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EXOGENOUS APPLICATION OF SODIUM NITROPRUSSIDE (NITRIC OXIDE DONOR) IMPROVES YIELD POTENTIAL AND SEED QUALITY OF *BRASSICA NAPUS* L.

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SUMMARY

The localised application of nitric oxide (NO) donor-sodium nitroprusside (SNP-100, 200 and 500 µg ml^{-1}) given to the terminal inflorescence (TI) of *Brassica napus* L. GSL-1 at floral initiation stage greatly influenced its growth and yield parameters. SNP treatments (100 and 200 µg ml^{-1}) significantly increased TI length, number of open flowers and siliquae, seed number per siliqua, 1000-seed weight and seed yield per TI, however, 500 µg ml^{-1} SNP proved inhibitory. The improvement in seed yield due to SNP treatments was accompanied by simultaneous enhancement of photosynthetic efficiency of TI and siliqua wall measured in terms of chlorophyll content and Hill reaction activity of isolated chloroplasts and enhanced accumulation of dry matter and its partitioning towards developing siliquae. Seeds of SNP-treated plants also exhibited higher content of various biochemical reserves viz. total soluble sugars, starch, total soluble proteins, total free amino acids and oil with maximum enhancement occurring due to 100 µg ml^{-1} SNP treatment. SNP treatments also exerted profound influence on vascular tissue differentiation in TI.

***Keywords*: *Brassica napus*, nitric oxide, photosynthesis, seed yield, sodium nitroprusside**

INTRODUCTION

The oleiferous *Brassica* species constitute a major group among oilseed crops of the world including India. However, the gap between yield potential and average yield in these crops is quite large and yields are unstable. The maximum yield potential of *Brassica* spp. is primarily limited due to reproductive failures such as abscission of flowers and developing fruits (siliquae), incomplete seed development and abortion of developing seeds (Setia and Setia 1990). *Brassica* plants exhibit indeterminate growth habit with terminal inflorescence (TI) bearing more number of siliquae than the lateral ones. Unlike other monocarpic crops, the inflorescence development in *Brassica* spp. is fairly complex as it involves floral determination and axis elongation with concomitant flowering and siliqua development, thus, bearing reproductive structures with different developmental and metabolic sequence. It has been reported that inflorescence development in *Brassica* spp. is amenable to exogenous application of plant growth regulators (Kaur *et al.* 2001, Setia *et al.* 2003).

Recent studies on nitric oxide (NO) in plants has gained considerable attention due to its involvement in many aspects of plant growth and development, notably plant–pathogen defense reaction, programmed cell death, maturation and senescence, stomatal closure, seed germination, root development and the induction of ethylene emission (Setia and Setia 2006, Jin *et al.*2009). However, information is lacking regarding NO's specific role in mediating plant reproductive processes, such as flowering and fruit development. The present study was conducted to assess the effect of exogenously applied

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SNP (sodium nitroprusside - a NO donor) on growth, yield potential and seed quality of oilseed rape (*Brassica napus* L. cv. GSL-1).

MATERIALS AND METHODS

The seeds of *Brassica napus* L. (cv. GSL-1) were procured from Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana. The plants were raised in small plots in randomised block design (RBD) in three replications in the experimental area of the Department of Botany. The package of practices recommended by this University were followed for application of fertilisers, irrigation and aphid control treatments. At anthesis (floral initiation) stage, terminal inflorescence (TI) of plants was given localised treatment of sodium nitroprusside (SNP-100, 200 and 500 µg ml^{-1}) by dipping in a measuring cylinder containing aqueous solution of SNP. All the treatment solutions contained 0.05% (v/v) Triton-X-100 as surfactant, and the dipping treatments were repeated after one week interval. The TI of plants treated with water containing 0.05% (v/v) Triton-X-100 served as control. Data on length of TI, numbers of reproductive structures produced on TI viz. floral buds, open flowers and siliquae were recorded during the entire generative phase from randomly selected five plants growing in the central rows of each plot. The plants were harvested at maturity and the data on various yield parameters was recorded. The dry matter of TI and siliquae was recorded at weekly intervals starting from 26 days after anthesis (DAA) until maturity. Total chlorophyll content (Anderson and Boardman 1964) and Hill reaction activity of isolated chloroplasts (Cherry 1973) were determined in TI from 26 DAA till 56 DAA and siliqua wall from 10 days after flower opening (DAF) till 40 DAF stages of development. The mature seeds from control and treated plants were also analysed for total soluble sugars (Dubois *et al.* 1956), starch (Clegg 1956), total soluble proteins (Lowry *et al.* 1951), total free amino acids (Lee and Takahashi 1966) and oil content (Alexander *et al.* 1967). For comparing the structure of terminal inflorescence of control and SNP treated plants (100 and 200 µg ml^{-1}), segments of TI from middle portion of axis at 50 DAA stage were fixed in FAA and processed for microtomy (Sass 1958). For comparing the effects of treatments on various components, least square difference (LSD) was calculated using ANOVA for RBD (CPCS1 software package).

