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SUCROSE-INDUCED ENHANCEMENT OF NITRATE REDUCTASE ACTIVITY PROMOTES *IN VITRO* GROWTH OF POTATO

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Nitrate reductase (EC 1.6.6.1-3) being an imperative key regulator of nitrogen metabolism acts as the rate limiting step in plant growth. Here, we present sucrose as an inducer of nitrate reductase activity (NRA) enhancing *in vitro* potato growth. This study explores physiological relationship between NRA and sucrose concentration affecting *in vitro* micropropagation in two potato varieties, 'Desiree' and 'Cardinal' followed by quantitation of various physio-chemical attributes of *in vitro* grown plants. MS medium with (3% W/V) sucrose was used as control. It was found that higher sucrose concentration (9%) enhanced nitrate reductase activity (79.34 µ mol NO_2 h^{-1} g^{-1}) and total soluble protein contents (2.22 mg g^{-1}) resulting in higher shoot length (15 cm) in genotype Desiree. Similarly, 9% sucrose appeared to enhance nitrate reductase activity (86.92 µ mol NO_2 h^{-1} g^{-1}) and total soluble proteins (3.14 mg g^{-1}) with overall shoot length upto 19 cm in genotype Cardinal. Based on results, it was concluded that addition of sucrose to optimum concentrations led to enhanced nitrate reductase activity due to which an increase in quantity of total soluble proteins was observed. Consequently, higher protein contents brought about an improvement in overall plant growth.

Keywords: *Solanum tuberosum*, N-assimilation, Total soluble proteins, Micropropagation, Plant growth.

Abbreviations: NR: Nitrate reductase, NiR: Nitrite reductase, NRA: nitrate reductase activity, Fds: ferredoxins, f.Wt.: Fresh weight, TR: room temperature, TSP: total soluble proteins, OD: Optical density.

INTRODUCTION

Nitrogen is a macronutrient, vital for plant growth as well as metabolism and is mostly absorbed in the form of nitrate (Li *et al.*, 2010). Nitrate is primarily reduced into nitrite via cytosolic Nitrate Reductase (NR) catalyzed reaction. This reaction is a rate-limiting and crucial phase in nitrogen assimilation pathway (Chamizo-Ampudia *et al.*, 2017). Later, nitrite is carried to chloroplasts through membrane transport where it is reduced to NH_4^+ through plastidic nitrite reductase (NiR) catalyzed reaction. The resultant ammonium ions are employed to various pathways for the synthesis of amino acids, other secondary metabolites and various nitrogenous compounds (Rad *et al.*, 2013). This nitrate assimilation pathway is known to utilize more than 25% of the total energy fixed through photosynthesis (Solomonson and Barbar, 1990). That's why; fast-growing plants like potato are supplied with higher amounts of nitrogen fertilizers in order to provide more nitrates which are reduced to nitrites and nitrates in their leaves where main part of reducing power arises directly from light via ferredoxins (Fds) (Beevers and Hageman, 1980). The ferredoxins (Fds) are small Iron-Sulfur containing cellular proteins that primarily move electrons from photoreduced Photosystem-I to Photosystem-II. In addition to C-assimilation via photosynthesis, Fds partition electron to several Fds-mediated enzymes for fixation as well as assimilation of nitrogen and sulfur (Fukuyama, 2004). When nitrate assimilation occurs in other plant parts like tubers, high amounts of photosynthesis products (sugars)should be transported to those parts and oxidized there to supply necessary reductants, energy and carbon skeleton. It is also known that NR is a substrate inducible enzyme whose catalytic activity depends upon several environmental, hormonal or metabolic factors (Hoff *et al.*, 1994; Kaiser and Huber, 1994). Hence, it can be hypothesized that higher the quantity of photosynthates higher will be the nitrate reductase activity (NRA) resulting in abundant amino acids and proteins formation. Here, the physiological relationship between sucrose (most important photosynthates) and activity of NR (rate limiting enzyme in N-assimilation pathway) has been explored.

Sucrose is often employed in plant tissue culture as a source of carbon as well as energy. It gets hydrolyzed into glucose and fructose. This process of hydrolysis has made sucrose the best carbohydrate being used as C source in plant tissue culture (Yu *et al.*, 2000; Wazir *et al.*, 2015). Potato (*Solanum*

tuberosum L.) is one of the most extensively consumed vegetable which is being eaten as staple food in many countries (Rauscher *et al.*, 2006). Compared to other foods, it provides maximum amount of carbohydrates per day per unit of mass (Fufa and Diro, 2013). It is one of the important edible crops (Rafique *et al.*, 2004) and is cultivated thrice a year in our country. Pakistan is ranked at number seven among potato growing countries (Humera and Iqbal, 2010). In Pakistan, potato growers spend more than 47% of total production cost for the acquisition of imported virus free seed from Holland and other countries. Owing to different climatic conditions of seed producing countries, we are unable to get quality crop according to the yield potential of foreign introductions (Fatima *et al.*, 2005). Hence, high-quality disease-free potato seed production is direly needed to feed ever increasing population of the country.

