Plant Archives Vol. 18 No. 1, 2018 pp. 737-744 ***ISSN 0972-5210***

EFFECT OF NITRIC OXIDE AND ABSCISIC ACID ON GROWTH DETERMINANTS IN *BRASSICA JUNCEA* CULTIVARS SUBJECTED TO PEG-INDUCED WATER STRESS

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Abstract

The aim of the present study was to evaluate the effect of different concentrations of sodium nitoprusside [SNP (10, 50, 100, 150 and 200 µM)], and abscisic acid [ABA (0.1, 1, 10, 50 and 100 µM) in absence (-) and presence (+) of polyethylene glycol 6000 [PEG 6000 (10%)]-induced water stress on growth determinants, in 7 days old hydroponically grown two *Brassica juncea* cultivars (Pusa Jagannath and Varuna), subjected to 96 hrs exposure. Results revealed that the growth determinants viz., shoot-root length, shoot-root fresh and dry weight and other root development related parameters decreased under PEG, as compared to control. The effect of SNP and ABA with or without PEG was concentration dependent. In particular, SNP100µM and ABA10µM treatments were optimum, and had efficient effect on promoting the maximum growth determinants of both *Brassica* cultivars in absence (-PEG) as well as in the presence of (+PEG) of water stress. However, the application of SNP150µM, SNP200µM, ABA50µM and ABA100µM had no obvious alleviating effect on PEG toxicity, as well as could not enhance growth in both cultivars when applied even alone.

Key words : PEG, water stress, NO, ABA, *Brassica juncea* L.

Introduction

Drought is one of the most severe environmental stress conditions that restrict plant growth and yield (Chaves *et al*., 2009). Drought stress alone affected major losses in economic yield in agricultural sector (Farooq *et al*., 2009; Suzuki *et al*., 2014). Indian mustard (*Brassica juncea* L.) is one of the most important and economic oilseed crops, and vulnerable to drought stress due to growing mainly in the arid and semiarid areas. It is an amphidiploids species (AABB) with a chromosome number 18 (2N=36), is developed by the inter-specific hybridization of two parental diploid (or primary) *Brassica* species, namely *B. nigra* (AA) (n=8) and *B. rapa* (BB) (n=10). The extent of drought stress is accompanied by the decrease in osmotic potential. PEG 6000 is a non-penetrating osmotic agent and, generally, it is very commonly used reliable marker modify the osmotic potential and to induce controlled water stress in the laboratory condition similar to that imposed by a drying soil in the field condition (Verslues *et al*., 2006; Benesova *et al*., 2012).

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Nitric Oxide (NO) is a small, highly diffusible gaseous redox molecule and produces endogenously in plants. It is nitrogen based chief reactive nitrogen species (RNS). Being a free radical (NO*), it has the potential in regulating multiple biological signalling responses in a variety of plants (Sahay *et al*., 2017). Studies using exogenous NO donors showed both promoting and inhibiting effect depending upon high and low concentration, respectively (Qiao *et al*., 2008; as reviewed by Santisree *et al*., 2015). Increasing NO concentration has deleterious effects resulted from imbalance production of NO along with other RNS against antioxidants, causes nitrosative stress. Interplay between NO and associated RNS and ROS radicals overlap together in imbalance manner, resulted in combination of both nitrosative and oxidative stress, collectively known as nitro-oxidative stress. Therefore, higher NO concentration may act in similar ways as that of ROS in plants.

The plant hormone abscisic acid (ABA) is ubiquitous in all flowering plants, and recognised as primary stress phyto-hormone. It has been documented that ABA coordinates an array of functions such as root development, stomata regulation, seed germination and dormancy, leaf senescence etc (Harris, 2015; Sah *et al*., 2016). Additionally, ABA application regulates the resistance to abiotic stresses including drought stress and enable plants to develop tolerance against stress condition. Several studies have revealed that exogenous ABA treatment enhance plant growth and development associated with drought tolerance/adaptation by modulation in morphological and physiological parameters (Zhang *et al*., 2012; Wei *et al*., 2015). However, the modulating role of ABA is dependent on the level of ABA applied to the plants. As such, high concentration of external ABA reduced the growth and yield of various crop plants (Morgan, 1980; as reviewed by Saradadevi *et al*., 2017). Therefore, the screening study of various types of treatment along with their concentrations, duration etc in respect to natural plant genotypes variability may useful to find out the suitable combination of the these multiple factors at which plants may have their optimum performance to growth and development. The present study was designed with different concentrations of SNP and ABA to determine how their concentrations affect plant growth in two *Brassica* cultivars (Pusa Jagannath and Varuna) and combating the drought stress at its optimum.

