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Influence of sodium nitroprusside, nitric oxide donor, on growth and development of fruit in *gobhi sarson (Brassica napus*)

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ABSTRACT

Foliar spraying of sodium nitroprusside (SNP), nitric oxide (NO) donor, on *gobhi sarson* [*Brassica napus* (cv. GSL 1)] plants, at green floral bud stage of terminal inflorescence ,exerted a profound effect on growth and metabolism of fruits(siliquae).SNP at all concentrations (50, 100, 200 and 400 µg/ml)caused significant increase in siliqua length and seed number/siliqua. In SNP-treated plants the siliquae were darker green, and during active phases of growth the siliqua wall and seed had higher total chlorophyll (chl) content, altered chl a/b ratios and greater Hill reaction activity of chloroplasts in comparison to control. This in turn resulted in increased accumulation of dry matter in siliquae of SNP-treated plants than controls. SNP enhanced the level of total soluble sugars, starch, total soluble proteins and free amino acids in both siliqua wall and seed during different developmental stages as compared to controls. The seed oil content increased by 2.27 percentage points with a treatment of 100 µg/ml SNP.

Key words: *Brassica napus*, Chlorophyll, Fruit, Nitric oxide, Siliqua, Sodium nitroprusside

NO has emerged as an important syncrhonising molecule in plant systems which mediates various pathophysiological and developmental processes, viz. expression of defense-related genes and programmed cell death, stomatal closure, seed germination and root development (Misra *et al*. 2011). In the past studies, research has provided evidences for the signaling role of this molecule at several unrelated biochemical nodes to control various aspects of growth and development (Ferreira and Cataneo 2010). Sodium nitroprusside (SNP), nitric oxide (NO) donor, is widely used to investigate the effects of NO in both plants and animals (Arasimowicz-Jelonek *et al*. 2011). Until now most of the studies with SNP were primarily aimed to explore the effects of NO at molecular or cellular levels but very little information is available about the role of NO at whole plant level. Thus an attempt was made to investigate the effects of NO using SNP on growth and development of plants at morphological, biochemical and physiological levels. The earlier studies using SNP on oilseed *Brassicas* revealed that SNP increased plant height, enhanced branching and improved yield contributing characters, with a consequent enhancement in seed yield (Jhanji *et al.* 2011) However, no detailed information is available regarding the influence of SNP on growth and metabolism of *Brassica* fruit. Therefore, the present investigation was carried out to study the impact of foliar spray of SNP at the green floral bud stage, on the fruit (siliqua) development in *Brassica napus* cv. (GSL-1) grown under field conditions.

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MATERIALS AND METHODS

The experiments were conducted in the field areas of the Department of Botany during the winter (*rabi*) seasons for two consecutive years (2007–09). The plants of *gobhi sarson* [*Brassica napus* (cv. GSL1)] were raised in randomized block design using three replicates. At the green floral bud stage of the terminal raceme, the plants were sprayed with SNP solutions at 50, 100, 200 and 400 µg/ml concentrations containing 0.05% (v/v) Triton X-100 as a surfactant. Three more spray treatments of different concentrations of SNP were repeated each at one week interval. The plants sprayed with water containing Triton X-100 served as controls.

The growth of siliquae was followed from flowering (i e opening of flower) until maturity (which was attained in about 55 days after flowering and the siliquae became yellowish brown) by collecting the siliquae at different stages of development from the middle flowering positions (13th, 14th and 15th from base) of the main inflorescence (terminal raceme). To ascertain the age of siliquae the flowers growing on these positions were tagged for several days in control and SNP-treated plants. Sampling of the tagged siliquae was carried in two ways, the first one at five days intervals for

recording data on siliquae length and the second one at 10 days intervals for recording data on dry matter changes and also for biochemical analysis of siliqua wall and seeds. The siliqua wall and seed ages are given as days after flowering (DAF). For the analysis of each growth parameter an average of 10 samples were observed from the pooled material for each growth stage. The length of siliqua was measured from base to tip with centimeter scale. The siliquae were carefully dissected at maturity to count the number of seeds/siliqua. Dry matter of siliquae was recorded after oven drying at 70°C for 72 hr.

