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REGULATION OF NITRATE REDUCTASE ACTIVITY IN POPLAR (*POPULUS DELTOIDS*) LEAVES BY BENZYLADENINE WITH *CALCIUM, EGTA (*CALCIUM CHELATOR) AND *LACL*$_3$ (CALCIUM CHANNEL BLOCKER

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ABSTRACT
Cytokinin (BAP) and calcium along with EGTA, a calcium chelator and $LaCl_3$, a calcium channel blocker were used to elucidate the NR activity of *In vitro* and *In vivo* leaves segment of *Populus deltoids*. Nitrate reductase activity was enhanced at lower concentration of BAP (25µM) which causes increment of protein content and total organic nitrogen. 25µM BAP along with calcium (0.1,0.5,1.0mM) works in a synergistic manner and enhanced NR activity but reduced when calcium chelator EGTA (0.1 and 1.0mM) along with 25µM BAP and 1.0 mM calcium was used, EGTA specifically reduced the availability of cellular Ca^{2+} ions. Two contrasting concentrations of calcium channel blocker $LaCl_3$ (1.0 and 10.0mM) along with 1.0mM calcium and 25μM BAP reduced the activity of nitrate reductase. The inhibitory effect of $LaCl_3$ with calcium and BAP on NR activity was assumed due to blockage of calcium channels. Hence, calcium seems to be an elicitor for nitrate reductase activity.

INTRODUCTION

Nitrate assimilation is a fundamental process of higher plants, algae, fungi as well as bacteria. At a global level plants assimilate 100 fold more nitrogen from nitrate than biological fixation of nitrogen. It involves the influx, transport and reduction of nitrate to ammonia, which is assimilated by glutamate synthase cycle and finally to form organic compounds. Enzyme nitrate reductase revives nitrite ion during first step of nitrogen assimilation in plants (Cheng *et al.,*2014). It is a rate limiting step for assimilation of nitrogen and always requires higher level for better growth and development of plants. Studies revealed that the level of nitrate reductase in plant tissues can be enhanced by the application of plant growth regulators and various nutrients as sodium, potassium *etc* alone or rarely in combination of calcium. Cytokinin induced enhancement of nitrate reductase has been first demonstrated in the embryo of *Agrostemma githago* even when they were grown in nitrate free medium, however it is usually higher in the presence of nitrate than the absence (Borris, 1967., Kende *et al.,* 1971). Since then lot of studies have been performed to understand the effect of cytokinins on nitrate reductase activity in plants. A marked increase in nitrate reductase activity of dark grown cotton seeding was found when treated with kinetin (90%) and benzylaminopurine (70%) (Behl *et al.,* 1993). Cytokinin application also showed an additive effect to nitrate assimilation by inducing enzyme activity as in Fenugreek cotyledons (Rijvan and Prakash, 1971), cucumber cotyledons (Knylp, 1973), maize leaves (Rao *et al.,* 1984), sunflower (Goswami and Srivastava, 1989) and in barley seedlings (Gaudinova, 1990). However, in some cases nitrate reductase activity and leaf nitrate content have shown to decreased with an increase in BAP and potassium nitrate levels as in *Nascissus tazetta* (Matin *et al.,* 2015).

Cytokinins are well known regulators of cell division processes (Skoog and Armstrong, 1970), and accelerate the growth of plant parts (Davis, 1988). They also play a major role in plants nutrient mobilization and delay aging and work synergistically with various nutrients like calcium and potassium *etc.* (Fathi and Esmaeel, 2009). Cytokinin considered as important physiological signal in stimulating flowering in various cases. The application of BAP increased the percentage of inflorescence production, induced earlier flowering, and contributed to the differences in inflorescence length and the number of leaves and flowers produced in Dendrobium Angel White however this was not able to influence of the size of the flower (Nambiar *et al*., 2012). *In vitro* flowering of *Lilium rubellum* by combining BAP with the appropriate temperature is also reported (Ishimori *et al.,* 2009). In a recent study, two cutivars of ground nut namely, SG99 and M13 were sprayed with indole acetic acid (5 and 7.5ppm) followed by a second spray of Ethrel (25ppm), sequential spray of mepiquat chloride (125ppm) and mepiquat chloride (125ppm) and Ethrel

(25ppm) under field conditions. It was observed in the study that cultivar M13 increased nitrate reductase activity in leaves with IAA (7.5ppm) and Ethrel (25ppm). Both the concentrations of IAA increased the nitrate reductase activity in the nodules of SG99 (Sharma and Sardana 2012).