Materials and Methods

A hydroponic experiment was carried out in a controlled growth culture room with a 16 hrs light/8 hrs dark photoperiod, the temperature of 25 ± 2°C and the relative humidity of 70%. Healthy seeds of two Indian mustard (*Brassica juncea* L.) cultivars *viz*., Pusa Jagannath and Varuna were procured from Indian Agricultural Research Institute (IARI), Pusa, New Delhi, India. Seeds were surface sterilized in 30% ethanol (v/v) for a few minutes (3-4 min) followed by washing several times with distilled water. Sterilized seeds were soaked in distilled water for overnight. Equal numbers of seeds (10 seeds) were transferred on the moist cotton bed in petri-plates and kept for germination in dark at 25^0C. Germinated seedlings were transferred into small plastic pots containing 5% Hoagland medium (HM) solution and allowed to grow up to a week. After a week (seven days), seedlings were treated with different concentrations of sodium nitroprusside [SNP (10, 50, 100, 150 and 200 μM)] and abscisic acid [ABA (0.1, 1, 10, 50 and 100 μM)] in absence (-) and presence (+) of polyethylene glycol 6000 [PEG 6000 (10%)] for 96 hrs. PEG 6000 at 10% was added to HM solution to mimic water stress by the gradual decrease in its osmotic potential. Among different concentration (10-50%) of PEG 6000, 10% was screen out which was believed to induce low drought toxicity on the basis of germination percentage (data not shown). SNP, a NO donor, $[Na_2\{Fe(CN)_5NO\}.2H_2O]$ was used to release NO^+ ions in growth medium. At harvest, untreated and treated seedlings were measured for their growth variables such as shoot length (SL), root length (RL), shoot fresh weight (SFW), root fresh weight (RFW), shoot dry weight (SDW) and root dry weight (RDW). Length was measured using metric scale (cm). Fresh weight (g) was recorded just after harvesting of seedlings, while dry weight was recorded after drying the seedlings in an oven. Root development related parameters such as, root volume (RV), root length ratio (RLR= RL/Plant DW), root mass ratio (RMR= RDW/ Plant DW), root fitness (RFit= RL/RV), root tissue density (RTD= RDW/RV), root water content (RWC= RGW-RDW/RFW × 100) were also measured as described by Pandey *et al*. (2016). The experiment was set up and treatments were arranged according to complete randomized block design (CRBD).

Statistical analysis

Data are presented as mean ± SE of three different replicates (n = 3). All the data were analysed using ANOVA test using SPSS17.0. Duncan multiple range test (DMART, p≤ 0.05) was performed to compared the significance difference between treatment means, marked by different letters in figures. The graphs were plotted using scientific software ORIGIN 6.1.

Results and Discussion

The shoot and root growth parameters decreased significantly under PEG-induced water stress, while the effect of different concentrations of SNP and ABA in absence and presence of PEG-induced water stress was concentration dependent in both *Brassica* cultivars (figs. 1-6, table 1).

PEG-induced water stress decreased SL, SFW, SDW, RL, RFW, RDW, RV, RLR, RMR, RFit and RTD by 27.2%, 57.7%, 40.0%, 17.1%, 37.5%, 28.6%, 45.6%, 26.3%, 48.5%, 17.0% and 18.1%, respectively in Pusa Jagannath cultivar, while 52.0%, 55.1%, 60.0%, 61.4%, 47.3%, 28.6%, 47.0%, 12.0%, 39.0%, 27.0% and 30.0%, respectively in Varuna cultivar, over their respective control. The results are similar to earlier reports in other plants such as *Arabidopsis*, barley, wheat, Indian mustard etc exposed to drought stress or other abiotic stress such as arsenic stress (Shi *et al*., 2014; Chen *et al*., 2015; Pandey *et al*., 2016; Zhao *et al*., 2016). Among the different concentration of SNP in absence of PEG [SNP(-