For biochemical assays the siliquae, at different stages of development, were separated into siliqua wall and seed and analysed for chlorophyll(Anderson and Boardman 1964), the Hill reaction activity of chloroplasts (Cherry 1973), total soluble sugars(Dubois *et al.*1956) and starch(Clegg 1956), total free amino acids(Lee and Takahashi 1966) and proteins(Lowry *et al.*1951). Oil content in seed was determined by the method of Alexander *et al.* (1967). The fatty acid composition of oil was determined by the method Uppstorm and Johansons (1978).

The data were analyzed for analysis of variance by CPCS1 software. The treatment means were compared using Duncan's Multiple Range Test (DMRT) at 5%level of probability.

RESULTS AND DISCUSSION

Siliqua length

The siliqua length increased rapidly during early growth and at a slower rate thereafter (Table 1). The SNP treatments-significantly enhanced the siliqua length at all the stages of development and this change was apparent from early stage of siliqua growth. SNP at 50, 100, 200 and 400 µg/ml increased siliqua length at maturity with a tune of 10, 12, 13 and 9%, respectively. This dimensional change in siliquae with SNP might be due to the reported role of NO in elongation growth of plants by influencing cell division and cell enlargement processes (Gabaldon *et al.* 2005). NO donors, like SNP, have also been reported to induce elongation of maize root tip cells and cucumber explants (Fernández-Marcosa *et al.*2011).

Seed number/siliqua

The average number of seed which continued to grow until maturity in control plants was 18/siliqua (Fig 1). In 50, 100, 200 and 400 µg/ml SNP-treated plants the average number of seeds/siliqua significantly increased to 20, 21, 24 and 19 respectively (Fig 1).

The significant increase in number of seeds/siliqua in SNP treated plants is seemingly the result of reduced ovule/seed abortion that possibly occurred due to enhanced availability and translocation of assimilates towards developing seeds (Pahwa *et al.*2009a) This, further, might be the result of crosstalk between NO and plant growth regulators

Table 1 Influence of sodium nitroprusside (SNP-50, 100, 200 and 400 µg/ml) on length of siliqua (cm) in *Brassica napus* (cv. GSL 1) at different stages of development

Treatment (T)	Days after flowering (F)									
	5	10	15	20	25	30	35	40	45	50
Control	1.04±0.05 Ge	2.86±0.09 Fe	3.96±0.07 Ee	4.66±0.07 Dd	5.07±0.14 Ce	5.49±0.15 Be	5.58±0.10 Ae	5.59±0.11 Ae	5.60±0.09 Ae	5.60±0.10 Ae
50	1.17±0.07 Gc	3.73±0.17 Fc	4.22±0.12 Ec	5.24±0.11 Db	5.57±0.07 Cc	5.97±0.11 Bc	6.16±0.10 Ac	6.18±0.09 Ac	6.20±0.09 Ac	6.20±0.09 Ac
100	1.24±0.05 Gb	3.92±0.07 Fb	4.34±0.10 Eb	5.45±0.19 Da	5.64±0.12 Cb	6.15±0.13 Bb	6.24±0.15 Ab	6.26±0.16 Ab	6.27±0.15 Ab	6.27±0.15 Ab
200	1.31±0.18 Ga	4.02±0.13 Fa	4.44±0.14 Ea	5.45±0.10 Da	5.74±0.10 Ca	6.18±0.09 Ba	6.40±0.24 Aa	6.42±0.23 Aa	6.43±0.23 Aa	6.43±0.25 Aa
400	1.10±0.04 Gd	3.59±0.13 Fd	4.06±0.13 Ed	5.15±0.06 Dc	5.49±0.16 Cd	5.90±0.06 Bd	6.03±0.06 Ad	6.07±0.05 Ad	6.09±0.06 Ae	6.09±0.08 Ad