Calcium is known for various metabolic functions as maintenance of cell walls and membrane integrity, prevention in stress induced decline of cell elongation and to improve the important salts status as potassium (Shabala, 2000). Foliar spray of calcium also influence the vegetative growth, flowering, longevity of spike as well as corms production in ornamental plants like gladiolus (Sharma *et al*., 2013). However very limited information's are available regarding the effect of calcium on nitrogen assimilation. But it is assumed that application of calcium, a regulator of many metabolic activities should also improve the activity of enzymes during physiological processes of cell. It was reported in many cases that calcium in combination of other salts and plant growth regulators showed synergistic effect on nitrate reductase activity however alone this salt is not more effective to improve the activity of nitrate reductase (Sacala *et al*., 2005). In *Oryza sativa,* application of nitrogen and calcium promotes both the nitrate reductase and nitrite reductase activity with an increase in nitrate reductase mRNA levels (Ali *et al*., 2007). In many cases where plants grown on calcium free medium decreased the activity of nitrate reductase, such type of results were observed in wheat leaves (Ali *et al*., 1987). Treatment of calcium in *Lablab purpuris.L* increased the nitrate reductase activity, total chlorophyll and carotenoid content (Khan *et al*., 2005).

EGTA (ethyleneglycol-bis-(*â*-aminoethyl ether) N,N2 tetraacetic acid, a Ca^{2+} chelator and $LaCl_3$ (lanthanium chloride), a calcium channel blocker are specifically used to study the effect of calcium on cell metabolism. In a study different concentration of calcium chelator EDTA (0.1 to 1.0mM) and calcium blocker $LaCl_3$ (1.0 to 10.0mM) along with calcium (1.0mM) decreased nitrate reductase activity of *Populus deltoids* leaves. EGTA specially reduced the availability of extracellular Ca^{2+} ions. It is concentration dependent. *In vitro* and *in vivo* nitrate reductase activities declined with lower to higher concentrations (0.1mM to 1.0 mM) of EGTA with 1mM calcium and 25 mM ABA (Mishra *et al*., 2012).

Therefore, keeping in view the above facts an experiment was conducted to evaluate the effect of cytokinin (benzyladenine) and calcium with a *calcium chelator,* EGTA *and calcium channel blocker lacl*$_3$ *on* poplar (*Populus deltoids*) leaves.

MATERIALS AND METHODS

The excised leaves (4-6mm^2) segment of three years old *Populus deltoids* leaves were incubated in half strength Hoagland's solution (pH 6.5) containing 10mM KNO_3- and BAP (25, 50 and 100µM) for 24 hours in light (70wm^{-2}) at 25 ± 2ºC. Before incubation the leaves were surface sterilized with 0.1% bleaching powder ($CaOCl_2$) solution for five minutes and thereafter washed thoroughly with distilled water. Effect of different concentration of BAP (25, 50 and 100µM), signal molecule calcium (0.1,0.5,1.0 and 2.0mM), calcium chelator EGTA (0.1 and 1.0mM) and calcium channel blocker $LaCl_3$ (1.0 and 10mM) on nitrate reductase activity were examined in the leaves of *Populus deltoids* under laboratory condition.

Determination of enzyme (Nitrate reductase activity)

In vitro assay

In vitro nitrate reductase activity was estimated by the method of Srivastava and Ormrod (1984). Floated poplar leaves were homogenized in 2.0ml of extraction medium in a prechilled pestle and mortar.. Extraction medium was prepared by mixing 0.1M phosphate buffer (pH 7.8), 0.1% casein, 0.01M cystein and 0.03M ethylene diamine tetraacetic acid (EDTA), the homogenate was centrifuged at 20,000 rpm for 20 minutes at 4ºC and supernatant was used for the assay of enzyme activity.