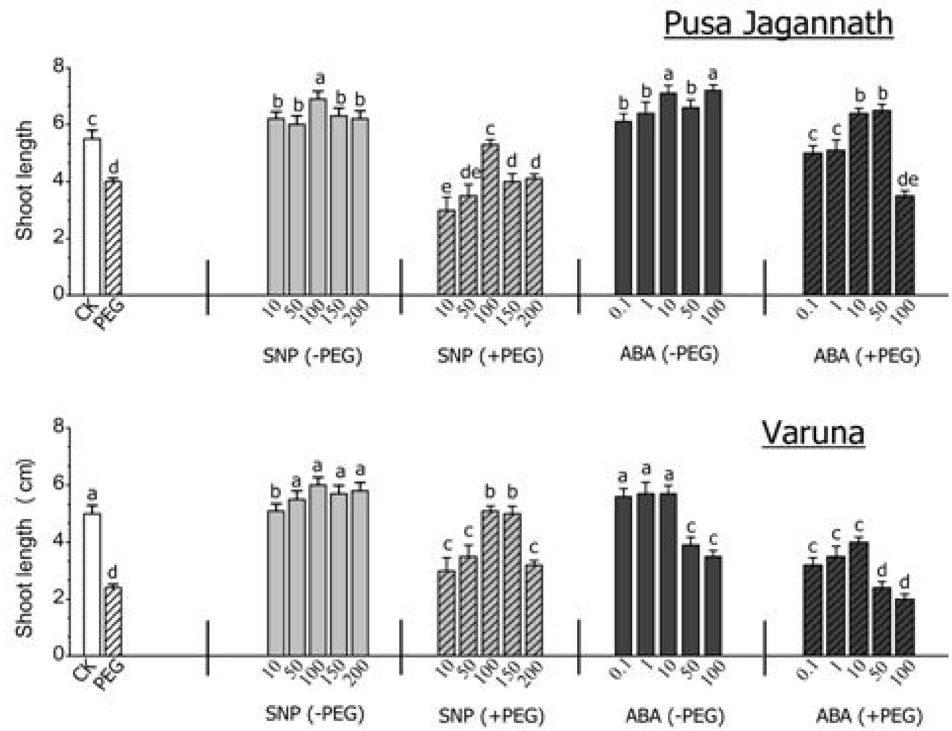

Fig. 1 : Effect of different concentrations of sodium nitroprusside (SNP, 10-200µM) and abscisic acid (ABA, 0.1-100µM) with and without polyethylene glycol (PEG, 10%) induced water stress on shoot length of two *Brassica* cultivars, Pusa Jagannath and Varuna. Different letters represents the significance differences between control and treatment means. CK= Control.

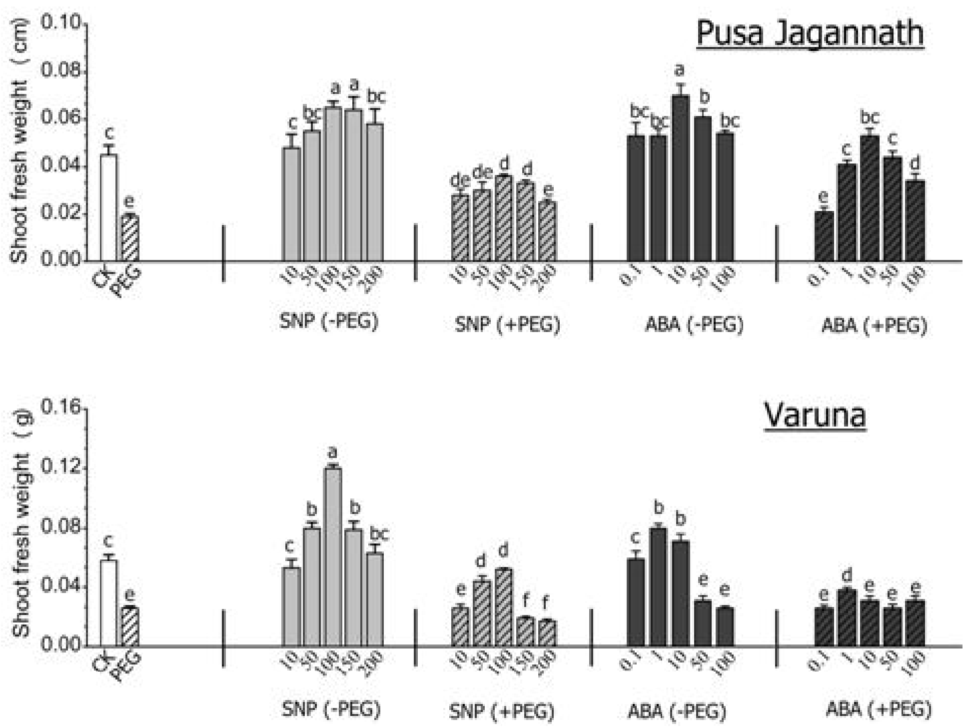

Fig. 2 : Effect of different concentrations of sodium nitroprusside (SNP, 10-200µM) and abscisic acid (ABA, 0.1-100µM) with and without polyethylene glycol (PEG, 10%) induced water stress on root length of two *Brassica* cultivars, Pusa Jagannath and Varuna. Different letters represents the significance differences between control and treatment means. CK= Control.