Micropropagation is a reliable method for disease free potato seed production. *In vitro* micropropagation of potato is executed using various explants cultured on micropropagation media augmented with different plant growth regulators (Rabbani *et al.*, 2001; Zaman *et al.*, 2001). BAP, IAA or GA_3 help to develop adventitious shoots (Borna *et al*, 2019). *In vitro* microtuberization in potato can be used as a rapid system for the production of uninfected seeds to meet requirements of potato farmers. It offers additional benefits of convenient transport, long storage times and round the year availability for various purposes (Hoque, 2010). The novelty of present study is that we have explored the relationship between sucrose concentration and the activity of endogenous nitrate reductase during micropropagation of potato and found that increasing sucrose concentration to an optimum enhances NRA activity that ultimately improves plant growth.

MATERIALS AND METHODS

***Plant material and explants*:** Tubers of two potato varieties 'Desiree' and 'Cardinal' were provided by Punjab Seed Corporation, Sahiwal for obtaining explants. Surface sterilized tubers were stored in craft paper bags for one month at 24ºC for emergence of etiolated buds. Those buds were cultured onto MS medium having 3% sucrose for shoot proliferation. The resultant *in vitro* shoots were used in further experiments.

***Explant sterilization*:** Etiolated buds were surface sterilized following (Kanwal *et al.*, 2006) with certain modifications. Buds were washed for 10 minutes, dipped in 70% ethanol for 1.5 minutes and agitated in 10% *Clorox solution* (5.25% NaOCl as the active ingredient) for 10 minutes. Finally, buds were rinsed with autoclaved distilled H_2O water under sterile conditions and cultured *in vitro*.

***Media preparations*:** Tissue culture medium augmented with varying sucrose concentrations (3%, 6%, 9%, and 12%) was used for the assessment of growth response. Sucrose @ 3% was used as control. The media were autoclaved at a temperature of 121 ºC for 15 min after maintaining pH at 5.7.

***Estimation of Nitrate reductase activity (NRA)*:** Activity of Nitrate reductase (I.U.B.:1.9.6.1 and EC 1.6.6.1-3) was computed following (Sym, 1984). The dried and powdered plant material was weighed (0.1 g) and mixed in five mL of phosphate buffer containing 0.02M KNO_3, incubated at 32 ºC for 1 h. Then 0.5 ml of 1% sulphanilamide and 0.5 ml of (1-Naphthyl)-ethylene diamine dihydrochloride were added per ml of the above extract, incubated at room temperature (RT) for 20 minutes. Then, optical density (OD) was determined at 542 nm using spectrophotometer. Standard curve developed by employing serial dilutions of NO_2 was used. The enzyme activity was represented as µ mol $NO_2\ h^{-1}\ g^{-1}$ fresh weight.

***Estimation of total soluble proteins (TSP)*:** Estimation of total soluble proteins (TSP) was carried out as described by Lowry *et al.*, (1951). For this purpose, 0.1 g (fresh weight) was grinded in 2 ml of sodium phosphate buffer and filtered. Later, 0.2 mL of filtrate was further diluted up to 2.0 mL by adding required volume of distilled H_2O. Then, 2.0 mL of reagent C (Alkaline Copper solution) was added, the reaction mixture was incubated at RT for 10 minutes. Then, 0.1 mL of Foline phenol reagent was added and incubated at RT for 30 min. The OD was measured at 620nm using spectrophotometer. Standard curve was developed by employing serial dilutions of Bovine serum albumin (BSA). Total soluble proteins were estimated according to following formula:

$$\text{Total soluble Proteins (mg /g F.Wt.)} = \frac{\text{O. D. X Vol. of Sample X Dilution Fcator}}{\text{Weight of Fresh Tissue X 100}}$$

The data for NRA and TSP estimation was also subjected to statistical analyses as described earlier.

***Data collection and statistical analysis*:** Data for physical growth parameters including (a) Shoot length (b) Root length and (c) Number of leaves were collected on weekly basis up to 5 weeks. The Completely Randomized Design (CRD) was applied with 3 replications (5 observations per replication) for data analysis (Hinkelmann and Kempthorne, 2008).

RESULTS AND DISCUSSION

Present study was aimed to explore the physiological relationship between sucrose concentrations (6%, 9% and 12%) and NRA for two potato varieties 'Desiree' and 'Cardinal' during *in vitro* micropropagation. Sucrose @ 3% was used as control treatment. It was found that increase in sucrose concentration resulted in increased NRA and subsequent higher *in vitro* plant growth. Increased plant growth was depicted by longer shoots, deeper roots and more number of leaves. This observation led to conclude that increased NRA is somehow responsible for enhanced *in vitro* plant growth as discussed in this section.