The assay mixture consisted 0.2ml of 0.2M sodium phosphate buffer (pH 7.4), 0.4ml of 0.2M KNO_3, 0.3ml of freshly prepared buffer (pH 7.4),2mM NADH (Nicotinamide adenine dinucleotide, reduced form). The incubation of assay mixture was carried immediately at 30ºC for 30 minutes and the reaction was terminated by adding 1.0 ml of 1.5% sulphanilamide in 1.5N HCl, 1.0 ml of napthyl ethylene diamine dihydrochloride (0.02% w/v in distilled water). The absorbance was recorded at 540nm, and calculated the amount of nitrite with the help of the standard curve of nitrite and enzyme activity was expressed in terms of NO_2^- produced $g^{-1}h^{-1}$ fresh weight. A control was also maintained parallel to it by omitting NADH from the reaction mixture**.**

In vivo assay

Nitrate reductase (*in vivo*) was determined by the method of Srivastava (1974). About 1-2mm wide leaf segments (0.5g) were placed in a medium containing 8.0 ml of 0.1M sodium phosphate buffer (pH 7.8), 1.0 ml of 0.5 M KNO_3 and 1.0ml of 25% isopropanol and incubated in dark at 30ºC for 30 minutes. The 1.0ml aliquot was drawn out of the incubation medium and 1.0ml of 1% sulphanilamide (prepared in HCl) and 1ml of 0.02% N-(1-Naphthyl) ethylene diamine dihydrochloride (NED) were added to it. The pink colour developed was measured spectrophotometrically at 540nm. The nitrite was calculated with the help of a standard curve of nitrite.

RESULTS AND DISCUSSION

Effect of BAP on nitrate reductase activity

In vivo the leaves of *Populus deltoids* showed maximum (5.39µmole $NO_2^-g^{-1}h^{-1}$fr.wt.) NR activity when treated with 25µM BAP. However, it declines with increasing concentration of BAP when compared with control. At 100µM BAP concentration nitrate reductase activity was declined by 22%,

Table 1: Effect of BAP on nitrate reductase activity in poplar leaves. Excised leaves were incubated in ½ strength Hoagland's solutions containing 10mM nitrate and desired concentration of the Benzyladenine for 24 h in light at 25 ± 2ºC. The values in bracket indicate relative to control (minus BAP) value.

Concentration of BAP(µM)	Nitrate reductase activity (ìmole NO2> g-1h-1fr wt *In vivo*	*In vitro*
0.0	4.03 ± 0.171 (100)	0.948 ± 0.024 (100)
25	5.39 ± 0.01 (133)	1.051 ± 0.033 (110)
50	3.98 ± 0.81 (98)	0.891 ± 0.43 (94)
100	3.13 ± 0.035 (78)	0.701 ± 0.054 (74)

Table 2: Effect of Calcium and BAP on nitrate reductase activity in poplar leaves. Excised leaves were incubated in ½ strength Hoagland's solutions containing 10mM nitrate and desired concentration of the Calcium and BAP for 24 h in light at 25 ± 2ºC. The values in bracket indicate relative to control (minus calcium) value.

Concentration	Nitrate reductase activity (μmole NO_2^- g^{-1} h^{-1} fr wt)	
	In vivo	*In vitro*
0.0	5.39 ± 0.014 (100)	1.051 ± 0.033 (100)
0.1mMCa + 25μM BAP	5.58 ± 0.056 (103)	1.186 ± 0.024 (112)
0.5mMCa + 25μM BAP	5.82 ± 0.055 (107)	1.312 ± 0.024 (124)
1.0mMCa + 25μM BAP	6.43 ± 0.14 (119)	1.569 ± 0.031 (149)
2.0 mMCa + 25μM BAP	4.66 ± 0.045 (86)	0.920 ± 0.17 (87)

Table 3 : Effect of Calcium and EGTA with BAP on nitrate reductase activity in poplar leaves. Excised leaves were incubated in ½ strength Hoagland 's solutions containing 10mM nitrate and desired concentration of the Calcium and EGTA with BAP for 24 h in light at 25 ± 2ºC. The values in bracket indicate relative to control (minus EGTA) value.

Concentration	Nitrate reductase activity (μmole NO_2^- g^{-1} h^{-1} fr wt)	
	In vitro	*In vivo*
0.0	1.569 ± 0.031 (100)	6.43 ± 0.14 (100)
1.0mMCa + 0.1mMEGTA + 25μM BAP	1.112 ± 0.02 (70)	5.45 ± 0.20 (84)
1mMCa + 1.0mMEGTA + 25μM BAP	0.559 ± 0.127 (35)	2.19 ± 0.31 (41)

Table 4: Effect of Calcium and $LaCl_3$ with BAP on nitrate reductase activity in poplar leaves. Excised leaves were incubated in ½ strength Hoagland's solutions containing 10mM nitrate and desired concentration of the Calcium and $LaCl_3$ with BAP for 24 h in light at 25 ± 2ºC. The values in bracket indicate relative to control (minus $LaCl_3$) value.