Table 1 : Effect of different concentrations of sodium nitroprusside (SNP, 10-200iM) and abscisic acid (ABA, 0.1-100iM) with and without 10% polyethylene glycol (PEG, 10%) induced water stress on roots traits viz., root volume, root length ratio, root mass ratio, root fitness and root tissue density of two *Brassica* cultivars, Pusa Jagannath and Varuna. Different letters represents the significance differences between control and treatment means. CK= Control.

Treatments		Root volume (cm^3)		Root length ratio ($cm\ g^{-1}$)		Root mass ratio ($g\ g^{-1}$)		Root fitness ($cm\ cm^{-3}$)		Root tissue density ($g\ cm^{-3}$)	
		Pusa Jagannath	**Varuna**	**Pusa Jagannath**	**Varuna**	**Pusa Jagannath**	**Varuna**	**Pusa Jagannath**	**Varuna**	**Pusa Jagannath**	**Varuna**
	CK	0.092^{c}	0.085^{c}	2372.5^{c}	1228.0^{e}	0.200^{d}	0.200^{d}	91.6^{c}	82.3^{d}	0.010^{b}	0.011^{d}
	PEG10%	0.050^{e}	0.045^{e}	1746.6^{e}	1080.0^{f}	0.103^{g}	0.122^{i}	76.0^{e}	60.0^{e}	0.007^{e}	0.009^{f}
	SNP10	0.091^{c}	0.065^{d}	1956.2^{d}	1282.6^{c}	0.230^{c}	0.149^{g}	87.5^{c}	90.7^{c}	0.006^{f}	0.009^{f}
	SNP50	0.110^{ab}	0.095^{b}	2615.3^{b}	1450.4^{d}	0.259^{b}	0.188^{e}	97.0^{c}	94.7^{c}	0.005^{g}	0.011^{d}
-PEG	SNP100	0.120^{a}	0.105^{b}	3411.7^{a}	1475.7^{d}	0.294^{a}	0.230^{c}	116.3^{b}	98.0^{c}	0.010^{b}	0.014^{b}
	SNP150	0.112^{ab}	0.102^{b}	1891.6^{d}	1040.8^{f}	0.183^{e}	0.230^{c}	87.9^{c}	50.0^{f}	0.008^{d}	0.008^{g}
	SNP200	0.110^{ab}	0.063^{d}	1734.1^{e}	864.8^{f}	0.189^{e}	0.180^{e}	91.1^{c}	63.4^{e}	0.006^{f}	0.010^{e}
	SNP10	0.060^{e}	0.060^{d}	1842.1^{d}	1025.6^{g}	0.210^{c}	0.130^{h}	74.7^{e}	53.3^{f}	0.010^{b}	0.008^{g}
	SNP50	0.080^{d}	0.080^{c}	2162.1^{de}	787.2^{h}	0.230^{d}	0.180^{e}	81.8^{d}	46.2^{f}	0.010^{b}	0.011^{d}
+PEG	SNP100	0.100^{b}	0.094^{b}	2615.3^{b}	1384.6^{d}	0.230^{c}	0.230^{c}	113.0^{b}	63.2^{e}	0.012^{a}	0.013^{c}
	SNP150	0.091^{c}	0.068^{d}	1865.3^{de}	1102.5^{f}	0.189^{e}	0.183^{e}	75.8^{e}	58.0^{ef}	0.007^{e}	0.012^{cd}
	SNP200	0.079^{d}	0.062^{d}	1945.9^{d}	786.8^{h}	0.189^{e}	0.154^{fg}	63.6^{f}	51.0^{f}	0.008^{d}	0.009^{f}
	ABA0.1	0.110^{ab}	0.092^{bc}	1783.7^{e}	1777.7^{c}	0.200^{d}	0.215^{cd}	60.0^{f}	80.0^{d}	0.006^{f}	0.011^{d}
	ABA1	0.110^{ab}	0.090^{bc}	1870.3^{de}	2586.2^{b}	0.210^{d}	0.259^{b}	91.8^{c}	91.4^{c}	0.007^{e}	0.010^{e}
-PEG	ABA10	0.125^{a}	0.120^{a}	2592.8^{b}	2800.0^{a}	0.259^{b}	0.310^{a}	112.0^{b}	166.6^{a}	0.012^{a}	0.019^{a}
	ABA50	0.122^{a}	0.062^{d}	2000.0^{d}	1509.8^{d}	0.210^{d}	0.200^{d}	135.8^{a}	112.0^{b}	0.006^{f}	0.012^{cd}
	ABA100	0.053^{e}	0.062^{d}	2057.1^{d}	1382.9^{d}	0.142^{f}	0.148^{g}	62.2^{f}	77.0^{d}	0.009^{c}	0.011^{d}
	ABA0.1	0.057^{e}	0.060^{d}	1763.1^{e}	1250.0^{c}	0.152^{f}	0.166^{f}	78.5^{e}	73.9^{de}	0.008^{d}	0.009^{f}
	ABA1	0.105^{b}	0.082^{c}	2440.0^{c}	1460.0^{d}	0.189^{e}	0.200^{d}	63.8^{f}	75.0^{de}	0.007^{e}	0.011^{d}
+PEG	ABA10	0.121^{a}	0.100^{b}	2925.9^{b}	1743.5^{c}	0.259^{b}	0.230^{c}	107.0^{b}	81.1^{d}	0.009^{c}	0.014^{b}
	ABA50	0.110^{ab}	0.058^{d}	2250.0c	847.8^{h}	0.180^{e}	0.152^{g}	71.8^{f}	67.7^{e}	0.006^{f}	0.010^{e}
	ABA100	0.109^{ab}	0.042^{e}	1557.3^{f}	711.8^{i}	0.166^{f}	0.130^{h}	49.5^{g}	62.9^{e}	0.003^{g}	0.009^{f}

