Plant Environment Development 8(1): 1-8, 2019 (January)

Print ISSN 1994-1501
Online ISSN 2311-3529

©Department of Botany, University of Rajshahi

http://plantenvdev.com
e-mail: plant.environ.dev@ru.ac.bd

Research Paper

Jasmonic acid induced microtubers of potato and its estimation of reducing sugar, total sugar, starch and vitamin C

M. H. Rahman, R. Karim and M. Hossain

Department of Botany, University of Rajshahi, Rajshahi-6205, Bangladesh
Email: hasanur7@yahoo.com

Abstract

Potato is one of the most important vegetable crops in Bangladesh. Potato microtubers are pea-like tiny tubers produced under *in vitro* culture which can provide adequate information for the physiological and metabolic studies of tuberization and the screening of potential potato genotypes. This study investigates the effects of Jasmonic acid (JA) induced microtubers on the accumulation of reducing sugar, total sugar, starch and vitamin C in microtubers of five potato cultivars. There was a significant impact among the JA levels (0, 5 and 10 µM) in accumulation of reducing sugar, total sugar and starch in microtubers. The 5 µM JA induced microtubers, significantly stimulated the accumulation traits. Microtubers treated with JA at 0 and 10 µM showed low performance and no significant difference existed between them in accumulation features. Among the cultivars tested, significant differences were noticed in accumulation of reducing sugar, total sugar and starch in microtubers except Diamant, All Blue and Shilbilaty in accumulation of starch. Diamant microtubers performed the best followed by Shilbilaty in all the accumulation traits. The estimation of vitamin C in microtubers showed that freshly harvested microtubers (5 µM JA induced microtubers) gaineds high amount of vitamin C accumulation but it was gradually declined in the extended storage periods. At 8-week storage, microtubers decreased in vitamin C content ranges from 18.7 to 10.82 mg/ 100 g found in different cultivars. Just after harvest , the vitamin C content of Diamant microtubers was high (24.4 mg/100 g) which gradually decreased by 16.2 mg/100 g at 8-week storage. The lowest amount of vitamin C content (10.82 mg/100mg) was noted in Atlanta microtubers after 8-week storage period. The present investigation may provide a clue for elite microtuber production (with an optimization of JA in potato microtuberization process) with a view to accumulate optimum level of soluble total sugar, reducing sugar, starch and vitamin C in microtubers for quality tuber production in screen house. It may also indicate a new avenue for the potato processing industry especially to avoid low temperature sweetening of potato tubers.

Keywords: Jasmonic acid, Potato microtubers, total sugar, reducing sugar, starch and Vitamin C.

INTRODUCTION

Potato microtubers are small tubers raised from micropropagation process. Quality seed potato production is quite dependent on micropropagation of virus-free plant materials. The end product of potato micropropagation is either plantlets or microtubers. The microtubers are more convenient than plantlets in respect to handling, long term storing and transporting. So, microtuber could be an ideal propagation material for seed potato production and also suitable for both short and long term germplasm storage (Wang and Hu, 1982, Rosell *et al.*, 1987, Tovar *et al.*, 1985). Besides, microtubers can be produced year round without seasonal constraints. Potato microtubers are generally considered to be too small to be efficiently used as seed tubers for the production of minituber to generate nuclear stocks.

Some of the micro-environmental factors such as photoperiod, temperature, nitrogen level, growth regulators, and sucrose play an important roles in the potato microtuberization process. However, no unequivocal tuberizing factor has been identified till date

and therefore, the recognition of a tuber inducing signal still remains as an elusive goal (Jackson, 1999). Tuberonic acid (TA) and tuberonic acid glucoside (TAG) with strong tuber inducing activities were isolated from potato leaves process (Koda and Okazawa, 1988; Yoshihara *et al*., 1989) which was later found to be structurally related to jasmonic acid (JA) having similar levels of tuber inducing activity in vitro (Koda *et al.,* 1991). In potato, plantlets cultured *in vitro* from stolons and nodal cuttings, JA stimulated microtuberization (Koda *et al*., 1991; Pruski *et al*., 2001, 2002; Abdala *et al*., 1996). It was demonstrated that 5 µM JA in microtuber media proved to be more potent growth regulator in microtuber formation (Rahman *et al,* 2013). Pelacho and Mingo-Castel (1991a) showed that JA was the stronger regulator of tuberization *in vitro* than kinetin. Koda (1997) reported that potato tuberization is regulated by a balance between the levels of jasmonates and gibberellin in the potato tissue cultured *in vitro.*

Potatoes are one of the most important sources of vitamin C in those countries where they are produced and consumed. Among vegetables and fruits, concentration of vitamin C in potatoes is moderate to low as listed by Mosure (2004) citing the dietary guidelines advisory committee. So, it is to be realized that the amount of vitamin C retention in potato tuber is very important for regular intake of potatoes. In Bangladesh where potato is regarded as one of the prime vegetable for regular intake coming into the market from long term cold-storage of about 8-9 months. The status of vitamin C retention in tuber during storage or after storage is still a great demand for research. According to FAO (2002) report, the vitamin C content of conventional tubers during storage has been deteriorated considerably. With a view of this information, the present experiment was also planned and studied to find out the status of vitamin C content in freshly harvested microtubers of five potato cultivars.

