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Research Article

EFFECT OF NITRATE NUTRITION DURING *IN VITRO* PHASE OF POTATO MICROTUBER PRODUCTION

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Abstract- The effects of nitrogen nutrition modification in Murashige and Skoog's medium during in vitro phase on the production of microtuber was studied to find out whether it enhance their effects on the weight and size of microtuber. The results reported that the reduction in total nitrogen by nitrate concentration in media gave better response for microtuberisation. The reduced level in nitrogen helps to obtain maximum weight and size of microtuber. Varietal differences were also reported and Kufri Chipsona-1 gave highest weight of its microtuber followed by Kufri Pukhraj and Kufri Badshah in the reduced level of nitrogen treatment. However, average effect showed that Kufri Pukhraj was best followed by Kufri Chipsona-1.

Keywords- Nitrate nutrition, *In vitro* phase, *Sollanum tuberosum,* Microtuberisation, Microtuber.

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Introduction

Potato is a good, cheap source of carbohydrate, vitamins, minerals and proteins .It has multipurpose use in daily consumption and also industrial purpose [1]. The physiological quality and safety of seed tubers are one the most important factors influencing potato yield [2].Generally, in the common methods, seed potato tubers are used for multiplication and production [3]. Some of these are low rate of multiplication, low output and high risk of concentration by different microbial infections which require aggressive control and a high number of field multiplication [4,5]. A method for in vitro culturing of potato tubers since 1953, Baker's demonstrations [6]. *In-vitro* cultures and microtuberisation of potato (*Sollanum tuberosum* L.), has been studied by many authors [7-11]. Sarkar and Naik reported that the mineral constituents, the form and concentration of nitrogen significantly affects the in vitro tuberisation process [12]. Considering the importance of mineral nutrition in induction and development of microtuber, the research study was carried out to find out the effect of nitrogen nutrition for potato microtuber production.

Material and Methods

In-vitro axenic cultures were produced from nodal explants and then tuber induction in resultant in vitro plantlets was studied [13]. The effect of nitrate concentration on in microtuberisation were studied in the following treatment where the different inorganic nitrogen nutrition in MS medium while others nutrient were kept as such in MS (1962)[14].

N_1 =MS (1900 $mgl^{-1}KNO_3$ + 1650 mgl^{-1} NH_4NO_3) + 5 mgl^{-1} BA + 500 mgl^{-1} CCC + 8 % Sucrose + 0.8% Agar

N_2=MS (3800 $mgl^{-1}KNO_3$ + 3300 $mgl^{-1}NH_4NO_3$) + 5 mgl^{-1} BA + 500 mgl^{-1} CCC + 8 % Sucrose + 0.8% Agar

N_3 =MS (950 $mgl^{-1}KNO_3$ + 825 $mgl^{-1}NH_4NO_3$) + 5 mgl^{-1} BA + 500 mgl^{-1} CCC + 8% Sucrose + 0.8% Agar

N_4=MS (475 $mgl^{-1}KNO_3$ + 412 mgl^{-1} NH_4NO_3) + 5 mgl^{-1} BA + 500 mgl^{-1} CCC + 8% Sucrose + 0.8% Agar

N_5=MS (1900 $mgl^{-1}KNO_3$ + 1650 $mgl^{-1}NH_4NO_3$) + 5 mgl^{-1} BA + 250 mgl^{-1} CCC + 8 % Sucrose + 0.8% Agar

N_6 =MS (3800 $mgl^{-1}KNO_3$ + 3300 mgl^{-1} NH_4NO_3) + 5 mgl^{-1} BA + 250 mgl^{-1} CCC + 8 % Sucrose + 0.8% Agar

N_7=MS (950 $mgl^{-1}KNO_3$ + 825 $mgl^{-1}NH_4NO_3$) + 5 mgl^{-1} BA + 250 mgl^{-1} CCC + 8% Sucrose + 0.8% Agar

N_8 =MS (475 $mgl^{-1}KNO_3$ + 412 $mgl^{-1}NH_4NO_3$) + 5 mgl^{-1} BA + 250 mgl^{-1} CCC + 8% Sucrose + 0.8% Agar

Result and Discussion

Effect of nitrate concentration on *in vitro* microtuber production

Study the effect of nitrate concentrations on *in vitro* microtuber production was conducted and the results are discussed here as under.