PEG)], SNP100µM gave maximum enhancement in all the growth parameters as compared to other SNP treatments over control, similar to earlier report by Gan *et al*. (2015). SNP100µM significantly increased SL by 25.4% and 20.0%, SFW by 44.4% and 106%, SDW by 150.0% and 20.0%, RL by 57.1% and 47.1%, RFW by 150.0% and 36.0%, RDW by 85.7% and 57.1%, RV by 30.4% and 20.0%, RLR by 43.8% and 20.1%, RMR by 47.0% and 15.0%, RFit by 26.9% and 19.0%, and RTD by 27.2% and 0.0% in Pusa Jagannath and Varuna, respectively over their respective control. Similarly, in the presence of PEG, SNP100µM treatment proved better treatment by maximally overcome the PEG-induced loss in all the growth parameters. SNP100µM (+PEG) showed an increase by 32.5% and 112.5% in SL, 89.4% and 100% in SFW, 233.3% and 150.0% in SDW, 67.2% and 77.7% in RL, 260.0% and 110.0% in RFW, 14.0% and 12.0% in RDW, 100% and 133.3% in RV, 49.7% and 28.2% in RLR, 123.3% and 88.5% in RMR, 48.6% and 5.33% in RFit, and 44.4% and 71.4% in RTD for Pusa Jagannath and Varuna, respectively over their respective PEG alone (figs. 1-6, table 1). The above results suggest that SNP (a NO donar) is modulating the adverse effect of PEG-induced water stress, which might be due to that NO may act as scavenger of toxic reactive oxygen species produced in stress response. The involvement of nitric oxide donar in alleviating water stress and enhance growth biomass

