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Effect of sodium nitroprusside on morphological characters under chilling stress in chickpea (*Cicer arietinum* L.)

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Abstract

An experiment was conducted with chilling tolerant (IC-424234) and sensitive (PBG-1) chickpea (Cicer arietinum L.) genotypes to study the effect of sodium nitroprusside (SNP) – nitric oxide donor applied as foliar spray of 150 and 300 μM concentrations at 45 DAS (vegetative stage), 85 DAS (flowering stage) and 125 DAS (post flowering stage). Both the concentrations of SNP (150 and 300 μM) resulted in significant increase in all the morphological characters viz. plant height, number of leaves plant^{-1}, leaf area plant^{-1} and leaf area index (LAI) over the control at all the stages, though lower concentration (150 μM) was more effective. Chilling sensitive (CS) genotype PBG-1 responded more effectively to SNP treatment. Electrolyte leakage percentage was effectively reduced by SNP treatments in both the genotypes at low temperature (15 DAA). Chilling sensitive genotype PBG-1 treated with SNP (150 μM) recorded significantly higher yield contributing characters viz. number of pods plant^{-1}, number of seeds pod^{-1}, seed yield plant^{-1}(g), pod setting percentage (%), 100 seed weight (g) and yield (kg ha^{-1}) over the chilling tolerant (IC-424234)

Key words

Chickpea, Sodium nitroprusside, Nitric oxide, Chilling stress

Introduction

Chickpea (*Cicer arietinum* L.) is the third most important legume crop in the world after dry bean and field pea. In India, chickpea is mainly grown in Northern and Central India, where there is wide fluctuation in maximum and minimum temperatures at the time of the flowering, which leads to yield instability. Moreover, low temperature at the time of flowering results in heavy flower drop. Yield instability in chickpea has been chiefly attributed to biotic and abiotic stresses (Kaur *et al.*, 2007). During the last decade, the foliar application of plant growth regulators and biomolecules has become an established procedure in crop production to increase yield and quality of the crop under abiotic stresses. Recently, nitric oxide has been implied as a key regulator of diverse physiological processes under various abiotic stresses in plants (Lamattina *et al.*, (2003). Nitric oxide produced in plants at low concentration may rapidly eliminate lipid peroxyl radicals, alter the species and components of reactive oxygen species (ROS), induce expression of antioxidant gene and the activity of antioxidant enzymes (Cheng *et al.*, 2002) and protect plants from abiotic stress (Neill *et al.*, 2003). Beneficial effects of nitric oxide/SNP application on increasing plant height, node number and pod number have been reported in mungbean (Kaur *et al*., 2006). However, the effect of nitric oxide/ SNP application in chickpea under Punjab conditions has not been demonstrated. Hence, the present experiment was conducted to study the effect of exogenous application of sodium nitroprusside (SNP - nitric oxide donor) on morphological characteristics in relation to yield of chickpea genotypes under chilling stress.

Materials and Methods

A field experiment was conducted with two chickpea (*Cicer arietinum* L.) genotypes in the experimental area of Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana. Seeds were sown in the field on 1st November, 2007 and crops were harvested on 18th April, 2008. The crop was raised in randomized block design with three replicates for each treatment in plot size of 4m X 1.2 m = 4.8 m^2, with rows 30 cm apart and plant to plant spacing was maintained at 15 cm. Sodium nitroprusside- (SNP- nitric oxide releasing compound) with the concentrations of 150 and 300 µM was applied as foliar spray at 30 days after sowing (DAS) which was repeated after 10 days interval till maturity and control plants were sprayed with distilled water. Five plants in each replication were selected randomly in order to record their various morphological, biochemical and yield contributing attributes. Morphological attributes *viz.* plant height (cm), number of leaves/ plant, leaf area (cm^2 $plant^{-1}$) and leaf area index (LAI) were studied at 45 (vegetative stage), 85 (flowering stage) and 125 (post flowering stage) DAS in selected plants of chilling tolerant (CT) IC-424234 and chilling sensitive (CS) PBG-1 genotypes. Leaf area index was calculated according to the formula of Frageria *et al.* (2006). Electrolyte leakage (%) was recorded from the 4th leaf of the selected plants at 15 and 40 days after anthesis (DAA). Yield contributing characters *viz.* number of pods $plant^{-1}$, number of seeds pod^{-1}, seed yield $plant^{-1}$(g), pod setting percentage (%), 100 seed weight (g) and yield (kg ha^{-1}) was recorded from seeds at harvest. The data on genotypes and various morphological, biochemical and yield contributing parameters were statistically analyzed, using random block design (Gomes and Gomes, 1984) and expressed at 5% level of significance.