Concentration	Nitrate reductase activity (μmole NO_2^- g^{-1} h^{-1} fr wt)	
	In vivo	*In vitro*
0.0	6.43 ± 0.14 (100)	1.569 ± 0.031 (100)
1mMCa + 1.0mM $LaCl_3$ + 25μM BAP	5.39 ± 0.089 (83)	1.12 ± 0.12 (70)
1mMCa + 10.0mM$LaCl_3$ + 25μM BAP	2.17 ± 0.060 (33)	0.695 ± 0.32 (44)

however no significant difference was observed at 50µM BAP. Similar trend was found in *In vitro* condition where 10% enhancement in comparison to control (1.051µmole $NO_2^-g^{-1}h^{-1}$fr. wt.) was found at 25µM BAP however it was declined by 22% at 100µM BAP (Table 1). The increase in enzyme activity by a certain level of cytokinins may be due to its accelerated uptake and intracellular mobilization.

On the basis various studies cytokonins are increasingly considered as potential messengers of the nitrogen status in most of the parts of the plant (Beck, 1994). Concentration of cytokinins in xylem tissues of roots responds to the availability of nitrogen in terms of either total nitrate availability (Samuelson and Larsson, 1993) or of differential distribution of nitrate along the lateral roots of barley (Samuelson *et al*., 1995). In etiolated barley leaves nitrate reductase activity was also increased with increasing concentration of benzyladenine, may be due to transcriptional level changes (Lu *et al.,* 1990). Another example is the poplar where increased nitrogen content in excised poplar leaves was found upon the application of benzyladenine, this might be also linked to the enhanced transcriptional level followed by protein synthesis and promotes the growth (Skoog and Armstrong, 1970). In case of chicory roots, enhanced transcription level of the NR gene was reported in presence of benzyl aminopurine which helps reactivating the activity of cell (Vuylsterer, 1997). A detailed study was performed to understand the effect of cytokinin on NR activity in stem pith parenchyma of kale, intact wheat and barley seedlings and isolated cucumber cotyledons. Out of these most profound effect on nitrate reductase activity was found in barley and wheat seedlings where they were sprayed with 100 IxM 6-benzylaminopurine (BAP) for three subsequent days. Nitrate reductase activity in wheat and barley leaves was increased by 57 % and 202 %, respectively however, additive effect of nitrate and BAP on nitrate reductase activity was shown in cucumber cotyledons in light (Gaudinova, 1990).

Effect of calcium and BAP on nitrate reductase activity

As in above experiment 25µM BAP enhanced the activity of nitrate reductase in the excised leaves of *Populus*. It was also tested along with different concentrations of calcium *i*.e. 0.1, 0.5, 1.0 and 2.0mM to elucidate the activity of nitrate reductase. Calcium was absent in the ambient medium of control, however, solutions were made in 10mM nitrate containing Hoagland's solution.*In vivo* nitrate reductase activity was gradually increased with increasing concentrations of calcium upto the level of 1.0mM calcium along with 25µM BAP thereafter a slight decrease was found at 2.0mM calcium. NR activity was found maximum (6.43µmole $NO_2^-g^{-1}h^{-1}$fr.wt.) at 1.0mM calcium and increased by 19% when compared with control and the minimum (4.66 µmole $NO_2^-g^{-1}h^{-1}$fr. wt.) was found at 2.0mM calcium along with 25µM BAP which was decreased by 14% and found less than the activity in control. Similar trend was found in *In vitro* condition, maximum NR activity in populus leaves (1.569µmole $NO_2^-g^{-1}h^{-1}$fr. wt.) was found at 1.0mM calcium with 25µM BAP and increased by 49% as compared to control however a gradual decline (13%) was found at 2.0 mM calcium with 25µM BAP when compared with control (Table 2). Calcium is an important signaling

molecule for various metabolic activities and might be act as a common component in nitrate signaling pathways as a secondary messenger (Pang *et al.,* 2007). As we discussed earlier that BAP alone acted at inducer for transcription and reactivate cellular activity. It showed synergistic effect with calcium and enhanced the activity of nitrate reductase. In a study when BAP was replaced by ABA with Ca, a slight increase (2%) in nitrate reductase activity (*in vivo*) was found when 0.1mM calcium was used along with 25mM ABA. However it was decreased by 22 to 40 % in 0.5mM to 1.0mM calcium with 25mM ABA (3.25 and 2.49 mmole $NO2^{-}$g-1h-1fr. wt.). More or less similar trend was found *In vitro* populous leaves (Mishra *et al.,* 2012).