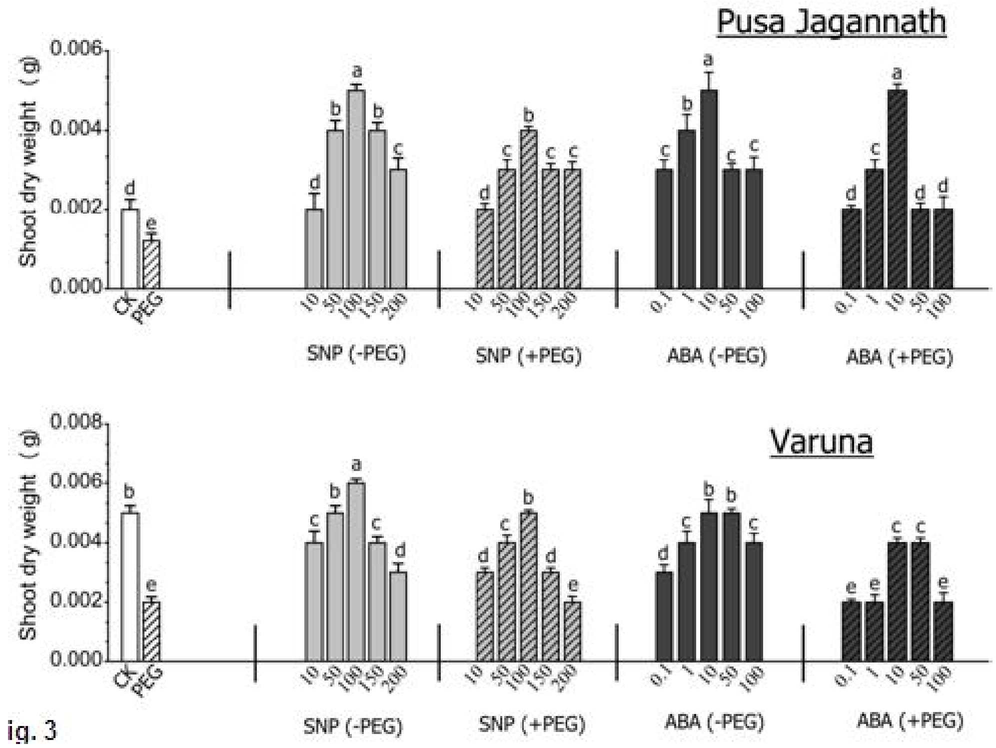

Fig. 3 : Effect of different concentrations of sodium nitroprusside (SNP, 10-200μM) and abscisic acid (ABA, 0.1-100μM) with and without polyethylene glycol (PEG, 10%) induced water stress on shoot fresh weight of two *Brassica* cultivars, Pusa Jagannath and Varuna. Different letters represents the significance differences between control and treatment means. CK= Control.

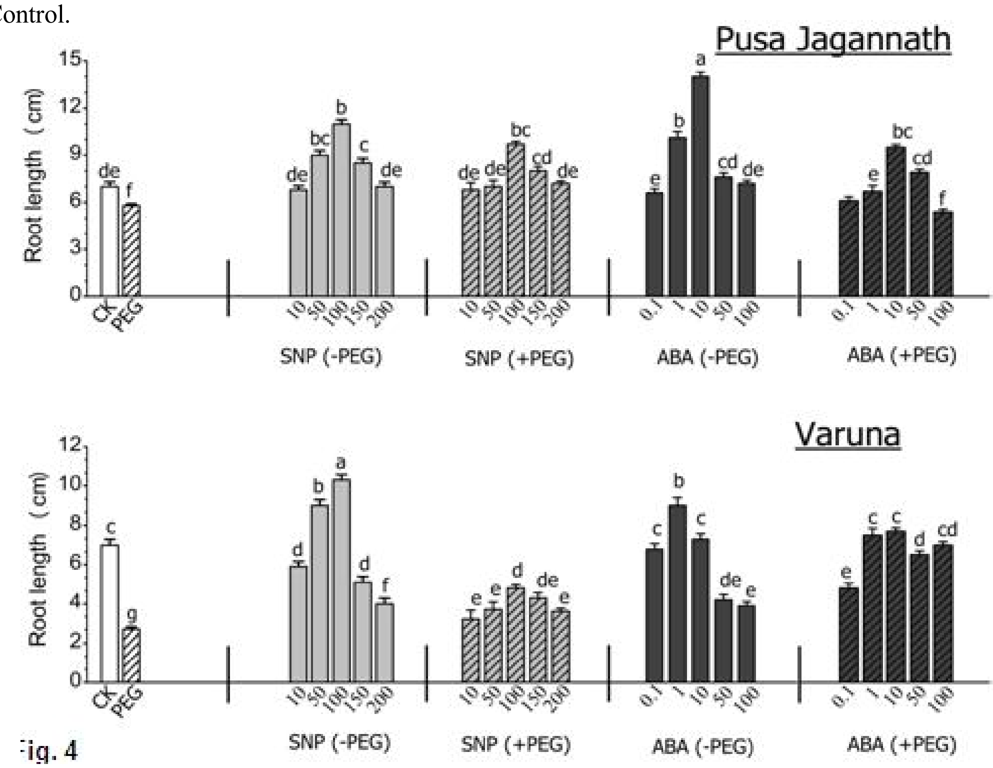

Fig. 4 : Effect of different concentrations of sodium nitroprusside (SNP, 10-200μM) and abscisic acid (ABA, 0.1-100μM) with and without polyethylene glycol (PEG, 10%) induced water stress on root fresh weight of two *Brassica* cultivars, Pusa Jagannath and Varuna. Different letters represents the significance differences between control and treatment means. CK= Control.