Results and Discussion

In the present study, both the chickpea genotypes IC-424234 (CT) and PBG-1 (CS) recorded significant increase in all the morphological parameters *i.e* plant height, number of leaves $plant^{-1}$, leaf area $plant^{-1}$ and LAI by the foliar application of SNP at different stages of growth as compared to control. The magnitude of increase was different in both the genotypes. The effect was more pronounced in chilling sensitive (CS) PBG-1 at all the growth stages. Both the concentrations of SNP (150 and 300 µM) resulted in increase in all the morphological characters over the control at all the stages though lower concentration (150 µM) was more effective. Maximum plant height was recorded with low concentration of SNP (150 µm) at all the stages of growth *i.e.* vegetative (45 DAS), flowering (85 DAS) and post-flowering (125 DAS) stages which was more than the control in PBG-1 (CS) (Table 1). Kaur *et al*. (2006) also observed that plant height increased with both the concentrations (100 and 200 µM of SNP) as compared to control at all the three growth stages (25, 45 and 65 DAS) in mungbean. The number of leaves $plant^{-1}$ also increased due to SNP application at all the growth stages (Table 1). Maximum increase in number of leaves $plant^{-1}$ was recorded in PBG-1 (CS) at 125 DAS (29%) with SNP (150 µM) treatment over that of control. Lateral bud growth is regulated principally by ratio of auxins to cytokinins and high ratio favours apical dominance. Nitric oxide action in plants has been closely linked to activities of traditionally known plant hormones. Cytokinins have been shown to induce NO synthesis in tobacco, parsley and *Arabidopsis* (Tun *et al.,* 2001). Kaur *et al*. (2006) also reported that number of nodes increased with the application of SNP -100 µM at 25 and 40 DAS. In the present study, due to increase in number of nodes, number of leaves also increased by SNP treatment.

There was a sharp increase in leaf area $plant^{-1}$ with application of SNP (150 and 300 µM). Maximum increase in leaf area was recorded in PBG-1 (CS) with foliar application of SNP (150 µM) at post flowering stage (37%) (Table 2). Chilling tolerant genotype (IC-424234) also responded by increase in leaf area at all the stages of growth with both the concentrations of SNP but the increase was lesser than PBG-1 (CS). Leaf area index also increased in both the genotypes IC-424234 (CT) and PBG-1 (CS) with foliar application of SNP. Maximum increase was recorded in chilling sensitive genotype PBG-1 (2.95) with 150 µM SNP treatment at 125 DAS.

Maximum number of pods $plant^{-1}$ (67) were observed with 150 µM SNP treatment as compared to 300 µM SNP treatment, while minimum number (50) was observed with control in chilling tolerant genotypes IC-424234 (Table 3). Similar trend was observed in chilling sensitive genotypes PBG-1 where foliar spray application of 150 µM SNP increased the number of pods $plant^{-1}$ by 35.9% over that of control. Pod setting percentage was highest (60.1%) with 150 µM SNP as compared with that of 300 µM SNP treatment and control in chilling tolerant genotype (IC-424234). Similar trend was observed in chilling sensitive genotype (PBG-1). The use of plant growth regulators has been found to greatly affect the reproductive development partitioning coefficient, pod setting percentage, seed yield per plant and seed quality (Setia *et al.,* 1996). Similar effects obtained with SNP treatment seem to be the result of modifications at the hormonal levels at crucial developmental stages which possibly trigger various metabolic processes to support reproductive development.