RESULTS AND DISCUSSION

Table 1 shows the effect of different SNP treatments on length of TI, yield and yield components. The length of TI improved in response to 100 and 200 µg ml^{-1} SNP treatments by about 2 and 11 per cent over control. The average number of floral buds per TI formed during the entire generative phase were not significantly influenced by different SNP treatments. However, number of buds developing into flowers and

Table 1. Influence of sodium nitroprusside (SNP-100, 200 and 500 µg ml^{-1}) on growth and yield parameters (per terminal inflorescence axis basis) of *Brassica napus* cv. GSL-1. The values are mean of 5 replicates ± SE

Characters	Control	SNP-100	SNP-200	SNP-500	$LSD_{0.05}$
Length of TI axis (cm)	77.2±2.08	78.6±3.14	85.6±2.38	45.9±3.07	0.05
Number of floral buds	76±0.92	78±0.42	79±0.64	80±0.51	n.s.
Number of open flowers	64±1.11	68±0.82	74±0.78	47±0.61	1.78
Number of total siliquae	56±2.42	65±0.63	69±1.11	43±0.58	1.78
Number of mature siliquae	50±2.11	60±1.69	66±0.98	41±0.81	1.27
Number of immature siliquae	6±1.51	5±1.21	3±1.39	2±0.94	n.s.
Seed number per siliqua	19±2.21	27±1.09	25±1.36	24±1.49	3.35
1000-seed weight(g)	3.05±0.34	3.30±0.42	3.19±0.31	3.15±0.21	0.11
Seed yield (g)	3.32±0.19	4.33±0.31	4.52±0.41	2.68±0.61	0.03

mature siliquae were significantly enhanced in response to 100 and 200 μg ml^{-1} SNP treatments, but 500 μg ml^{-1} SNP proved to be inhibitory. At final harvest, the per cent increase in the number of mature siliquae per TI in response to 100 and 200 μg ml^{-1} SNP treatments was about 20 and 32, respectively, over control. On the contrary, the seed number per siliqua and 1000-seed weight was significantly increased by all the tested concentrations of SNP with maximum enhancement being caused by 100 μg ml^{-1} SNP. Siliqua number and seed yield per TI was also increased with 100 and 200 μg ml^{-1} SNP treatments (30 and 36 per cent over control).

SNP treatments also significantly enhanced total dry matter of TI and its components during various stages of development (Fig. 1). Following 100, 200 and 500 μg ml^{-1} SNP treatments, the average increase in dry matter of TI and siliquae at final harvest was about 50, 25 and 4 per cent, respectively, over control. The increased dry matter accumulation in SNP- treated siliquae was contributed by an increase in seed number per siliqua and 1000-seed weight. Due to indeterminate growth habit, maximum seed yield in *Brassica* species is greatly influenced during initiation or progression of seed development (Clarke 1979). Further, sink strength of developing fruits and seeds is greatly influenced by hormones. The increase in number of mature seeds per siliqua and 1000-seed weight (seed size) by SNP seems to be the result of reduced ovule/seed abortion that possibly happened due to enhanced availability and translocation of assimilates towards developing seeds. It suggests possible involvement of this chemical not only in maintenance of sufficient supply of assimilates in these structures but also influences cell division and metabolic activities of growing embryos. Localised application of different plant growth regulators have been found to exert a profound influence on assimilate transport and enhance crop yields (Patrick and Mulligan 1987). Total chlorophyll content and Hill reaction activity of isolated chloroplasts increased during development and peaked maximum at 36 DAA in TI and 30 DAF in the siliqua walls followed by a decline thereafter (Figs. 2 and 3). SNP treatment at 100 μg ml^{-1} concentration was found to be most effective in eliciting this increase. Further, the loss in chlorophyll content during later stages of development was comparatively less in treated plants as compared to control. The dry matter production is intimately linked to the photosynthetic efficiency of crop plants. In *Brassica* species, besides leaves, siliqua wall and plant axis is photosynthetically very active (Setia and Setia 1990). The improvement in seed yield and alterations in yield contributing traits observed in the present study in response to SNP treatments are possibly the result of improving the photosynthetic activity and partitioning of assimilates towards reproductive sinks. NO donors have been reported to increase chlorophyll content in pea, potato, *Arabidopsis*, lettuce and dark