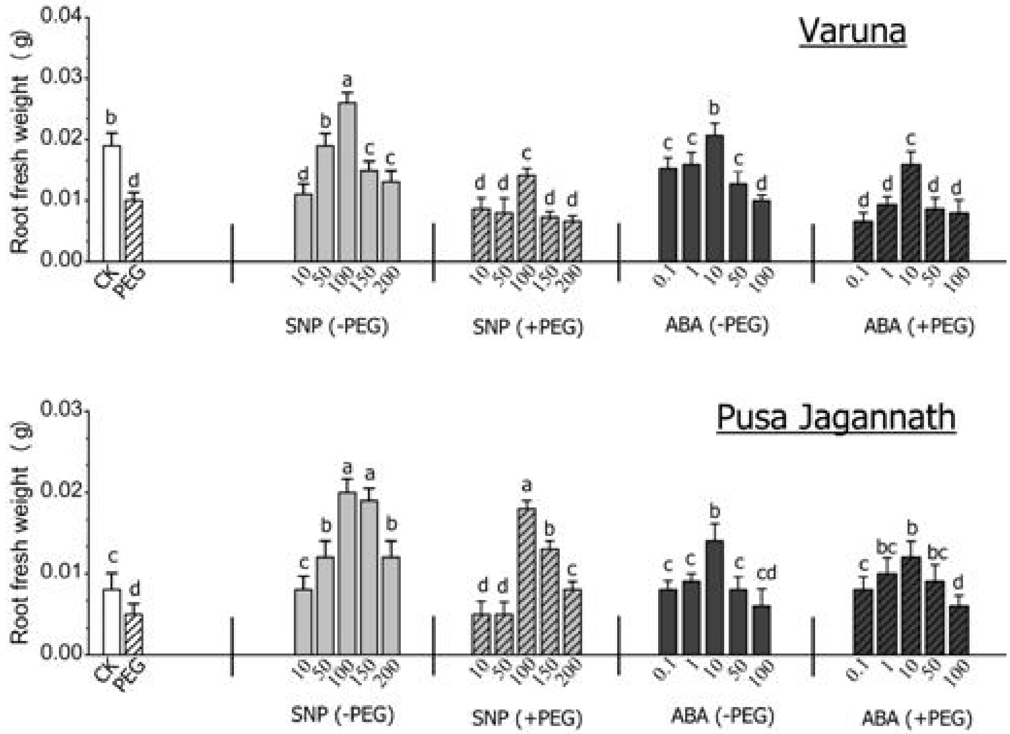

Fig. 5 : Effect of different concentrations of sodium nitroprusside (SNP, 10-200µM) and abscisic acid (ABA, 0.1-100µM) with and without polyethylene glycol (PEG, 10%) induced water stress on shoot dry weight of two *Brassica* cultivars, Pusa Jagannath and Varuna. Different letters represents the significance differences between control and treatment means. CK= Control.

