

## Effect of Exogenous Nitric Oxide on Chlorophyll Content and Hill Reaction Activity in Leaves of *Brassica napus* L.

SHALINI PAHWA, R. C. SETIA\* AND NEELAM SETIA

*Department of Botany, Punjab Agricultural University  
 Ludhiana 141004, India*

*E-mail : rc\_setia@yahoo.co.in*

*\*Correspondence*

### Abstract

In this investigation the effects of foliar applications of nitric oxide donor, sodium nitroprusside (SNP), were studied on chlorophyll content and Hill reaction activity of chloroplasts in leaves of *Brassica napus* (cv GSL 1) at different stages of development. Different SNP treatments (50, 100, 200 and 400 µg/ml) increased the level of total chlorophyll and that of chlorophyll *a* and chlorophyll *b* but decreased chl *a/b* ratio. Increase in the Hill reaction activity of chloroplasts was also recorded in SNP treated leaves. The maximum alteration in these parameters occurred in response to 100 µg/ml SNP. The reported changes in the chlorophyll and chloroplast characteristics due to SNP application indicate improved photosynthetic and metabolic activities in leaves of *B. napus*.

**Key words :** *Brassica napus*, Nitric oxide, Sodium nitroprusside, Chlorophyll, Hill activity.

In recent years, nitric oxide (NO) has emerged as an endogenous and potent regulator of plant growth and development (1, 2). It is a small highly diffusible gas and an ubiquitous molecule which has been shown to mediate several developmental and physiological processes in plants including xylogenesis, programmed cell death, pathogen defense, photomorphogenesis, root growth, leaf expansion, seed germination pigment synthesis, senescence, stomatal closure and gravitropism (2 — 4). However, the role of NO in photosynthesis is not completely revealed as indicated by a number of studies with conflicting results (5). The present investigation was undertaken to study the effect of exogeneous application of No, using sodium nitroprusside (SNP) as donor, on some chlorophyll characters and Hill reaction activity of chloroplasts in the leaves of *Brassica napus*.

### Methods

Seeds of *Brassica napus* (cv GSLI) were procured from the Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana. The plants were grown in the field area of the Department of Botany of the university during 2005-06 in a randomized block design, following the recommended practices for fertilizer application, plant protection and

irrigation. At rosette stage the plants were sprayed with SNP solutions at 50, 100, 200 and 400 µg/ml concentrations containing 0.01%. Triton X-100 was used as surfactant. Three more spray treatments of each concentration of SNP were repeated after one week's interval. The plants sprayed with water containing triton-X served as control. The leaves collected from the sixth node from the base of control and SNP treated plants were analyzed for chlorophyll content and Hill reaction activity of chloroplast at 20-day intervals, starting from 85 DAS upto 145 DAS; the period between 105 to 125 DAS being the active reproductive phase of the plant development. The total chlorophyll content was estimated by the method of Anderson and Boardman (6) and Hill reaction activity of chloroplasts was measured according to Cherry (7).

### Results and Discussion

Table 1 shows changes in leaf chlorophyll content of control and SNP treated plants at different developmental stages of plants. The total chlorophyll content of leaves showed a consistent increase from 85 to 125 DAS followed by a decline thereafter. Compared to control, the leaves of SNP treated plants exhibited higher level of total chlorophyll at all the stages. At 125 DAS, a maximum increase in total chlo-

**Table 1.** Influence of sodium nitroprusside (SNP 50, 100, 200 and 400  $\mu\text{g/ml}$ ) on chlorophyll (chl) characters (Total chl, a, chl b and chl a/b ratio) in leaf of *Brassica napus* (cv GSL 1) at different stages of development.

Stage (DAS)	Treatment SNP (kg/ml)	Total chl (mg/g FW)	Character		
			Chl a (mg/g FW)	Chl b (mg/g FW)	Chl a/b ratio
85	Control	0.44	0.27	0.15	1.8
	50	0.46	0.29	0.18	1.6
	100	0.52	0.34	0.22	1.5
	200	0.51	0.32	0.20	1.6
	400	0.49	0.30	0.19	1.5
105	Control	0.74	0.52	0.25	2.1
	50	0.78	0.55	0.28	1.9
	100	0.86	0.62	0.32	1.9
	200	0.85	0.60	0.30	2.0
	400	0.78	0.59	0.29	2.0
125	Control	0.92	0.69	0.28	2.5
	50	0.97	0.71	0.32	2.1
	100	1.07	0.77	0.35	2.2
	200	1.01	0.75	0.33	2.2
	400	0.99	0.72	0.32	2.3
145	Control	0.30	0.19	0.11	2.7
	50	0.35	0.25	0.13	1.9
	100	0.40	0.29	0.15	1.9
	200	0.39	0.28	0.11	2.5
	400	0.37	0.26	0.12	2.1

rophyll content was observed in leaves of 100  $\mu\text{g/ml}$  SNP treated plants which was about 16% over control. Following 50, 200 and 400  $\mu\text{g/ml}$  SNP treatments the enhancement in leaf content was about 5, 10 and 8%, respectively, over control. An increase in content of chlorophyll *a* and chlorophyll *b* in response to different SNP treatments was also observed (Table 1), with a maximum increase being recorded in 100  $\mu\text{g/ml}$  SNP treated plants which was about 57 and 27%, respectively, over control. However, there was decrease in the chlorophyll *a/b* ratio (Table 1). The decrease in chlorophyll *a/b* ratio observed following 50, 100, 200 and 400  $\mu\text{g/ml}$  SNP treatments was 34, 34, 14 and 27% respectively, as compared to controls.