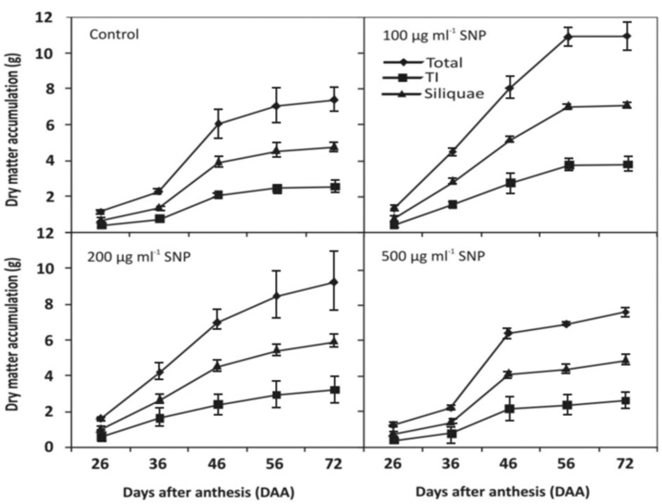

Fig. 1. Influence of sodium nitroprusside (SNP-100, 200 and 500 μg ml^{-1}) on dry matter accumulation in terminal inflorescence and its components during different stages of development in *Brassica napus* L. cv. GSL-1. Vertical bars represent the standard error

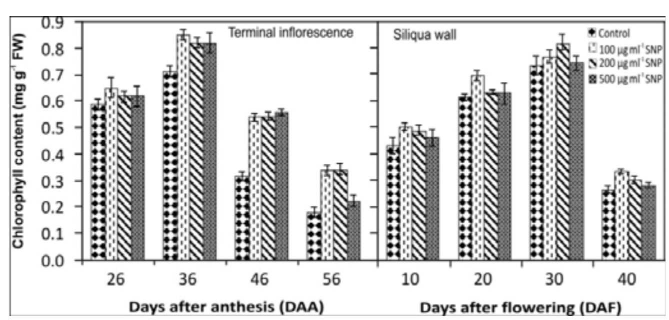

Fig. 2. Influence of sodium nitroprusside (SNP-100, 200 and 500 μg ml^{-1}) on chlorophyll content in terminal inflorescence axis and siliqua wall during different stages of development in *Brassica napus* L. cv. GSL-1. Vertical bars represent the standard error

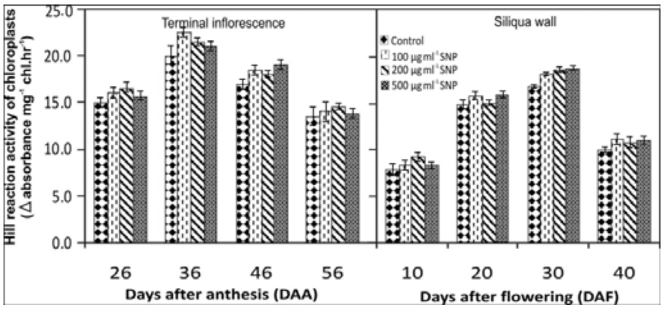

Fig. 3. Influence of sodium nitroprusside (SNP-100, 200 and 500 µg ml^{-1}) on Hill reaction activity of isolated chloroplasts in terminal inflorescence axis and siliqua wall during different stages of development in *Brassica napus* L. cv. GSL-1. Vertical bars represent the standard error

grown wheat seedlings (Beligni and Lamattina 2000) and retard chlorophyll loss in potato leaves infected with *Phytophthora* (Leshem 1996). According to Bambeyer and Mayer (1990) NO is required to accomplish synthesis of cytokinin induced pigment synthesis and may function as a gaseous plant hormone.