± indicates standard error

Different lower case letters indicate statistically significant differences between SNP treatments for the same stage of development and different uppercase letters show differences between stage of development for the same SNP treatment ($P < 0.05$)

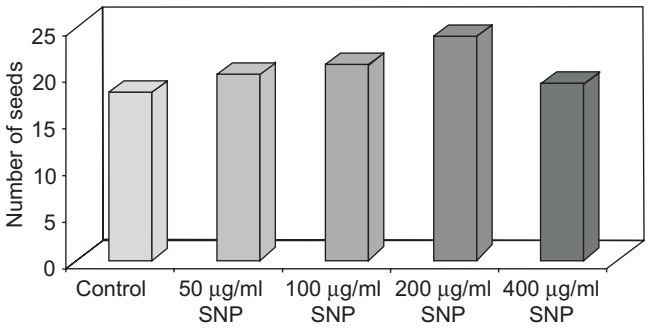

Fig 1 Influence of sodium nitroprusside (SNP-50, 100, 200 and 400 µg/ml) on number of seeds/siliqua at final harvest in *Brassica napus*(cv. GSL 1).

at several metabolic cascades to attain cellular homeostasis (Ferreira and Cataneo 2010)).

Chlorophyll content and hill reaction activity of chloroplasts in siliqua

The total chl content in siliqua wall significantly increased till 20 DAF and then decreased whereas it increased till 30 DAF in seed and then declined till maturity (Table 2). SNP enhanced the total Chl content of the siliqua wall and seed as compared to controls during all stages of development. Results demonstrated that 100 µg/ml SNP treatment had maximum Chl content in siliqua wall (0.82 mg/g fresh weight) and seed(0.99 mg/g fresh weight) at 20 and 30 DAF, respectively. Assays for Chl a and Chl b indicated that the content of chl a was more than chl b both in siliqua wall and seed (Table 2). The higher ratio of chla/b with SNP indicates greater photosynthetic efficiency of treated plants than control. Following SNP treatments, there was significant increase in Hill reaction activity of chloroplasts during stages of development with maximum effect at 20 DAF in siliqua wall

Table 2 Effect of sodium nitroprusside (SNP ,50, 100, 200, 400 µg/ml) on chlorophyll (Chl) content (mg/g fresh weight) and Hill activity (D mg chlorophyll/hr) in siliqua wall and seed at different stages (DAF) Of siliqua development in *Brassica napus* (cv GSL 1)