Total number of seeds pod^{-1} increased with 150 and 300 µM SNP treatments in both chilling tolerant and sensitive genotypes. Maximum number of seeds pod^{-1} were recorded with 150 µM SNP treatment in both the chilling tolerant (IC-424234) and sensitive (PBG-1) chickpea genotypes, respectively. Seed yield $plant^{-1}$ was also observed maximum with SNP (150 µM) treatment in both chilling tolerant IC-424234 (35.0 g) and sensitive PBG-1 (45.0 g) chickpea genotypes as compared to their respective controls (22.1 and 32.3 g). Application of 150 µM SNP also increased 100 seed weight in both the chilling tolerant and sensitive genotypes. Chilling sensitive PBG-1 recorded the highest seed yield (2225 kg ha^{-1}) when treated with 150 µM SNP as compared to 300 µM SNP and control. Kaur *et al*. (2006) reported that exogenous application of 150 µM SNP increased the total yield, since SNP has potential for conversion of flowers to pods. This may be achieved through changes in endogenous hormonal levels leading to favourable allocation of photoassimilates. Yield characters *viz.* total pods $plant^{-1}$, pod setting percentage, number of seeds pod^{-1}, seed yield $plant^{-1}$, 100 seed weight and yield (kg ha^{-1}) increased with SNP treatment indicating stimulating the efficiency

Table - 1: Effect of sodium nitroprusside (SNP) on plant height (cm) and number of leaves plant^{-1} of chilling tolerant (IC-424234) and sensitive (PBG-1) chickpea genotypes

Parameters	Plant height (cm)						Number of leaves plant^{-1}					
Genotypes	IC-424234(CT)			PBG-1(CS)			IC-424234(CT)			PBG-1(CS)		
treatments	45 DAS	85 DAS	125 DAS	45 DAS	85 DAS	125 DAS	45 DAS	85 DAS	125 DAS	45 DAS	85 DAS	125 DAS
SNP (150µM)	22.2±0.9	35.4±1.0	47.8±1.1	16.3±0.8	63.5±1.9	70.7±2.0	44.1±0.9	63.4±1.1	75.5±1.5	36.0±1.3	104.0±1.0	120.3±0.5
SNP (300µM)	20.4±0.8	30.2±1.1	43.4±0.6	14.9±1.0	50.5±1.0	66.4±1.4	41.3±1.0	60.3±1.1	73.2±0.7	34.0±2.0	100.8±1.0	115.4±1.0
Control	17.9±0.7	25.6±0.9	32.1±0.8	13.7±1.0	45.4±0.8	47.8±0.9	30.4±0.5	48.1±1.8	57.4±0.8	28.5±1.5	85.8±1.4	93.0±1.7
CD at 5%	**0.5**	**0.6**	**1.4**	**0.6**	**1.3**	**2.4**	**1.4**	**2.0**	**2.9**	**1.2**	**2.0**	**2.4**

The values are mean of three replicates ± SD. CD values are critical differences among the mean values at 5% significance level

Table - 2: Effect of sodium nitroprusside (SNP) on leaf area (cm^2 plant^{-1}) and leaf area index of chilling tolerant (IC-424234) and sensitive (PBG-1) chickpea genotypes

Parameters	Leaf area (cm^2 plant^{-1})						Leaf area index					
Genotypes	IC-424234(CT)			PBG-1(CS)			IC-424234(CT)			PBG-1(CS)		
treatments	45 DAS	85 DAS	125 DAS	45 DAS	85 DAS	125 DAS	45 DAS	85 DAS	125 DAS	45 DAS	85 DAS	125 DAS
SNP (150µM)	23.3±1.0	50.3±1.5	63.4±1.1	20.2±1.0	88.5±1.6	110.1±1.1	0.98±0.1	1.68±0.01	2.78±0.32	0.73±0.03	1.80±0.2	2.97±0.02
SNP (300µM)	23.0±1.3	47.1±1.0	60.6±1.4	20.1±0.9	83.4±1.5	105.3±1.0	0.95±0.03	1.65±0.01	2.60±0.30	0.72±0.02	1.77±0.01	2.95±0.04
Control	22.0±1.0	38.3±1.6	46.3±0.7	19.0±1.0	68.1±1.9	87.5±1.0	0.80±0.01	1.40±0.06	1.60±0.50	0.74±0.04	1.48±0.01	1.78±0.02
CD at 5%	**NS**	**3.4**	**2.0**	**NS**	**3.0**	**2.6**	**0.01**	**0.05**	**0.08**	**0.02**	**0.03**	**0.11**