***Nitrate reductase activity (NRA)*:** The data were recorded for the NRA (µ mol NO_2 $hr^{-1}g^{-1}$) with different concentrations of sucrose provided in growth media for potato. ANOVA (Table 1.0) indicated highly significant variation among varieties, no. of weeks and concentration of sucrose. Interaction of week, variety and concentration also showed highly significant results. Maximum value (86.92) was observed in 5th week for variety cardinal at 9% sucrose, while lowest value (9.23) was computed in first week for Desiree at 12% sucrose concentration (Table 2).

Table 1. Mean squares from ANOVA of various potato growth attributes measured *in vitro.*

SoV	df	Mean squares				
		NRA	TSP	SL	RL	NOF
Week (A)	4	4494.29**	3.5731**	471.882**	142.518**	235.596**
Variety (V)	1	13018.30*	49.6465**	1.344**	28.227**	45.633**
A x V	4	766.21**	0.2606**	7.325**	0.138^{NS}	3.571**
Concentration (C)	3	4226.92**	3.3905**	105.508**	67.87**	47.267**
A x C	12	35.89**	0.3542**	4.647**	1.656**	0.385^{NS}
V x C	3	68.11**	0.1062**	20.153**	29.67**	4.589**
A x V x C	12	45.40**	0.2684**	2.248**	0.467**	0.304^{NS}
Error	80	0.27	0.0001	0.062	0.056**	0.792

Where ANOVA=Analysis of variance, SoV=Source of variance, df= degree of freedom, NRA= Nitrate Reductase activity (µmol$hr^{-1}g^{-1}$ of fresh weight), TSP=Total soluble proteins (mg g^{-1} of fresh weight), SL=Shoot length, RL=Root length, NOL=Number of leaves, ** = Highly significant (P<0.01) and NS=not significant. The values carrying same letter have no significant differences.

Table 2. Week x Variety x Concentration interaction of different potato growth attributes *in vitro*.

Growth attributes	V×C	Week					V×C Means
		1st	2nd	3rd	4th	5th	
NRA	V1 (3%)	9.86 z	19.56 w	31.97 t	42.64 pq	58.35 j	32.48 G
	(6%)	14.96 y	24.86 v	41.85 q	56.97 k	62.40 i	40.21 F
	(9%)	19.25 w	34.85 r	51.25 m	69.53 e	79.34 b	50.84 D
	(12%)	9.23 z	17.83 x	25.93 u	35.62 r	43.35 p	26.39 H
	V2 (3%)	42.14 q	45.92 o	49.27 n	58.24 j	62.55 i	51.62 C
	(6%)	49.94 n	53.34 l	62.09 i	64.94 h	68.25 f	59.71 B
	(9%)	66.62 g	72.14 d	76.14 c	79.12 b	86.92 a	76.19 A
	(12%)	33.17 s	42.66 pq	45.97 o	49.34 n	57.46 jk	45.72 E
TSP	V1 (3%)	0.59	0.88]	1.16 yz	1.42 w	1.75 t	1.16 G
	(6%)	0.82 ^	1.15 z	1.53 v	1.62 u	1.86 r	1.40 F
	(9%)	1.05 [	1.40 x	1.84 s	1.98 p	2.22 n	1.70 E
	(12%)	0.47 .	0.70 -	0.93 /	1.17 y	1.52 v	0.96 H
	V2 (3%)	2.01 o	2.26 n	2.70 i	2.84 g	2.99 d	2.56 C
	(6%)	2.32 l	2.58 j	2.91 f	2.94 e	3.05 c	2.76 B
	(9%)	2.55 k	2.84 g	3.05 c	3.09 b	3.15 a	2.94 A
	(12%)	1.93 q	2.21 n	0.66 ,	2.76 g	2.94 e	2.10 D
SL	V1 (3%)	2.30 t	4.53 o	6.73 kl	9.10 i	11.93 e	6.92 F
	(6%)	2.60 st	5.77 m	9.10 i	11.50 f	13.90 c	8.57 C
	(9%)	3.90 p	6.80 k	10.33 gh	12.70 d	15.00 b	9.75 B
	(12%)	1.40 u	3.10 qr	5.20 n	7.60 j	10.00 h	5.46 G
	V2 (3%)	1.17 u	3.20 qr	6.57 kl	10.47 g	14.67 b	7.21 E
	(6%)	3.37 q	5.50 mn	9.40 i	14.00 c	19.00 a	10.25 A
	(9%)	2.80 rs	4.30 op	6.40 kl	10.17 gh	13.90 c	7.51 D
	(12%)	1.50 u	2.60 st	4.50 o	6.30 l	9.47 i	4.87 H
RL	V1 (3%)	1.40 rs	3.40 n	5.20 hi	7.47 e	9.60 b	5.41 C
	(6%)	3.90 m	5.77 g	7.50 e	9.70 b	11.30 a	7.63 A
	(9%)	1.50 rs	2.80 op	4.30 klm	5.30 h	6.40 f	4.06 E
	(12%)	0.80 t	1.50 rs	2.80 op	4.33 kl	5.20 hi	2.93 G
	V2 (3%)	0.33 u	1.10 st	3.10 no	5.03 hij	6.70 f	3.25 F
	(6%)	1.77 qr	3.10 no	4.80 ij	6.50 f	8.10 d	4.85 D
	(9%)	2.47 p	4.17 lm	5.90 g	7.37 e	8.83 c	5.75 B
	(12%)	0.20 u	1.10 st	2.00 q	3.50 n	4.70 jk	2.30 H
NOL	V1 (3%)	1.33	3.00	5.00	7.67	10.00	5.40 C
	(6%)	1.67	3.33	6.00	8.33	11.00	6.07 B
	(9%)	2.67	4.00	6.67	9.33	11.33	6.80 A
	(12%)	0.00	1.67	4.00	6.33	7.67	3.93 E
	V2 (3%)	0.33	2.00	3.67	5.67	7.33	3.80 E
	(6%)	2.00	4.33	6.00	7.67	9.67	5.93 B
	(9%)	1.33	3.00	5.00	6.33	8.67	4.87 D
	(12%)	0.00	1.00	2.00	4.00	6.33	2.67 F