Stage of development (D)	Treatment (T)	Siliqua wall				Seed			
		Total chlorophyll	Chlorophyll a	Chlorophyll b	Hill activity	Total chlorophyll	Chlorophyll a	Chlorophyll a	Hill activity
10 DAF	Control	0.30±0.03	0.21±0.01	0.10±0.02	7.52±0.52	0.13±0.02	0.07±0.01	0.05±0.01	5.53±0.51
	50	0.35±0.01	0.24±0.03	0.11±0.02	13.8±0.86	0.26±0.02	0.15±0.01	0.11±0.02	10.00±0.64
	100	0.48±0.02	0.35±0.03	0.13±0.02	18.77±0.76	0.40±0.01	0.23±0.03	0.19±0.02	10.20±0.20
	200	0.45±0.02	0.33±0.03	0.12±0.02	16.24±0.75	0.37±0.01	0.20±0.01	0.17±0.02	9.88±0.61
	400	0.32±0.02	0.23±0.02	0.09±0.01	11.48±0.78	0.37±0.02	0.18±0.01	0.17±0.02	7.48±0.46
20 DAF	Control	0.49±0.03	0.31±0.02	0.17±0.01	16.72±1.11	0.43±0.02	0.23±0.02	0.19±0.02	16.27±0.56
	50	0.65±0.04	0.44±0.03	0.21±0.03	18.89±0.69	0.52±0.03	0.35±0.03	0.17±0.02	19.47±0.51
	100	0.82±0.05	0.54±0.05	0.28±0.03	24.93±0.66	0.77±0.04	0.51±0.04	0.26±0.03	22.06±0.16
	200	0.75±0.01	0.49±0.02	0.26±0.01	21.82±0.74	0.71±0.04	0.46±0.04	0.25±0.02	20.01±0.33
	400	0.60±0.03	0.41±0.04	0.19±0.03	17.17±0.52	0.63±0.04	0.40±0.04	0.23±0.03	17.07±0.24
30 DAF	Control	0.35±0.01	0.25±0.01	0.11±0.02	10.61±0.56	0.72±0.04	0.49±0.03	0.23±0.02	21.59±0.52
	50	0.46±0.03	0.29±0.02	0.17±0.02	14.87±0.76	0.79±0.05	0.55±0.04	0.24±0.03	24.56±1.27
	100	0.67±0.02	0.46±0.03	0.21±0.03	19.37±0.35	0.99±0.07	0.69±0.06	0.28±0.03	30.14±0.67
	200	0.59±0.04	0.40±0.03	0.18±0.01	20.86±0.69	0.92±0.06	0.65±0.06	0.28±0.03	27.41±0.73
	400	0.47±0.04	0.32±0.01	0.15±0.01	13.51±0.57	0.86±0.05	0.61±0.05	0.24±0.03	22.85±0.15
40 DAF	Control	0.17±0.02	0.15±0.01	0.02±0.01	4.03±0.53	0.27±0.03	0.14±0.01	0.12±0.01	10.17±0.45
	50	0.25±0.01	0.18±0.01	0.07±0.01	7.23±0.30	0.42±0.03	0.27±0.03	0.15±0.01	12.19±0.32
	100	0.35±0.03	0.21±0.02	0.14±0.01	9.70±0.50	0.59±0.05	0.38±0.03	0.19±0.02	14.20±0.32
	200	0.28±0.01	0.17±0.01	0.11±0.02	8.73±0.19	0.54±0.03	0.35±0.03	0.18±0.02	13.03±0.13
	400	0.22±0.01	0.15±0.01	0.06±0.01	6.37±0.56	0.48±0.03	0.30±0.02	0.18±0.02	11.18±0.45
	LSD (0.05)								
	T	0.01	0.02	0.02	0.50	0.01	0.02	0.02	0.43
	D	0.01	0.01	0.01	0.45	0.01	0.01	0.01	0.38
	T × D	0.03	0.03	0.03	1.01	0.03	0.03	0.03	0.86

and 30 DAF in seed with 100 µg/ml SNP treatments.

The increased level of Chl content and delayed reduction in loss of chlorophyll during later stages of development in siliqua of SNP treated plants is indicative of the role of NO in delaying senescence and, therefore, prolonging availability of assimilates in developing siliquae (Procházková and Wilhelmová 2011). NO donors stimulated de-etiolation and increased chlorophyll content in lettuce, potato, Arabidopsis and dark-grown wheat seedlings (Beligni and Lamattina 2000). Increased chlorophyll content, altered chl a and chl b content and enhanced Hill reaction activity of chloroplasts in the siliqua of SNP-treated plants indicate increased photosynthetic and metabolic activities (Pahwa *et al.* 2009b).

Accumulation of dry matter in siliqua

The increase in dry matter of total siliqua occurred at a slow rate up to 15 DAF but rapidly between 15 and 45 DAF and then again slowly until maturity, thus presenting a sigmoidal pattern (Fig 2). At maturity the significant increase in dry matter recorded with 50, 100, 200 and 400 µg/ml SNP treatments was about 22, 64,79 and 12%, respectively, over controls.