The values are mean of three replicates ± SD. CD values are critical differences among the mean values at 5% significance level

Table 3 : Effect of sodium nitroprusside (SNP) on yield attributes and yield (kg ha^{-1}) of chilling tolerant (IC-424234) and sensitive (PBG-1) chickpea genotypes

Genotypes	IC-424234						PBG-1					
treatments	Total no. of pods plant^{-1}	Pod setting (%)	No. of seeds pod^{-1}	Seed yield plant^{-1} (g)	100 seed weight (g)	Yield (kg ha^{-1})	Total no. of pods plant^{-1}	Pod setting (%)	No. of seeds pod^{-1}	Seed yield plant^{-1} (g)	100 seed weight (g)	Yield (kg ha^{-1})
SNP(150 µM)	67±1.5	60.1±1.5	2.2±0.7	35.0±2.0	35.8±1.0	1775±4.0	87±2.0	65.4±1.6	2.7±0.6	45.0±3.0	36.0±1.2	2225±4.0
SNP(300 µM)	60±1.0	58.9±1.7	2.0±1.0	31.0±0.6	30.2±2.0	1768±3.1	78±1.0	60.9±1.7	2.5±1.5	40.1±1.0	32.6±2.0	2212±3.3
Control	50±1.0	48.4±1.2	2.0±0.2	22.1±1.0	20.5±0.6	1268±3.0	64±1.5	53.3±1.3	2.0±0.3	32.3±1.5	19.7±1.6	1856±4.3
CD at 5%	**4**	**5.4**	**NS**	**2.5**	**3.5**	**8**	**6**	**5.4**	**NS**	**5.0**	**4.3**	**10**

The values are mean of three replicates ± SD. CD values are critical differences among the mean values at 5% significance level

Table 4 : Effect of sodium nitroprusside (SNP) on electrolyte leakage (%) of chilling tolerant (IC-424234) and sensitive (PBG-1) chickpea genotypes

Parameter	Electrolyte leakage (%)			
Genotype	IC-424234		PBG-1	
treatment	15 DAA	40 DAA	15 DAA	40 DAA
SNP (150 μM)	48 ±2.0	40 ±1.0	63±2.0	55±1.0
SNP (300 μM)	55 ±1.0	44 ±1.0	65±0.9	60±0.6
Control	60 ±1.0	50 ±1.3	75±3.0	65±2.0
CD at 5%	2.4	1.0	3.0	2.0

The values are mean of three replicates ± SD; CD values are critical differences among the mean values at 5% significance level

of NO in developing sinks to utilize more assimilates. Our results corroborated the findings of Arasimowicz and Wieczorek (2007) who observed that treatment of SNP plays a crucial role in plant growth and development starting from germination to flowering, ripening of fruit and senescence of organs. Leshem (2001) has proposed that this bioactive molecule (NO) should be classified as a phytohormone. Several studies documented that NO interacts with different plant hormones (IAA, GA, ABA, CKs and ethylene) at different steps of signaling cascades to evoke various responses and finally increase the yield (Gracia-Mata and Lamattina, 2002). Sodium nitroprusside treatments enhanced the accumulation of total dry matter and partitioning towards different plant parts. The enhanced dry matter accumulation in seeds of SNP treated chickpea plants suggests that this chemical has altered source: sink ratio. Nitric oxide increased the translocation of photoassimilates from source (leaves) to sink (seeds) efficiently. The interaction between source and sink play an important role in yield production system.

Sodium nitroprusside treatments (150 μM and 300 μM) effectively reduced the electrolyte leakage (%) in both the genotypes over that of control at 15 and 40 DAA stages (Table 4). Maximum reduction in electrolyte leakage was recorded with SNP (150 μM) treatment in PBG-1 (29%) in comparison with control at 15 DAA (low temperature). Huaifu *et al*. (2007) also observed that exogenous application of SNP reduced the membrane lipid peroxidation and prevented the electrolyte leakage, suggesting that NO possessed the functions of repairing and protecting the cell membranes. Similarly, application of 10 mM putrescine to chilling stressed plants reduced the electrolyte leakage by 29%, elevated the cellular respiration by 40% and resulting in increase in floral retention, pod set, total number of pods and finally increasing the yield (Nayyar, 2005).

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