Where V1: Desiree, V2: Cardinal, NRA: Nitrate Reductase activity (µmol$hr^{-1}g^{-1}$ of fresh weight), TSP: Total soluble proteins (mg g^{-1} of fresh weight), SL: Shoot length, RL: Root length, NOL: Number of leaves, Means sharing similar letters are statistically non-significant. Capital letters indicate the difference between main effects and small letters show the difference for interaction means.

Gradual increase in NRA took place in both cultivars but at higher concentration (12%) proportionate increase in activity did not exist. Varietal comparison showed a remarkable difference in NRA. Cardinal showed better performance than Desiree. Data depicted that NRA increased with increase in sucrose concentration upto 9% but further increase in sucrose concentration resulted in decreased NRA. Nitrate Reductase activity also increased with increase in the age of plantlets. Data showed that 9% sucrose was best for maximum NRA in both Desiree and Cardinal but in case of Cardinal, NRA was higher than Desiree (Fig.1). The aim of this study was to unveil the impact of sucrose concentration on NRA activity in plants especially in Potato. Though, 3% sucrose is used in studies pertaining to *in vitro* regeneration and micropropagation in plants, yet there are various studies showing use of higher concentrations as well (van Rensburg and Vcelar, 1989; Saha *et al*., 2013; Elazab and Shabaan, 2015; Verma *et al*., 2016; Zahara *et al*., 2017). The present study unveiled a relation between sucrose concentration and NRA. It was found that sucrose concentration higher than 3% caused an increase in NRA and subsequent plant growth.

The concentration of sugars modulates nitrate reductase gene expression at transcription as well as post-translational levels. Lower sugar concentrations inactivate nitrate reductase protein at post-translational level. On the other hand, some other studies also revealed that transcription of nitrate reductase enhances when starved leaves are fed with high sugar concentrations. These studies used detached leaves to