The dry matter production is intimately linked with photosynthetic efficiency of plants. As discussed earlier,

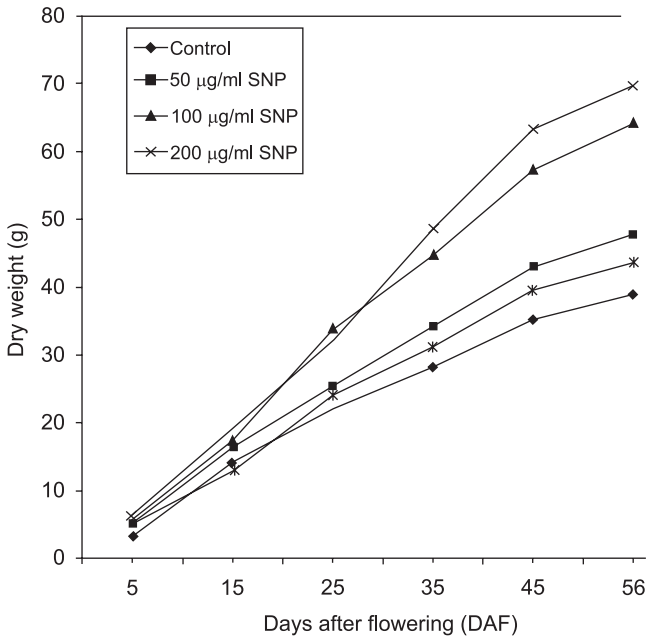

Fig 2 Influence of sodium nitroprusside (SNP-50, 100, 200 and 400 µg/ml) on dry weight of siliquae at different stages of development in *Brassica napus*(cv. GSL 1)

Table 3 Influence of sodium nitroprusside (SNP-50, 100, 200 and 400 µg/ml) on total soluble sugars (mg/g FW), starch (mg/g FW), total soluble proteins (mg/g FW) and total amino acids (mg/g FW) in siliqua wall at different stages of development in *Brassica napus* (cv. GSL 1)