Weight of microtuber

Treatment N_3 (inorganic nitrogen was reduced to half in MS medium i.e. MS (950 mgl^{-1} KNO_3 + 825 mgl^{-1} NH_4NO_3 + 5 mgl^{-1} BA + 500 mgl^{-1} CCC + 8% sucrose + 0.8% Agar) found to be best responsive for the obtaining the higher weight of microtuber [Table-1].

It was observed that when the levels of growth retardants was reduced from 500 mgl^{-1} CCC to 250 mgl^{-1} CCC the trend was almost similar and the lower levels of inorganic nitrogen gave better response for the harvesting of higher weight of microtuber. The treatment N_3 and N_7 gave best results in which Kufri Chipsona-1 has highest weight of microtuber was obtained. [Table-1]. It was also reported that the 500 mgl^{-1} CCC was better as compared to 250 mgl^{-1} CCC in the treatments.

Table-1 *Effect of nitrate concentration on weight of microtuber*

Treatment	Variety			Mean (M) gm
	V_1 Kufri Badshah	V_2 Kufri Chipsona-1	V_3 Kufri Pukhraj	
N_1	0.022	0.010	0.034	0.022
N_2	0.038	0.033	0.035	0.035
N_3	0.042	0.081	0.060	0.061
N_4	0.019	0.032	0.059	0.037
N_5	0.033	0.017	0.034	0.028
N_6	0.015	0.031	0.031	0.025
N_7	0.034	0.067	0.044	0.048
N_8	0.044	0.046	0.043	0.044
Mean (V)	0.031	0.040	0.043	
	V	N	V x N	
S.Em. ±	0.002	0.003	0.005	
C.D.	0.005	0.008	0.014	
C.V. %				33.37

The result indicates that when inorganic nitrogen was increased to double then also positive response and the weight increased. When variety was compared the Kufri Chipsona-1 gave highest weight of its microtuber followed by Kufri Pukhraj and Kufri Badshah in the best treatments N_3 and N_7.However, average effect showed that Kufri Pukhraj was best followed by Kufri Chipsona-1.

Size of microtuber

The results indicate that when the level of inorganic nitrogen was reduced the size of microtuber reported to be increased. The maximum size of microtuber was reported in N_3 treatment followed by N_7 treatment [Table-2]. Both treatments contain the lower levels of nitrogen content. At the same time when the levels of inorganic nitrogen were reduced to one fourth then the size of microtuber were reduced.

After 90 days, N_3 treatment found to be best where the level of nitrogen was reduced. The higher levels of nitrogen in N_6 treatment where 250 mgl^{-1} CCC was incorporated then results were not responsive. Even though N_2 treatment where levels of nitrogen were higher with higher levels of CCC gave the positive response for microtuber formation. In N_7 treatment response was good may be due to the half levels of nitrogen and in N_8 treatment, the levels was one fourth also gave the positive response as compared to double levels of nitrogen.

Table-2 *Effect of nitrate concentration on size of microtuber*

Treatment	Variety			Mean (M) cm
	V_1 Kufri Badshah	V_2 Kufri Chipsona-1	V_3 Kufri Pukhraj	
N_1	0.325	0.278	0.288	0.297
N_2	0.387	0.275	0.317	0.326
N_3	0.397	0.487	0.440	0.441
N_4	0.320	0.333	0.390	0.348
N_5	0.303	0.210	0.358	0.291
N_6	0.182	0.298	0.268	0.249
N_7	0.370	0.460	0.343	0.391
N_8	0.349	0.387	0.368	0.368
Mean (V)	0.329	0.341	0.347	
	V	N	V x N	
S.Em. ±	0.010	0.017	0.029	
C.D.	NS	0.047	0.081	
C.V. %				21.0

In case of Kufri Chipsona-1 (KC) N_1 treatment was not very much effective. The N_3 treatment had reduced levels of nitrogen was effective in size and growth with higher levels of CCC i.e. 500 mgl^{-1}. The least level of nitrogen in N_4 treatment also gave positive response but size was reduced visually.

It was noted in the 500 mgl^{-1} CCC was better compare to 250 mgl^{-1} CCC in microtuber formation. We can say that not significantly differ but they are at par.

Higher dose of nitrogen was not good for microtuberisation in N_6 treatment. Reducing levels in N_7 treatment was found to be positive response for the microtuberisation. Same trend was found in Kufri Pukhraj variety.