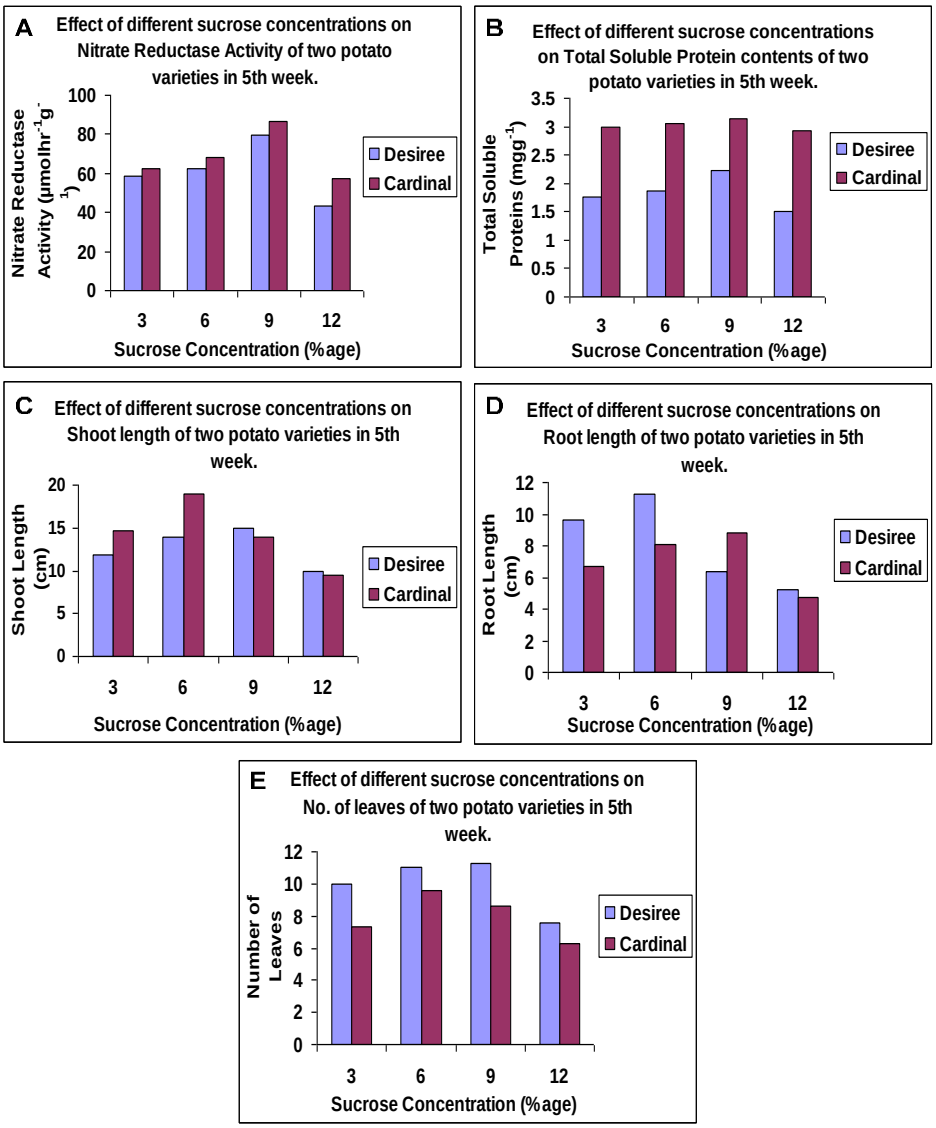

Figure 1. Effect of different sucrose concentrations on *in vitro* micropropagation of potato A. Nitrate Reductase activity B. Total soluble proteins C. Shoot length D. Root length E. No. of leaves.

corelate sugar concentration and NRA. However, in present study we have unveiled similar results for intact plants growing under *in vitro* conditions (Klein *et al.*, 2000).

Here it is evident that sucrose plays an important role in regulating NRA as increased concentration of sucrose increases NRA which in turn increases NH_4^+ production causing a subsequent promotion in plant growth. As depicted in Fig.2, the overall plant growth is due to the direct involvement of NRA in the synthesis of amino acid, secondary metabolites and various protein based primary metabolites. We also observed that NRA increased with increase in sucrose concentration upto 9% in Desiree as well as Cardinal. Nikitin and Izmailov (2016) reported a possibility to boost the seedling growth via utilization of sucrose formed as a result of degradation of reserve C substrates. Taghavi and Babalar (2007) reported NRA dependent growth in strawberry (*Fragaria ananassa* cv. Selva). Similarly, other studies (Haba *et al.*, 2001; Camacho-Cristóbal *et al.*, 2002) also revealed that sucrose concentration and the Nitrate Reductase activity are directly linked with each other.

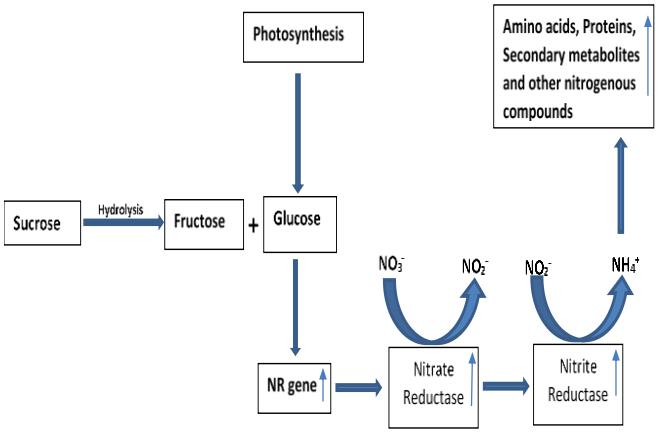

Figure 2. Diagrammatic sketch showing role of exogenously applied Sucrose on enhanced activity of endogenous Nitrate Reductase and subsequent increased plant growth.