Treatment	Biochemical reserve	Days after flowering (DAF)					
		5	15	25	35	45	56
Control	TSS	5.58±0.41(De)	26.22±0.21(Bd)	43.26±1.02(Ad)	19.63±0.72 (Ce)	5.61±0.54(Dd)	3.75±0.28 (Ec)
	Starch	5.38±0.24(Fe)	24.75±0.58(Ed)	65.77±0.71(Ae)	51.92±0.15(Be)	34.75±0.45(Cd)	30.47±0.44(De)
	Proteins	6.64±0.43(Fe)	23.01±0.15(Ce)	28.07±0.56(Be)	32.93±0.32(Ae)	15.85±0.22(Dd)	15.09±0.11(Ed)
	Amino acids	0.02±0.01(Ed)	0.18±0.01(Ce)	0.25±0.10(Ae)	0.23±0.02(Be)	0.11±0.01(De)	0.03±0.01(Ee)
50	TSS	6.65±0.35(Dd)	27.84±1.01(Bc)	45.22±0.68(Ac)	20.85±0.81 (Cd)	11.14±0.14(Ea)	5.59±0.44(Fb)
	Starch	6.46±0.06(Fd)	28.37±0.67(Ec)	73.38±0.34(Ad)	57.49±0.53(Bc)	39.15±0.38(Cc)	32.99±0.24(Dc)
	Proteins	7.06±0.08(Fd)	28.56±0.26(Cd)	30.50±0.65(Bd)	36.27±0.30(Ad)	22.36±0.22(Dc)	21.03±0.19(Eb)
	Amino acids	0.04±0.01(Dc)	0.27±0.01(Bd)	0.37±0.02(Ad)	0.27±0.04(Bd)	0.15±0.01(Cd)	0.05±0.02(Dd)
100	TSS	9.92±0.58(Da)	33.69±0.78(Ba)	53.50±1.02(Ab)	23.49±0.87(Cb)	8.19±0.34(Eb)	6.49±0.41(Fa)
	Starch	8.47±0.08(Fa)	36.41±0.48(Ea)	76.55±0.49(Aa)	59.61±0.63(Ba)	39.84±0.27(Ca)	38.83±0.25(Da)
	Proteins	10.04±0.47(Fa)	39.12±0.54(Ca)	44.18±0.40(Ba)	48.55±0.50(Aa)	28.92±0.48(Da)	21.55±0.56(Ea)
	Amino acids	0.07±0.01(Fa)	0.69 ±0.01(Ba)	0.98±0.02(Aa)	0.66±0.04(Ca)	0.32±0.03(Da)	0.14±0.03(Ea)
200	TSS	9.20±0.32(Eb)	32.79±0.26 (Bb)	53.44±0.70 (Ab)	21.63±0.58(Cc)	10.04±0.19 (Da)	5.75±0.30(Fb)
	Starch	9.30±.19(Fb)	33.71±0.15(Eb)	76.24±0.25(Ab)	59.21±0.39(Bb)	39.42±0.15(Cb)	35.88±0.70(Db)
	Proteins	8.08±0.17(Fc)	34.87±0.75(Cb)	35.96±0.19(Bc)	45.91±0.33(Ac)	28.15±0.67(Db)	21.48±0.44(Ea)
	Amino acids	0.06±0.02(Fab)	0.46±0.06(Bb)	0.62±0.10(Ab)	0.43±0.03(Cb)	0.21±0.02(Db)	0.09±0.02(Eb)
400	TSS	8.54±0.39(Dc)	32.61±1.31(Bb)	54.04±0.40(Aa)	26.33±0.45(Ca)	5.78±0.11(Ec)	5.27±0.30(Eb)
	Starch	7.57±0.15(Fc)	33.74±0.46(Db)	75.62±0.38(Ac)	56.82±0.37(Bd)	41.44±0.39(Cc)	31.52±0.49(Ed)
	Proteins	9.58±0.25(Eb)	38.24±0.31(Bc)	38.46±0.53(Bb)	46.56±0.33(Ab)	28.94±0.65(Ca)	20.41±0.51(Ec)
	Amino acids	0.05±0.01(Fbc)	0.42 ± 0.02(Bc)	0.59±0.02(Ac)	0.38 ±0.02(Cc)	0.18 ±0.01(Dc)	0.07±0.01(Ec)

* ± indicates standard error

*Different lower case letters indicate statistically significant differences between SNP treatments for the same stage of development and different uppercase letters show differences between stage of development for the same SNP treatment ($P < 0.05$)

SNP increased the chlorophyll content and Hill reaction activity of chloroplasts in siliqua wall and seed which increased the photosynthetic efficiency. This observation corroborates the enhanced dry matter accumulation in fruit.

Accumulation of reserves in siliqua total soluble sugars and starch

The levels of TSS and starch in siliqua wall (Table 3) and seed (Table 4) increased until 25 DAF and showed a decline thereafter until the end of siliqua growth. The levels of TSS and starch remained significantly higher in siliqua wall and seed of SNP treated plants at all stages of development, as compared to their respective controls. Compared to TSS, the level of starch remained high in siliqua wall and seed after 25 DAF. In mature seeds of 100 µg/ml SNP treated plants the increase in sugar and starch content was about 12 and 58%, respectively over the controls.

Proteins and free amino acids

In both siliqua wall (Table 3) and seed (Table 4) the level of total soluble proteins increased up to 25 DAF and then declined until the end of siliqua maturity. SNP significantly enhanced the protein content in both structures during different stages of development. In mature seeds ,the increase in protein content caused by 50, 100, 200 and 400 µg/ml SNP treatments was 21, 61, 47 and 47%, respectively, over the controls. Compared to other reserves, the level of total free amino acids in siliqua wall (Table 3) and seed (Table 4) remained very low. SNP treatments significantly increased the level of total free amino acids at all stages of development. The maximum enhancement in total free amino acids was 39 and 69% in siliqua wall and seed following 100 µg/ml SNP treatment.