SNP treatments also improved seed quality by enhancing the accumulation of various seed storage reserves viz. total soluble sugars, starch, total soluble proteins, total free amino acids and oil content and 100 µg ml^{-1} SNP treatment proved to be most effective followed by 200 and 500 µg ml^{-1} SNP treatments (Table 2). This increase in level of various reserves, including oil in response to SNP treatments seems the result of enhanced availability of assimilates towards developing sinks (siliquae/seeds). The role of plant growth regulators in improving the nutritional status of seeds is well established (Setia *et al.* 1995). SNP treatments also influenced anatomical features of TI (Plate 1). The total transectional area occupied by the vascular bundles and associated area of xylem and phloem was significantly increased in response to SNP treatments (Table 3). However, their influence was differential and maximum increase in size of vessel element was caused by 200 µg ml^{-1} SNP, and that of phloem cells by 100 µg ml^{-1} SNP. NO donors are reported to influence cellular differentiation, especially xylogenesis (Gabaldon *et al.* 2005). Apparently, physiological and anatomical changes induced by SNP treatments have exerted positive influence on development of siliquae on inflorescence axis and thereby contributed towards overall yield potential improvement in *Brassica napus*. Moreover, the enhancement in yield and quality parameters in response to SNP treatments could also be the result of modifications at the hormonal levels as NO has been

Table 2. Influence of sodium nitroprusside (SNP-100, 200 and 500 µg ml^{-1}) on seed quality of *Brassica napus* cv. GSL-1. The values are mean of 5 replicates ± SE

Characters	Control	SNP-100	SNP-200	SNP-500	$LSD_{0.05}$
Total soluble sugars (mg g^{-1} DW)	45±3.19	57±1.61	54±2.17	47±2.41	3.32
Starch (mg g^{-1} DW)	38±1.49	46±2.31	43±2.36	41±1.13	2.67
Total soluble proteins (mg g^{-1} DW)	158±4.63	166±4.13	164±4.31	162±3.42	8.69
Total free amino acids (mg g^{-1} DW)	6±0.64	8.5±0.71	8.0±0.84	7.0±0.91	1.51
Oil content (%)	38.7±2.07	41.5±3.01	39.8±2.01	40.0±1.19	2.36

Table 3. Influence of sodium nitroprusside (SNP-100 and 200 µg ml^{-1}) on anatomical characters of terminal inflorescence axis of *Brassica napus* cv. GSL-1. The values are mean of 5 replicates ± SE

Characters	Control	SNP-100	SNP-200	$LSD_{0.05}$
Area of vascular bundle (mm^2)	2.56±0.65	7.27±0.49	11.26±0.71	1.11
Area of xylem (mm^2)	1.13±0.32	1.60±0.23	2.13±0.11	0.96
Area of phloem (mm^2)	0.36±0.17	0.62±0.19	0.52±0.09	0.21
Cell size of vessel element (µm^2)	60x10^3±1.12	88x10^3±2.16	130x10^3±2.18	0.32
Cell size of phloem element (µm^2)	13x10^3±2.21	26x10^3±3.12	17x10^3±3.20	0.46

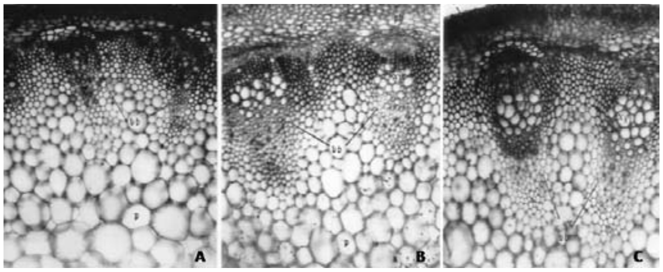

Plate 1. Transverse sections of terminal inflorescence (TI) of *Brassica napus* L. cv. GSL-1. (A) Control (B) 100 µg ml^{-1} SNP (C) 200 µg ml^{-1} SNP.

[Note: enhanced vascular tissue differentiation in 100 and 200 µg ml^{-1} SNP treated TI as compared to control (A – C x 150)]

reported to act as a second messenger in various metabolic processes to support plant development (Neill *et al.* 2003, Unsal and Arisan 2009).

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