***Total Soluble Proteins (TSP)*:** The data for TSP (mg g^{-1}) were recorded following Lowry *et al.* (1951). Statistical analysis revealed that amount of total soluble proteins depends upon the varieties, duration of treatment and concentration of sucrose (Table 1). Maximum value (3.15 mg g^{-1}) was observed in 5th week for variety cardinal at 9% sucrose concentration, while minimum value (0.47 mg g^{-1}) was observed in 1st week for Desiree at 12% sucrose concentration (Table 2).

Gradual increase in TSP took place in cultivars but at higher sucrose concentration (12%) proportionate increase in TSP was not observed. Genotypic difference also influenced the increase in TSP hence, Cardinal showed better performance than Desiree. Data showed that soluble protein contents improved by increasing sucrose concentration. Soluble protein contents also increased with increasing age of plantlet upto 5th week. Data showed that 9% sucrose was found to be the best for soluble protein production in both Desiree and Cardinal however total soluble protein contents were greater in Cardinal than in Desiree (Fig. 1B).

A correlation between TSP, NRA and yield responses in different cotton genotypes was reported by Ananthi and Vijayaraghavan, (2012). They highlighted that NR is rate limiting enzyme in nitrogen assimilation pathway and is a crucial point in the regulation of metabolic activities, plant growth and development. Similarly, Diémé *et al.* (2013) reported that both varietal difference and sucrose concentrations affect TSP contents in potato. It was found that TSP contents also increased with increasing sucrose concentrations.

***Physical growth parameters*:** Variation in physical parameters including shoot length, root length and number of leaves is a direct evidence of plant growth under given conditions. If a plant has longer shoots, deeper roots and more number of leaves, growth of such a plant would be considered better than others. Hence, similar parameters were recorded to corelate the growth of plants with NRA, total soluble proteins and sucrose concentrations. Following scenario was observed for growth parameters given below.

***Shoot Length*:** Shoot length was measured at different concentrations of Sucrose. Maximum shoot length (19.00 cm) was observed in 5th week for Cardinal variety at 6% sucrose concentration, while minimum shoot length (1.17 cm) was observed in 1st week also for Cardinal at 3% sucrose concentration (Table 2). Gradual increase in shoot length took place in both cultivars but at higher sucrose concentration (12%) proportionate increase in shoot length did not appear, which may be attributed to osmotic stress due to unsuitable high sucrose concentration (Fig.1). Shoot length was also found to be a genotype dependent and Cardinal showed better performance than Desiree. Data showed that 9% sucrose is best for maximizing shoot length in case of Desiree but 6% is best for increasing shoot length in case of Cardinal. Similar results were reported by Harvey *et al.* (1994), Al-Abdallat and Suwwan (2002). Zaman *et al.* (2015) also indicated that both genotypic difference and sucrose concentration affect shoot length significantly.

***Root Length*:** Root length was also measured at different sucrose concentrations for both genotypes. Maximum root length (11.30 cm) was observed in 5th week for Desiree at 6% sucrose concentration, while minimum root length value (0.20 cm) was observed in 1st week for cardinal at 12% sucrose concentration (Table 2). From analysis of variance table, it was obvious that highly significant results were observed for varieties, no. of weeks and sucrose concentration (Table 1). Interaction of week, variety and concentration also showed highly significant results.

Gradual increase in root length took place in both genotypes upto certain sucrose concentrations (6% for Desiree and 9% for Cardinal). Contrary to the shoot length, Desiree showed longer roots than Cardinal. Data indicated that root length increased with increase in sucrose concentration but increase in root length decreases proportionately at higher sucrose concentrations as discussed in case of shoot length. Root length also increased with increasing time scale (Fig.1D). Similarly, Harvey *et al*. (1994), Al-Abdallat and Suwwan (2002) and Zaman *et al.* (2015) also reported that both genotypic difference and sucrose concentration affect root length in potato.

***Number of Leaves*:** The data were recorded for number of leaves as well. Different concentrations of sucrose were used for comparison of two Potato varieties. Maximum number of leaves (11.33) was observed in 5th week for Desiree at 9% sucrose concentration, while minimum number of leaves (0) was observed in 1st week for both Desiree and Cardinal at 12% sucrose concentration (Table 2). From analysis of variance, highly significant results were observed for varieties, weeks and concentration of sucrose. Interaction of week, variety and concentration showed non-significant results (Table 1). Varietal difference exhibited a significant variation in number of leaves. Desiree developed more leaves than Cardinal. In case of Desiree, shoot length and intermodal distance were low whereas number of leaves was high. Data depicts that number of leaves increased with increase in sucrose concentration. Data showed that 9% Sucrose was the best for producing maximum leaves in case of Desiree but 6% was the appropriate one for Cardinal (Fig.1E). Similar results were reported by Zaman *et al.* (2015). It is also evident from these results that Desiree is relatively more resistant genotype towards osmotic stresses as compared to Cardinal.

