parámetros morfológicos.

a was revealed by Punjab-2011 with 10^{-4} M SNP priming under unchilled growth conditions. Comparatively, 10^{-4} M SNP primed Uqab-2002 showed maximum chlorophyll *a* under chilled GC. Minimum chlorophyll *a* was revealed by Lasani under chilled GC and 0 M SNP treatment during both trials.

Chlorophyll *b*. There was variation among chlorophyll *b* concentrations for the interaction of wheat varieties with varied SNP concentrations in different GC during both trials (Table 1). Almost all studied wheat varieties with all SNP treatments revealed maximum chlorophyll *b* concentrations under unchilled than chilled conditions. Maximum chlorophyll *b* was revealed under unchilled GC for 10^{-4} M SNP-treated Punjab-2011, followed by the same variety with 10^{-5} M SNP priming under the same GC. These values were non-significant to one another, but significant when compared with other interactions. Minimum chlorophyll *b* was revealed by 10^{-4} M SNP primed Uqab-2002 under chilling conditions during first trial, and 0 M SNP primed Uqab-2002 under the same growth condition during the second trial.

Carotenoids. Carotenoids were determined under both growth conditions from all the studied samples and considerable differences (P≤0.05) were observed between them. Interactions of wheat varieties and SNP priming under varied GC showed that GC can affect carotenoid contents in wheat varieties. However, SNP treatments could mitigate the effect of GC on wheat varieties. During the first trial, highly significant variations were observed for carotenoid contents, whereas these variations were nonsignificant during the second trial (Table 1). Maximum carotenoid concentrations were observed for Punjab-2011 with 10^{-4} M SNP priming, followed by the same variety with 0 M SNP and 10^{-4} M SNP priming under unchilled GC during first trial, and 0 M SNP primed Faisalabad-2008. Minimum carotenoids were revealed for 0 M SNP primed Lasani under chilled GC during the first trial, and it did not vary significantly during the second trial.

Anthocyanins. Analysis of variance for anthocyanins revealed non-significant variations (P>0.05) between the two trials data. However, all the factors (i.e., wheat varieties, SNP priming and growth conditions) varied highly significantly, independently during both trials. Anthocyanin concentration of wheat varieties was affected by SNP concentrations as well as GC (Table 1). Maximum anthocyanin concentration was revealed by Punjab-2011 treated with 10^{-4} M SNP under chilling GC, whereas Lasani with 0 M SNP priming showed minimum anthocyanin concentrations.

DISCUSSION

Leaf growth is an important vegetative character of plants that cause either increases or decreases in the plants' respiratory mechanisms in addition to the various metabolic activities, including photosynthesis and transpiration. Increase in leaf length, width and leaf numbers may increase such activities in plants. In the present project, a decrease in LL and LW was observed in plants by stress. Minimum LL and LW were revealed by the samples under chilling GC, and maximum values were obtained under controlled GC. NO donor, when applied at the concentration of 10^{-4} M, significantly enhanced leaf growth by reducing the injurious effects of chilling. Punjab-2011 and Faisalabad-2008 showed the maximum LL with SNP priming. The decreases in LL and differences among varieties due to temperature stress were reported by many previous studies (Yang & Hsiang 1992; Aghamolki et al., 2014; Lianopoulou et al., 2014). Chakwal-50 and Punjab-2011 showed the maximum LW values as compared to other studied wheat varieties (WV). This is because of the varied genetic potential of these WV. 10^{-4} M SNP priming in Narc-

2011, Aas-2011 and Punjab-2011 played an important role in reducing chilling stress consequently increasing LW. These results agree with the findings of Yang and Hsiang (1992), Aghamolki et al. (2014) and Lianopoulou et al. (2014). In the present study, LN did not vary much either among WV. Once again, this might be due to variation in their genetic potential. Such variations among varieties were also reported by many other scientists (Reddy & Vaid, 1983; Aghamolki et al., 2014). However, non-significant variations in LN because of varied GC and SNP concentrations are consistent with findings of Yang and Hsiang (1992). They reported that the total number of leaves remained the same despite varied conditions.

Temperature stress reduces pigment biosynthesis and disintegration of the membranes of the chloroplasts besides disrupting biochemical reactions of the photosystems (Havaux, 1998). Changes in photosynthetic pigments were observed in response to cold stress with and/ or without the addition of NO on different wheat varieties. We found a significant decline in the concentrations of chlorophyll *a*, chl *b*, carotenoids and anthocyanins under chilling stress compared to unchilled samples. However, NO seed priming increased the photosynthetic pigment composition markedly even under chilling stress growth conditions. Harris et al. (1999) observed that seed priming caused earlier emergence of seedlings and had a positive role in stress resistance. All priming alleviated the harmful effects of stress on pigment concentrations, as primed seedlings showed a vigorous growth as compared to unprimed seeds (Baxter et al., 1994) and vigorous growth is a proof of a strong photosynthetic activity of plants. Thus, it can be concluded from this study that priming plays an important task with the enzyme pool which is responsible of the photosynthetic processes that raise the potential of plants to overcome stress. Different wheat varieties showed varied rate of resistance to chilling stress as variations in pigment concentrations were revealed by them. This might be the result of variability on the genetic potential and seed vigor. Hayatu and Mukhtar (2010) observed variations on pigment concentrations between different genotypes of cowpea or rice after the same treatment and stress conditions.

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