The effect of nitrate concentration on *in vitro* microtuberisation was presented [Fig-1], which indicates treatment N3, and N_7 were best for Kufri Chipson-1 and Kufri Badshah and least effective for Kufri Pukhraj (in that particular treatment). [Fig-2] indicates the size of microtubers in three varieties of the experiments.

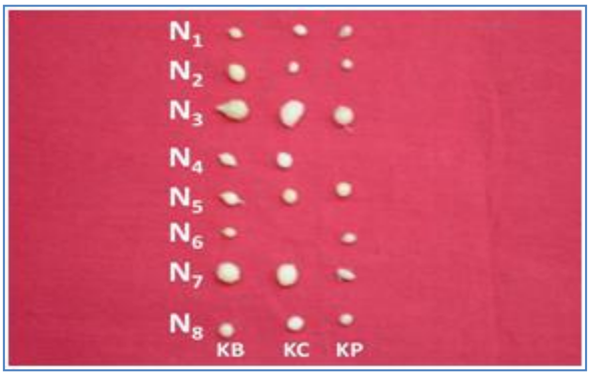

Fig-1 Effect of nitrate concentration on *in vitro* microtuberisation

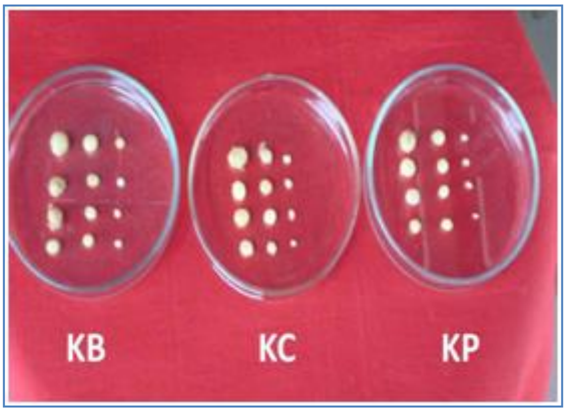

Fig-2 Different size of microtubers in three different varieties of potato

The size of microtuber is more (N_3 and N_7) which contains lower levels of nitrogen but the levels of inorganic nitrogen is reduced to lower i.e. one fourth concentration leads to lowering the size of microtuber. In N_6 the level of inorganic nitrogen was higher which found to be less effective. Even in Kufri Chipsona-1. It does not respond even.

From the overall discussion, it was reported that reduced nitrogen level found to be best for obtaining maximum weight and size of the microtuber. At the very same time, it was found best the half time reduced inorganic nitrogen as compared to one fourth reducing level. These our results were supported by Stallknecht and Farnsworth [13] as well as Wattimena [15] where they found that law nitrogen was best for coumarin induced tuberisation in potato while Sarkar and Naike [12] found that reduced nitrogen level in the medium caused better results during cytokinin induced tuberisation.

In present results, when the level of nitrogen was increased to double it was not found better as compared to the reducing level of nitrogen, the increased level had a inhibitory effect on micro tuberisation when compared to the reduced level of nitrogen. These results were supported by Zarrabeitia *et al.*, [16] as they reported that micro propagation in low nitrogen medium resulted in significantly higher tuberisation than micro propagation in high nitrogen containing MS medium. Sarkar and Naike [12] also found that high nitrogen concentration did not because total inhibition of cytokinin induced microtuberisation but the number of microtuber developed per gm biomass reduced. Stalknecht and Farnsworth [13] reported that no tubers were found when high nitrogen was present in the medium.

On the contrary, the inhibitory effect of reduced nitrogen level on microtuber was reported in potato when the microtubers were induced on media free growth regulating substances Garner and Blake [17].

In the present study the size and weight was positively influenced with reduced level of nitrogen nutrition. The results and discussion narrates that nitrogen nutrition is sensitive during cytokinin induced micro tuberisation. Nitrogen nutrition of intact potato plants influences synthesis and translocation of abscisic acid and cytokinins [18-20]. The growth retardants 500 mgl^{-1} CCC found to be more beneficial for microtuberisation, which was supported with the results of Rodrigues *et al*. [21] and Hossain [22].

Furthermore, nitrogen nutrition effect found to be significant for the varieties in case of weight of microtuberisation. This suggests that nitrogen nutrition also play role for better results for a specific cultivar. The reason may be a genetic potential of a genotype, which is directly or indirectly influenced by a number of factors. Many workers in potato reported that cultivar affected by different factors like nutrition, growth regulators, photoperiod, temperature etc.