***Conclusion and prospects*:** It has been concluded that sucrose being the chief C-source under *in vitro* conditions has a direct effect on NRA and increase in sucrose concentration to an optimum level leads to enhanced NRA. Subsequently, higher nitrate reduction resulted in increased level of TSP which brought about an improvement in overall plant growth. Hence, it can be said that improving NRA through modern biotechnological interventions like genome editing technology, we can improve plant productivity.

REFERENCES

Al-Abdallat, A. and M. Suwwan. 2002. Influence of sucrose and CO_2 enrichment on potato (*Solanum tuberosum*, L.) tuberization *in vitro*. Dirasat. Agricult. Sci. 29**:**87-96.

Ananthi, K. and H. Vijayaraghavan. 2012. Soluble Protein, Nitrate Reductase Activity and yield responses in Cotton genotypes under water stress. Insight Biochem. 2:1-4

Beevers, L. and R.H. Hageman. 1980. Nitrate and Nitrite reduction. In: B.J. Miflin. (Ed.) *The Biochemsitry of Plants* Vol.5. Academic Press, Inc. New York. pp. 166-169.

Borna RS, M. I. Hoque and R. H. Sarker. 2019. *In vitro* Microtuber induction and regeneration of plantlets from microtuber discs of cultivated Potato (*Solanum tuberosum* L.). Plant Tissue Cult. Biotech. 29:63-72

Camacho-Cristóbal, J.J., J.M. Maldonada and A. González-Fontes. 2002. Effects of phosphate on *in vitro* nitrate reductase activity from tobacco leaves. Plant Sci. 163:455-461

Chamizo-Ampudia, A., E. Sanz-Luque, A. Llamas, A. Galvan and E. Fernandez. 2017. Nitrate Reductase Regulates Plant Nitric Oxide Homeostasis. Trends Plant Sci. 22:163-174

Diémé, A., M.A.N. Sambe, E.C.A. Mame and O. Sy. 2013. Residual Effects of Sucrose and Hormonal Treatments of the Tuberization Medium on *in Vitro* Germination of Potato (*Solanum tuberosum* L.) Microtubers. Amer. J. Plant Sci. 4:1872-1878

Elazab, D.S. and Shaaban, M.M. 2015. The impact of sucrose concentration on root growth and development in Fig *(Ficus carica L.) in vitro*. Assiut J. Agric. Sci. 46: 67-75

Fatima, B., M. Usman, I. Ahmad and I.A. Khan. 2005. Effect of explant and sucrose on microtuber induction in potato cultivars. Int. J. Agri. Biol. 7:63-66

Fufa, M. and M. Diro. 2013. The Effects of Sucrose on *in vitro* Tuberization of Potato Cultivars. Adv. Crop Sci. Technol. 1:114. doi:10.4172/2329-8863.1000e114

Fukuyama, K. 2004. Structure and function of plant-type ferredoxins. Photosynthesis Res. 81:289-301.

Ghaffoor, A., G.B. Shah and K. Waseem. 2003. *In vitro* response of potato (*Solanum tuberosum* L.) to various growth regulators. Biotechnology 2:191-197.

Haba, P.D.L., E. Aguera, L. Benitez and J.M. Maldonaldo. 2001. Modulation of nitrate reductase activity in cucumber (*Cucumis sativus*) roots. Plant Sci. 161:231-237.

Harvey, B.M.R., G. Bowden, C. Reavey and C. Selby. 1994. Stimulation of *in vitro* root and shoot growth of potato by increasing sucrose concentration in the presence of Fluridone, an inhibitor of abscisic acid synthesis. Plant Cell Tiss. Org. Cult. 37:271-276

Hinkelmann, K. and O. Kempthorne (2008). Design and Analysis of Experiments. I and II (Second ed.). Wiley. ISBN: 978-0-470-38551-7.

Hoff, T., H.N. lhong and M. Caboche. 1994. The use of mutants and transgenic plants to study nitrate assimilation. Plant Cell Environ. 17:489-506.

Hoque, M.E. 2010. *In vitr*o tuberization in potato (*Solanum tuberosum* L.). Plant Omics J. 3:7-11.

Humera, A. and J. Iqbal. 2010. *In vitro* techniques and mutagenesis for the genetic improvement of potato cvs. Desiree and Diament. Pak. J. Bot. 42:1629-1637.

Kaiser, W.M. and S.C. Huber. 1994. Posttranscriptional regulation of nitrate reductase in higher plants. Plant Physiol.106:817-821.