Effect of calcium and EGTA with BAP on nitrate reductase activity

Two contrasting concentrations of calcium chelator EGTA *i.e.* 0.1 and 1.0 mM along with 1.0mM calcium and 25µM BAP (appropriate concentrations to induce the NR activity in the leaves) were used to elucidate the NR activity *In vivo* and *In vitro* both. Only EGTA was absent in control. *In vivo* and *In vitro,* the nitrate reductase activity of poplus leaves was declined gradually with increasing concentration of EGTA and found to be minimum (2.19µ mole $NO_2^-g^{-1}h^{-1}$fr.wt.) in *In vivo* and (0.559µmole $NO_2^-g^{-1}h^{-1}$fr. weight) *In vitro* at 1.0 mM EGTA along with 25µM BAP and 1.0mM calcium respectivily, it was declined by 16-59% *In vivo* and 30-65% *In vitro* at 0.1mM to 1.0mM EGTA along with 25µM BAP and 1.0mM calcium (Table 3). EGTA is a calcium chelator. An exogenous application of EGTA along with calcium and cytokinin showed antagonistic effect and reduced nitrate reductase activity. In our study nitrate reductase activity was found to be low with increasing concentration of EGTA (0.1 to 1.0 mM) with Ca and BAP. Almost similar effects of EGTA along with Ca^{2+} on NR activity in maize leaf tissue have been reported by Sakakibara and his coworkers. EGTA specifically reduced the availability of extra cellular Ca^{2+} ions and strongly inhibited the nitrate dependent expression of genes for nitrate reductase (Sakakibara *et al.,* 1997)**.** EGTA ions markedly inhibited the nitrate dependent accumulation of transcripts of genes from NR, NIR, GS2, Fd-GOGAT and NADH – GOGAT in maize tissues. These findings imply that uptake of extra cellular Ca^{2+} ions might be necessary for nitrate dependent expression of genes for nitrate reductase and nitrite reductase (Mishra *et al.,* 2012).

Effect of calcium and $LaCl_3$ with BAP on nitrate reductase activity

As EGTA did not show the synergistic effect with calcium and BAP and not able to increase the nitrate reductase activity of leaves. Lanthanum chloride, a calcium channel blocker was used in concentrations (1.0 and 10mM) along with 1.0mM calcium and 25 μM BAP to elucidate the NR activity. Half strength Hoagland's solution containing 10mM nitrate was used to prepare solutions, only calcium was used in control. *In vivo* and *in vitro* both NR activity was decreased with increasing concentration of $LaCl_3$ along with 1.0mM calcium and 25μM BAP. *In vivo* it was declined 17 and 67% (5.39 & 2.17µmole $NO_2^-g^{-1}h^{-1}$fr.wt.) at 1.0mM $LaCl_3$ and 10.0mM $LaCl_3$ along with 1.0mM calcium and 25μM BAP. Whereas *In vitro* leaves showed 30 to 56 % (1.12 to 0.695µmole $NO_2^-g^{-1}h^{-1}$fr.wt.) decline at 1.0mM $LaCl_3$ to 10.0mM $LaCl_3$ along with 1.0mM calcium and 25μM BAP (Table 4). Pretreatment of $LaCl_3$ showed inhibitory effect on nitrate related genes as it is a calcium channel blocker and block the passage of calcium for metabolic processes. It was due to inhibitory effect of $LaCl_3$ on nitrate dependent expression of the gene for uroporphyrinogen III methyl-transferase, which is involved in the synthesis of siroheme, a prosthetic group of NIR (Sakakibara *et al.,* 1996)**.** It was a long back research that calcium was implicated in nitrate signaling by reports that EGTA (a calcium chelator) and $LaCl_3$ (a calcium channel blocker) could inhibit the nitrate induction of gene expression (Sueyoshi *et al.,* 1999). In a recent study using aequorin transgenic *Arabidopsis* plants, a rapid and transient increase in intracellular calcium in response to nitrate treatment was detected, which was blocked by $LaCl_3$. During study it was found that four nitrate-responsive, nitrate assimilatory, and regulatory genes (NRT2.1, NRT3.1, NITRITE REDUCTASE, and TGA1) was inhibited by U732122 and by $LaCl_3$ (Riveras *et al.,* 2015).