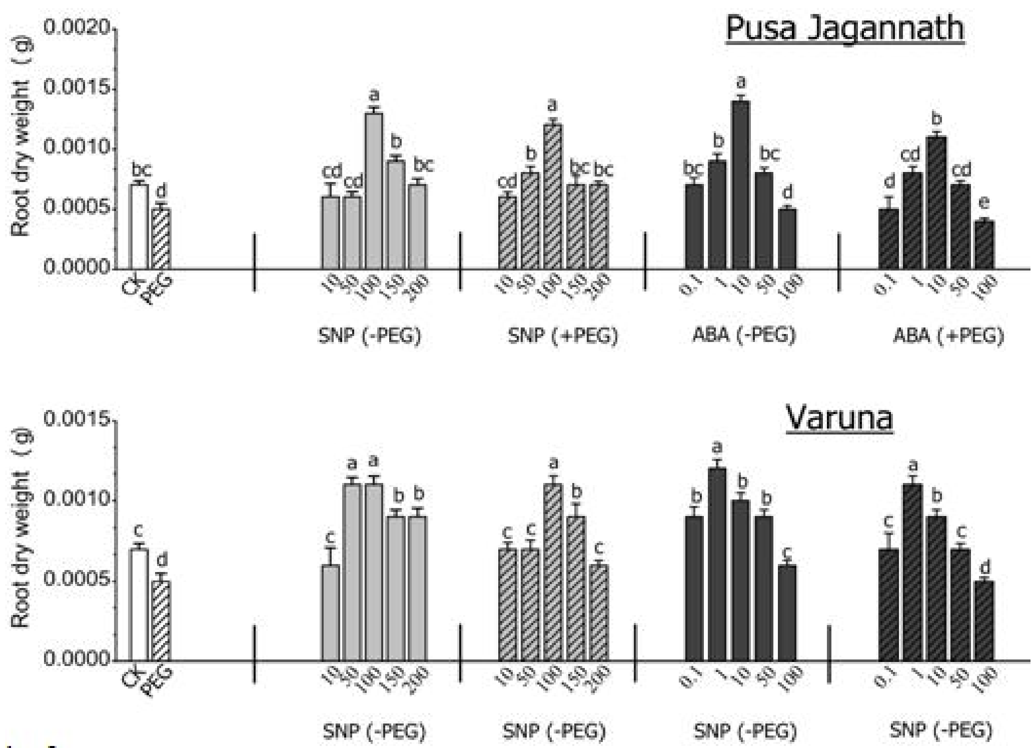

Fig. 6 : Effect of different concentrations of sodium nitroprusside (SNP, 10-200µM) and abscisic acid (ABA, 0.1-100µM) with and without polyethylene glycol (PEG, 10%) induced water stress on root dry weight of two *Brassica* cultivars, Pusa Jagannath and Varuna. Different letters represents the significance differences between control and treatment means. CK= Control.

has been reported in sugarcane by Silveira *et al*. (2016).

Regarding different concentrations of ABA, the growth parameters performed better at concentration ABA10, as compared to other treatments in both the cultivars. As such, ABA10μM enhanced SL, SFW, SDW, RL, RFW, RDW, RV, RLR, RMR, RFit and RTD by 29.0%, 55.5%, 150.0%, 100.0%, 75.0%, 28.6%, 9.27%, 29.5%, 22.2% and 72.7%, respectively in Pusa Jagannath cultivar, while by 14.0%, 22.5%, 20.0%, 28.5%, 63.1%, 71.6%, 41.1%, 128.0%, 55.0%, 102.4% and 20.0%, respectively in Varuna cultivar over their respective control (figs. 1-6, table 1). The supplementation of ABA with PEG showed differential effect depending on their concentration. In both Pusa Jagannath and Varuna cultivars, ABA10μM (+PEG) had the pronounced effect and gave an increase by 60.0% and 66.6% in SL, 178.9% and 19.2% in SFW, 316.2% and 100.0% in SDW, 63.7% and 185.1% in RL, 140.0% and 140.1% in RFW, 120% and 115.0% in RV, 67.5% and 61.0% in RLR, 151.4% and 88.5% in RMR, 40.7% and 35.1% in RFit, and 55.5% and 28.5% in RTD over their respective control. All the effect of ABA might be due to a central role of ABA to change the root system development according to surrounding environment (Harris, 2015). ABA stimulates the water flow into roots by decreasing the transpiration through leaves under abiotic stress including drought (Glinka and Reinhold, 1971; as reviewed in Sah *et al*., 2016). The growth promoting effect of exogenous applied ABA under PEG- induced drought stress has also been reported by Zhang *et al*. (2012) and Wei *et al*. (2015). The ABA50μM and ABA100μM with PEG was observed toxic as the growth parameters decreased at these treatment concentrations, compared to control, or could not enhance the PEG stressed decreased growth (figs. 1-6, table 1). The application of high concentration of external ABA reduced the growth and yield of various crop plants has been reported (Morgan, 1980; as reviewed in Saradadevi *et al*., 2017).

Conclusion

The involvement of NO and ABA was based on the selected concentration to enhance the growth determinates in both *Brassica* cultivars. Further, the application of NO and ABA at low concentration was effective to alleviated the PEG-induced toxicity on shoot and root growth parameters.

Acknowledgment

Thanks to University Grant Commission (UGC), Government of India, New Delhi to provide the financial assistance in the form of Post-Doctoral fellowship (grant numbers F./PDFSS-2015-17-UTT-12296) to SS.

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