Kanwal, A., Ali, A. and K. Shoaib.2006. *In Vitro* Microtuberization of Potato (*Solanum tuberosum* L.) Cultivar Kuroda-- A New Variety in Pakistan. Int. J. Agri. Biol. 8:337-340

Klein, D., R. Morcuende, M. Stitt and A. Krapp. 2000. Regulation of nitrate reductase expression in leaves by nitrate and nitrogen metabolism is completely overridden when sugars fall below a critical level. Plant, Cell Environ.23:863-871

Li, G.J., F.T. Peng, L. Zhang, X.Z. Shi and Z.Y. Wang. 2010. Cloning and characterization of a SnRK1 encoding gene from *Malus hupehensis* Rehd. and heterologous expression in tomato. Mol. Biol. Rep. 37:947-954.

Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall. 1951. Protein measurement with the Folin Phenol reagent. J. Biol. Chem. 193:265-275.

Nikitin, A.V. and S.F. Izmailov. 2016. Enzymes of Sucrose Dissimilation as Targets for Nitrate in Early Ontogenesis of Garden Pea. Russ. J. Plant Physiol. 63:152-157.

Rabbani, A., B. Askari, N.A. Abbasi, M. Bhatti and A. Quraishi. 2001. Effect of growth regulators on *in vitro* multiplication of potato. Int. J. Agri. Biol. 3:181-182.

Rad, J.S., M.S. Rad and A. Miri. 2013. Regulation of the Expression of Nitrate Reductase genes in Leaves of Medical plant, *Foeniculum vulgare* by Different Nitrate Sources. Int. J. Agri. Crop Sci. 5:2911-2916

Rafique, T., M.J. Jaskani, H. Raza and M. Abba. 2004. *In vitro* studies on microtuber induction in potato. Int. J. Agri. Biol. 6:375-377

Rauscher, G.M., C.D. Smart, I. Simko, M. Bonierbale, H. Mayton, A. Greenland and W.E. Fry. 2006 Characterization and mapping of RPi-ber, a novel potato late blight resistance gene from *Solanum berthaultii*. Theo. Appl. Genet. 112:674-687.

Saha, S., M. Ahmed, M.M. Islam, R.N. Remme and M.R. Ali. 2013. Effect of different levels of sucrose on microtuberization and different substrates on minituber production resulted from potato meristem culture. IOSR J Agri. Vet. Sci. 4:58-62.

Solomonson, L.P. and M.J. Barbar. 1990. Assimilatory Nitrate Reductase: Functional Properties and Regulation. Ann. Rev. Plant Physiol. 41:225-253.

Steel, R.G.D. and J.H. Torrie. 1980. Principles and Procedure of Statistics. A Biometrical Approach. McGraw Hill Book Co., Inc. New York.

Sym, G.J. 1984. Optimization of the *in-vivo* assay conditions for nitrate reductase in barley. J. Sci. Food Agri. 35:725-730.

Taghavi, T.S. and M. Babalar. 2007. The effect of nitrate and plant size on nitrate uptake and in vitro nitrate reductase activity in strawberry (*Fragaria X ananassa* cv. Selva). Sci. Hort. 112:393-398

van Rensburg, J.G.J. and B.M. Vcelar. 1989. The effect of the sucrose concentration on the initiation and growth of adventitious buds from leaf tissues of *Lachenalia*. S. Afr. J. Bot. 55:117-121.

Verma, S.K., A.K. Das, G.S. Cingoz, E. Uslu and E. Gurel. 2016. Influence of nutrient media on callus induction, somatic embryogenesis and plant regeneration in selected Turkish crocus species. Biotechnology Reports .10:66-74.

Wazir, A., Z. Gul, M. Hussain, Z.U. Khan, M. Saleem and I. Khurshid. 2015. Effect of sucrose on inducing *in vitro* microtuberization in potato without using any growth hormone. Int. J. Agron. Agri. Res. 7:118-124

Yu, W.C., P.J. Joyce, D.C. Cameron and B.H. Mc Cown. 2000. Sucrose utilization during potato microtuber growth in bioreactors. Plant Cell Rep. 19:407- 413.

Zahara, M., A. Datta, P. Boonkorkaew and A. Mishra. 2017. The Effects of Different Media, Sucrose Concentrations and Natural Additives on Plantlet Growth of *Phalaenopsis* Hybrid 'Pink'. Braz. Arch. Biol. Technol. 60:e1 71 60149.

Zaman, M.S., A. Quershi, G. Hasan, R.U. Din, S. Ali, A. Khabir and N. Gul. 2001. Meristem culture of potato (*Solanum tuberosum* L.) for production of virus free plantlets. Online J. Biol. Sci. 1:898-899.

Zaman, S., S.Z.A. Shah, M. Shehzad, F. Kayani, S. Erum S and N. Ahmad. 2015. Characterization of Potato (*Solanum tuberosum* L.) adquiridode Centro Internacional de la Papa. J. Agri. Technol. 11:1641-1647.