REFERENCES

Ali, A., Sivakumar, S. and Raghuram, N. 2007. Regulation of activity & transcript levels of NR in rice (*Oryza sativa*): Roles of protein kinase and coproteins. *Plant Science.* **172:** 406-413.

Ali, A. A., Ikeda, M. and Yamada. 1987. Effect of the supply of potassium, calcium and magnesium on the absorption, translocation and assimilation of ammonium and nitrate nitrogen in wheat plants. *Soil Sci Plant Nutr.***33(4):** 585-594.

Beck, E. 1994. The morphogenic response of plants to soil nitrogen: adaptative regulation of biomass distribution and nitrogen metabolism by phytohormones. In: Detlef-Schulze E, ed. Flux control in biological systems from enzyme to population and ecosystems. New York: Academic Press, pp 119-51.

Behl, R., Tischner, R. and Raschke, K. 1993. Induction of a high capacity nitrate mechanism in barley roots promped by nitrate uptake through constitutive low capacity mechanism. *Planta*.**176:** 236-240.

Borris, H. 1967. Ultrastructure uber die steuerung der enzymaktitat in pflanzlichenh embryonic durch cytokinine.*Wiss Z Univ Math Naturwiss Reihen.* **16:** 629-639.

Cheng-Wei, L., Sung ,Yu., Chen, B. and Hung-Yu, L.2014. Effects of nitrogen fertilizers on the growth and nitrate content of Lettuce (Lactuca sativa L.).*Inter J Environ Res Pub Health*.**11:** 4427-4440.

Davis, P. J. 1988. Plant hormones and their role in plant growth and development. Kluwer Academic Poblishers p. 432.

Fathi, Q. H. A. and Esmaeel, P. B. 2009. Plant growth regulators *Principles appl*.85-90.

Gaudinova, A. 1990. The effect of cytokinins on nitrate reductase activity. *Biol.Plant*.**32:** 89-96.

Goswami, B. K. and Srivastava, H. S. 1989. Effect of benzyl adenine on nitrate reductase enzyme in sunflower (Helianthus Annuus.L.) *Indian J. Plant Physiol.* **32(4):** 325-329

Ishimori, T., Niimi,Y. and Han, D.S. 2009. *In vitro* flowering of *Lilium rubellum* Baker. *Scie Hort*. **20:** 246-249.

Kende, H., Hahn, H. and Kays, S. E. 1971. Enhancement of nitrate reductase activity by benzyl adenine in *Agrostemma githago. Plant Physiol*.**48:** 702-706.

Khan, M., Masroor, A., Naeem, M. and Siddiqui, M. H. 2005. Calcium fertilization ameliorates growth, yield and quality of hyacinth bean (*Lablab purpureus L.*).Proceedings of the Ist International Edible Legume conference in conjunction with the *IVth World Cowpea*

Congress Durban South Africa. pp 1-9.

Knylp, J. S. 1973. Synergistic induction of nitrate reductase activity by nitrate and benzylaminopurine in detached cucumber cotyledons. *Zeitschrift Fur Pflanzenphyiogie*.**70 (1):** 1-11.

Lu, J. L., Ertl, J.R. and Chen, C.M.1990. Cytokinin enhancement of the light induction of nitrate reductase transcript levels in etiolated barley leaves. *Plant.Mol.Biol*.**14:** 585-594.

Matin, S. R. M., Mortazavi, S. N. and Heidari, M. 2015. Application of benzyladenine and potassium nitrate on growth ,nitrate content and nitrate reductase activity in Narcissus (Narcissus tazetta). *Inter Res J appl Basic Sci*.**9(6):** 824-830.

Mishra, R., Garg, S. K. and Senger, R. S. 2012.Regulation of nitrate reductase activity by Abscisic acid in Poplar(Populus deltoids) leaves. *Vegetos*. **25(2):** 57-61.