Conclusion

It was concluded that half level (i.e. 950 mgl^{-1} KNO_3 and 825 mgl^{-1} NH_4NO_3) of inorganic nitrogen in the MS media gave best results. When the level of inorganic nitrogen was reduced the size of microtuber reported to be increased. Further it was concluded that half level of inorganic nitrogen in the MS media gave best results with benefit of economic point of view also, because the inorganic nitrogen were used less quantity with better results. Higher dose of nitrogen was not good for microtuberisation. It was noted that in the 500 mgl^{-1} CCC was better compare to 250 mgl^{-1} CCC in microtuber formation.

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Abbreviations:

MS- Murashige and Skoog, KNO_3 - Potassium nitrate, NH_4NO_3-Ammonium nitrate, BA-6-Benzylaminopurine, benzyl adenine, CCC-Chlorocholine Chloride, V-variety, M-media, gm-gram, mg- milligram, ml-milliliters, cm- centimetre,

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors

Conflict of Interest: None declared

References

[1] Hoque M.E.(2010) *Plant Omics Journal* 3(1),7-11

[2] Wiersema S.G. (1984) The Production and Utilization of Seed Tubers Derived from True Potato Seed. PhD thesis, University of Reading, Department of Agriculture and Horticulture, Reading, UK, pp. 229

[3] Struik P.C. and Wiersema L. M.J. (1990) Production, storage and use of micro- and minitubers. Proceeding of the 11th Triennial Conference of the European Association for Potato Research (EAPR), Edinburgh, UK pp. 122-133.

[4] Beukema and Van der Zaag (1990) Introduction to potato production. Pudoc, Wageningen, The Netherlands, pp. 208.

[5] Struik P.C. and Wiersema I.G. (1999) Seed potato technology. Wageningen Pers Wageningen The Netherlands, 383 pp

[6] Baker W.G. (1953) *Science*, 118, 384-385

[7] Nistor A., Nicoleta C., Mihaela C. and Monica P. (2012) *Studia Universitatis "Vasile Goldiş", Seria Ştiinţele Vieţii* 22(4),543-547

[8] Koleva G.L.,Sasa M.,Trajkova F. and Llievski M.(2012*) Electronic Journal of Biology*, 8(3),45-49

[9] Macwan S.J., Subhash N., Vaishnav, P.R. and Shukla Y.M. (2010) *In* National conference of plant physiology and molecular approaches for crop improvement under changing environment, November 25-27 BHU, Varanasi, pp173

[10] Balali G.R., Hadi M.R., Yavari P., Bidram H., Naderi A.G.and Eslami A. (2008) *African Journal of Biotechnology*, 7,1265-1270.

[11] Ashan H., Jihad A. and Danielle J.D. (2004/5) *Potato Research*,47,139-150

[12] Sarkar D. and NaikeP.S (1998) *Potato Research* 41, 211 -217

[13] Stallknecht, G.F. and Farnsworth S. (1979) *American Potato Journal,* 56, 523-530.

[14] Murashige T. and Skoog F. (1962) *Physiol. Plant*, 15, 473-497

[15] Wattimena G.A. (1983) Micropropagation as an alternative technology for potato production in Indonesia. PhD Thesis, University of Wisconsin, Madison.

[16] Zarrabeitia A., Lejarcegui X., Veramendi J.& Mingo-Castel A.M. (1997) American *Potato Journal,* 74,369-378.

[17] Garner N. and Blake J.(1989) *Annals of Botany,* 63, 663-674.

[18] Sattelmacher B. and Marschner H. (1978) *Physiologia Plantarum,* 42,185-189

[19] Sattelmacher B. and Marschner H. (1978) *Physiologia Plantarum,* 44, 65-68

[20] Krauss A. (1985) Interaction of nitrogen nutrition, phytohormones, and tuberization. *In:* P.H.Li (Ed.), Potato Physiology. Academic Press, London, pp. 209-230.

[21] Rodriguez E., Martiney O., Alcantwa P., Quinones Y. and Izquierdo H. (2004) *Cutivols Tropicales,* 25(2), 23-27.

[22] Hossain M.J. (2005) *Plant Tissue Cult .& Biotech.*, 15(2), 157-166.