Table 2 shows the Hill reaction activity of chloroplasts in leaves of control and SNP treated plants during various stages of development. It was maximum at 125 DAS. SNP enhanced the Hill reaction activity of chloroplasts at all the stages of development. At 125 DAS, a maximum increase in Hill reaction activity of chloroplasts was observed in leaves of 100  $\mu\text{g/ml}$  SNP treated plants which was about 24% over the controls.

**Table 2.** Influence of sodium nitroprusside (SNP 50, 100, 200 and 400  $\mu\text{g/ml}$ ) on Hill reaction activity ( $\Delta\text{OD/mg/chl h}$ ) of chloroplasts of leaf of *Brassica napus* (cv GSL 1) at different stages of development.

Treatment SNP ( $\mu\text{g/ml}$ )	Hill reaction activity Stage (DAS)			
	85	105	125	145
Control	11.76	18.37	25.37	8.13
50	13.16	22.49	26.37	9.02
100	20.31	29.24	31.53	13.15
200	19.53	28.51	30.88	11.51
400	18.44	24.17	29.42	10.02

The increased level of chlorophyll content, especially during reproductive phase, and delayed reduction in loss of chlorophyll during later stages of development in leaves of SNP treated plants is indicative of the role of NO in delaying leaf senescence and, therefore, prolonging availability of assimilatory tissues. Promotive effects of NO on chlorophyll were reported earlier by He et al. (8) and Yentur et al. (9). NO donors stimulated de-etiolation and increased chlorophyll content in lettuce, potato, *Arabidopsis* and dark grown wheat seedlings (10) and in pea leaves particularly guard cells (11). Increased chlorophyll content, the decreased chlorophyll *a/b* ratio and enhanced Hill reaction activity of chloroplasts in the leaves of SNP treated plants indicate increased photosynthetic and metabolic activities (12, 13).

#### References

1. Lamattina L., C. Garcia-Mata, M. Granziano and G. Pagnussat. 2003. Nitric oxide : The versatility of an extensive signal molecule. *Ann. Rev. Pl.Biol.* 54 : 109—136.
2. Setia N. and R. C. Setia. 2006. Nitric oxide (NO) as a regulator and signaling molecule in plants. Pages 32—57 in P. C. Trivedi, editor. *Plant molecular physiology current scenario and future prospects*. Aavishkar Publ., Jaipur, India.
3. Lamotte O., K. Gould, D. Lecourieux, A. Sequeira-Le Grand, A. Lebrun-Garcia, J. Durner, A. Pugin and D. Wendehenne. 2003. Analysis of nitric oxide signalling functions in tobacco cells challenged by the elicitor cryptogrein. *Pl. Physiol.* 131 : 58—63.
4. Neill S. J., R. Desikan and J. T. Hancock. 2003. Nitric oxide signaling in plants. *New Phytol.* 159 : 11—35.
5. Wodala B., Z. Deak, I. Vass, L. Erdei, I. Altorjay and F. Horvath. 2008. *In vivo* target sites of nitric oxide in photosynthetic electron transport as studied by chlorophyll fluorescence in pea leaves. *Pl. Physiol.* 146 : 1920—1927.

6. Anderson J. H. and N. K. Boardman. 1964. Studies on greening of dark brown green plant VI. Development of photochemical activity. *Aust. J. Bio.* 17 : 93—101.
7. Cherry J. H. 1973. *Molecular biology of plants, a text manual.* Columbia Univ. Press, New York. USA.
8. He Y., R. H. Tang, Y. Hao, R. D. Stevans, C. W. Cook, S. M. Ahn, L. Jing, Z. Yang, L. Chen, F. Guo, F. Fionari, R. B. Jackson, N. M. Crawford and Z. M. Pei. 2004. Nitric oxide represses the *Arabidopsis* floral transition. *Science* 305 : 1968—1971.
9. Yentur S., N. Ylmazer, G. Cevahir, E. Aytamka and F. Erylmaz. 2005. The effect of nitric oxide, salicylic acid and hydrogen peroxide on the pigment content in excised cotyledons of red cabbage (*Brassica oleracea* L.). *Fresenius Environ. Bull.* 14 : 591—598.
10. Beligni M. V. and L. Lamattina. 2000. Nitric oxide stimulates seed germination and de-etiolation and inhibits hypocotyl elongation, three light inducible responses in plants. *Planta* 210 : 215—221.
11. Leshem Y.Y., E. Haramaty, D. Iluz, Z. Malik, Y. Sofer and L. Roitman. 1996. Effect of stress on nitric acid (NO) interaction between chlorophyll fluorescence, galactolipid fluidity and lipoxigenase activity. *Pl. Physiol. Biochem.* 35 : 573—579.
12. Fedtke C. 1973. Effect of herbicide methabenzthiazuron on the physiology of wheat plants. *Pestic. Sci.* 4 : 653—664.
13. Fedtke C. 1974. Influence of methabenzthiazuron on ATP-level and protein synthesis in wheat. *Pestic. Biochem. Physiol.* 4 : 386—392.