Nambiar, N., Siang, T. C. and Mahmood, M .2012. Nambiar, N., Siang, T.C. and Mahmood, M 2012. Effect of 6-Benzylaminopurine on flowering of a Dendrobium orchid. *Aust J Crop Sci*. **6(2):** 225-231

Pang, X., Halaly, T., Crane, O., Kellin, T., Keiserman, A.K., Ogrodovitch, A., Galbraith, D. and O. E. 2007. Involvement of calcium signaling in dormancy release of grape buds. *J Exp Bot*. **58(12):** 3249-3262.

Rao, L.V.M., Datta, N., Maha denam, M., Guha Mukharjee, S. and Sopory, S.K. 1984. Influence of cytokinines and phytochrome on nitrate reductase activity in etiolated leaves of maize *Photochem*. **23(9):** 1875-1879.

Rijven, A. H. G. C. and Prakash, V. 1971. Action of kinetin on cotyledons of *Fenugreek*. *Plant Physiol*.**47:** 59-64.

Riveras, E., Alvarez, J. M., Vidal, E.A., Oses, C., Vega, A. and Gutiérrez, R. A. 2015. The calcium ion is a second messenger in the nitrate signaling pathway of Arabidopsis. *Plant Physiol*. **169:** 1397-1404.

Sacala, E., Biegun, N., Demczuk, A and Grzys, E. 2005. Effect of Nacl and supplemental calcium on growth parameters and nitrate reductase activity in maize. *Acta societatis botanicorum poloniae*. **74(2):**119-123.

Sakakibara, H., Kobayashi, K., Deji, A. and Sugiyama, T. 1997. Partial characterization of the pathway for the nitrate dependent expression of genes for nitrogen assimilation enzymes using detracted maize leaves. *Plant cell Physiol*.**38(7):** 837-843

Sakakibara, H., Takai, H., Takahashi, K. and Sugiyama, T. 1996. Isolative characterization of a cDNA that encodes maize uropphyrinogen III an enzyme involved in the synthesis of siroheme which is a prosthetic group of nitrate reductase. *Plant J*. **10:** 883 -892

Samuelson, M. E., Campbell, W. H. and Larsson, C. M. 1995. The influence of cytokinins in nitrate regulation of nitrate reductase activity and expression in barley. *Physiol*. **93:** 533-539.

Samuelson, M. E., Larsson, C.M. 1993. Nitrate regulation of zeatin riboside levels in barley roots: effects of inhibitors of N assimilation and comparison with ammonium. *Plant Sci*. **93:** 77-84.

Shabala, S.2000. Ionic and osmotic components of salt stress specifically modulate net ion fluxes from bean leaf mesophyll. *Plant Cell Environ*. **23:** 825-837.

Sharma, J., Gupta, A. K., Kumar, C. and Gautam, R. K. S . 2013. Influence of zinc, calcium and boron on vegetative and flowering parameters of gladiolus cv. aldebran. *The Bioscan*. **8(4):** 1153-1158.

Sharma, P. and Sardana, V. 2012. Effect of growth regulating substances on the chlorophyll, nitrate reductase, leghaemoglobin content and yield in groundnut (*Arachis hypogaea*). *The Bioscan*. **7(1):** 13-17.

Skoog, F. and Armstrong, D. J. 1970. Cytokinins. *Ann Rev Plant Physiol*. **21:** 359-384.

Srivastava, H. S. 1974. *In vivo* activity of nitrate reductase in maize seedlings. *Indian.J .Biochem Biophys* .**11:** 230-232.

Srivastava, H. S. and Ormrod, D.P. 1984. Effect of nitrogen dioxide and nitrate nutrition on growth and nitrate assimilation in bean leaves. *Plant Physiol*.**78:** 418-423.

Sueyoshi, K., Mitsuyama, T., Sugimoto, T., Kleinhofs, A., Warner, R. L. and Oji, Y. 1999. Effects of inhibitors for signaling components on the expression of the genes for nitrate reductase and nitrite reductase in excised barley leaves. *Soil Sci Plant Nutr*. **45:** 1015-1019

Vuylsterker, C., Leleu, O. and Rambour, S. 1997. Influence of BAP and NAA on the expression of nitrate reductase in excised chicory roots. *J Exp Bot*. **48(310):** 1079-1085.