Seed oil content and fatty acid composition of oil

The oil content of seeds of SNP treated plants increased as compared to controls at maturity (Table 5). The SNP at a concentration of 100 µg/ml caused maximum enhancement in the seed oil content which was by 2.23 percentage points over control. The fatty acid composition of oil was slightly

Table 4 Influence of sodium nitroprusside (SNP-50, 100, 200 and 400 µg/ml) on total soluble sugars (mg/g FW), starch (mg/g FW), total soluble proteins (mg/g FW) and total amino acids (mg/g FW) in seed at different stages of development in *Brassica napus* (cv. GSL 1)

Treatment	Biochemical reserve	Days after flowering (DAF)					
		5	15	25	35	45	56
Control	TSS	9.90±0.40(Fc)	37.18±0.31(Be)	70.31±0.51(Ae)	25.04±0.58(Cd)	19.24±0.38(Dd)	16.41±0.44(Ee)
	Starch	5.10±0.19(Fe)	35.26±0.69(Ce)	82.02±0.19(Ae)	42.98±0.57(Bd)	27.14±0.48(Dd)	24.49±0.57(Ee)
	Proteins	13.04±0.39(Fe)	30.92±0.54(Ce)	41.03±0.49(Be)	49.03±0.08(Ad)	16.34±0.41(De)	15.77±0.33(Ee)
	Amino acids	0.38±0.05(Ed)	2.86±0.10(Bd)	3.08±0.09(Ad)	2.33±0.21(Ce)	0.36±0.01(Db)	0.12±0.02(Fc)
50	TSS	11.81±0.67(Fb)	43.53±1.13(Bd)	76.63±0.43(Ad)	25.22±0.29(Cc)	20.20±0.56(Dc)	17.11±0.22(Ed)
	Starch	8.40±0.37(Fc)	40.15±0.77(Dc)	93.87±0.61(Ac)	54.47±0.43(Bb)	42.81±0.39(Cb)	30.77±1.07(Ec)
	Proteins	16.15±0.70(Fc)	35.06±0.21(Cd)	43.97±0.21(Bd)	57.93±0.33(Ab)	23.11±0.33(Dd)	19.25±0.30(Ed)
	Amino acids	0.74±0.09(Cc)	3.04±0.05(Bc)	3.63±0.11(Ac)	2.83±0.05(Bd)	0.36±0.04(Db)	0.36±0.05(Da)
100	TSS	13.83±0.37(Fa)	47.94±0.72(Ba)	83.05±0.46(Aa)	46.43±0.50(Ca)	27.66±0.23(Da)	18.74±0.86(Ea)
	Starch	9.78±0.30(Fa)	42.77±1.03(Da)	98.58±0.86(Ab)	55.09±0.24(Ba)	47.35±0.35(Ca)	37.80±0.56(Ea)
	Proteins	19.15±0.17(Fa)	48.00±0.51(Ca)	52.95±0.67(Ba)	59.82±0.38(Aa)	30.86±0.39(Da)	27.47±0.50(Ea)
	Amino acids	1.06±0.09(Ca)	4.87±0.11(Ba)	5.68±0.17(Aa)	4.76±0.17(Ba)	0.54±0.04(Da)	0.24±0.04(Eb)
200	TSS	11.73±0.57(Fb)	46.83±0.66(Bb)	82.06±0.53(Ab)	32.76±1.21(Cb)	24.59±1.13(Db)	18.26±0.68(Eb)
	Starch	9.02±0.08(Fb)	41.01±0.32(Db)	95.14±1.09(Aa)	54.61±0.82(Bb)	43.04±0.55(Cb)	36.10±0.63(Eb)
	Proteins	17.31±0.43(Fb)	46.18±0.24(Cb)	49.49±0.46(Bb)	57.86±0.36(Ab)	28.46±0.44(Db)	23.16±0.61(Eb)
	Amino acids	0.90±0.07(Db)	3.95±0.19(Bb)	4.53±0.13(Ab)	3.47±0.12(Cb)	0.46±0.06(Ea)	0.24±0.05(Fb)
400	TSS	10.15±0.48(Fc)	44.87±0.14(Bc)	79.55±1.18(Ac)	25.55±0.61(Cc)	20.37±0.34(Dc)	17.74±0.25(Ec)
	Starch	6.76±0.51(Fd)	37.08±0.209Cd0	88.23±0.41(Ad)	48.24±0.53(Bc)	34.18±0.41(Dc)	30.14±0.45(Ed)
	Proteins	15.52±0.49(Fd0	42.00±0.39(Cc)	48.23±0.249Bc)	54.00±0.77(Ac)	26.33±0.45(Dc)	21.58±0.48(Ec)
	Amino acids	0.89±0.08(Db)	3.92±0.09(Bb)	4.46±0.07(Ab)	3.09±0.02(Cc)	0.52±0.01(Da)	0.24±0.02(Eb)

* ± indicates standard error

*Different lower case letters indicate statistically significant differences between SNP treatments for the same stage of development and different uppercase letters show differences between stage of development for the same SNP treatment ($P < 0.05$)

Table 5 Influence of sodium nitroprusside (SNP-50, 100, 200 and 400 µg/ml) on total oil content, fatty acid composition (% of total) of oil, oleic/linoleic ratio (O/L) and iodine value (IV) in mature seeds of *Brassica napus* (cv. GSL 1)

Treatment	Fatty acid composition of oil									
	% oil content	Palmitic acid (16:0)	Stearic acid (18:0)	Oleic acid (18:1)	Linoleic acid (18:2)	Linolenic acid (18:3)	Eicosenic acid (20:1)	Erucic acid (22:1)	Oleic/ linoleic ratio	Iodine value
Control	40.13	3.41	0.58	16.88	14.33	7.94	9.84	46.64	1.18	47.07
50 µg/ml SNP	41.06	3.54	0.45	17.58	14.336	8.77	9.73	45.96	1.22	47.58
100µg/ml SNP	42.40	3.57	0.73	18.74	14.23	8.06	9.39	45.14	1.32	48.14
200µg/ml SNP	41.73	3.66	0.33	18.31	14.04	8.45	9.82	45.37	1.30	47.86
400µg/ml SNP	41.60	3.56	0.58	17.95	14.01	7.85	10.13	45.61	1.28	47.66

altered with SNP treatments (Table 5). In control, the oleic/ linoleic (O/L) ratio was 1:18 which increased to 1.22, 1.32, 1.30 and 1.28 with 50, 100, 200 and 400 µg/ml SNP treatment, respectively. The iodine values also showed an increase from 47.07 in control to 47.58, 48.14, 47.86 and 47.66, respectively, in 50, 100, 200 and 400 µg/ml SNP treated plants.

The SNP enhanced the levels of TSS, starch, proteins and amino acids in siliquae (siliqua wall and seed). Increased carbohydrate levels in siliqua at early stages of development mean that there is increased energy to support seed development. High protein content in these structures during development is indicative of high metabolic status. Several studies in *Brassica* spp revealed that plant growth regulators affect accumulation of storage reserves in seeds via different metabolic pathways (Ghai *et al.* 1996). The SNP has been reported to influence glucose metabolism in mungbean leaves and pea leaves (Ganjewala *et al.* 2008).

Thus, spraying of SNP as a source of NO exerted a profound effect on siliqua growth and altered the levels of various seed reserves. The most significant effect on siliqua development was observed with 100 µg/ml SNP treatment. Though, NO has been shown to exert a variety of effects but little is known about their underlying mechanisms (Šírová *et al*. 2011). However, to understand the molecular basis of mechanism of action of NO in regulation of various morpho-physiological and structural traits, new high throughput gene expression analysis techniques and system-wide approaches are highly desired.

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