The present investigation was employed with two objectives 1) to assess the comparative efficacy of JA containing media, in accumulation of reducing sugar, total sugar and starch in microtubers and 2) the estimation of vitamin C content in microtubers obtained from 8% sucrose media which promoted microtuberization.

MATERIALS AND METHODS

Plant stocks for microtuberization

Five potato cultivars (Atlanta, Shepody, All Blue, Diamant and Shilbilaty) belonging to the Plant Breeding and Gene Engineering Lab at the Department of Botany, University of Rajshahi were used in the present study. Cultures from single-node cuttings were established from virus-free meristem culture stocks. The MS medium (Murashige and Skoog, 1962) consisted of 3% sucrose and liquid condition. Twelve single-node cuttings were cultured per vessel containing 10 ml of medium. The cultures were incubated at 25 ±1°C under a 16 h photoperiod with a light intensity of 600-800 lux. Regular subculture was maintained on similar fresh medium at an interval of 21 days and followed until required microplants for tuberization was obtained.

In vitro tuberization

The 21-day-old microplants grown under above conditions were used as explants for microtuber formation. They were refreshed by microtuber induction medium. The microtuber induction medium was of two parts. The first part of microtuber induction medium was added with 0, 5 and 10 µM JA and 8% sucrose to test in accumulation of reducing sugar, total sugar and starch in microtubers. The second part of microtuber induction medium was comprised with 8% sucrose without JA, to determine the vitamin C content of microtubers at different storage periods. The pH of all the media was adjusted to 5.8. JA was filter- sterilized and added to the microtuber media after autoclaving at 121°C for 20 min. All the microtuberization cultures were grown in 8 h photoperiod (with 200-300 lux) at 18±2°C for 16 weeks. Microtubers were harvested after 16 weeks and evaluated the following experiments.

Freshly harvested microtubers were directly used for determination of reducing sugar, total sugar and starch. The vitamin C estimation of microtubers was carried out at different storage periods (0, 2, 4, 6 and 8 weeks) at 4°C in refrigerator. Prior to storage, freshly harvested microtubers were kept in incubator at 15°C in the dark for 3-5 days. Every two weeks intervals, data were taken from microtubers placed in four replicates petridishes and each petridish contained ten microtubers.

Determination of total sugar

Total sugar content of fresh potato flesh was determined by spectrophotometry anthrone method as described in Laboratory Manual in Biochemistry (Jayaraman, 1981).

a) Anthrone reagent: The anthrone reagent was prepared by dissolving 2 g of anthrone in 1 liter of concentrated H_2SO_4.

b) Standard glucose solution: A standard solution of glucose was prepared by dissolving 10 mg of glucose in 100 ml of distilled water.

Extraction of sugar from potato flesh

Extraction of sugar from potato flesh was done following the methods described by Loomis and Shull (1937). Two grams of potato microtuber flesh were taken into 80% ethyl alcohol and allowed to boil for 5-10 min (5 to 10 ml of alcohol was used for every gram of potato flesh). The extract was cooled and crushed thoroughly in mortar with a pestle. Then the pasted extract was filtered through two layers of muslin cloth and the re-extracted the ground tissue for three minutes in hot 80% ethyl alcohol using 2 to 3 ml of alcohol for every gram of sample. The second extraction ensured complete removal of alcohol soluble substances. The extract was cooled and passed through muslin cloth. Both the extracts were filtered through Whatman No-41 filter paper. The volume of the extract was evaporated to about $1/4^{th}$ the primary volume over a steam bath and cooled. The reduced volume of the extract was then transferred to a 100 ml volumetric flask and made up to the mark (100 ml) with double distilled water. Then 1 ml of the diluted solution was taken into another 100 ml volumetric flask and made up to the mark of 100 ml with distilled water (working standard).

Procedure

One ml of the extract was pipetted into test tube and 4 ml of the anthrone reagent was added to each of this solution and mixed well. Glass marbles were placed on the top of each tube to prevent loss of water by evaporation. The test tubes were heated for 10 min in a boiling water bath and then cooled. A reagent blank was prepared by taking 1 ml of distilled water and 4 ml of anthrone reagent in a tube and treated similarly. The absorbance of the blue green solution was measured at 680 nm using as the blank in spectrophotometer. A standard curve of glucose was prepared by taking 0, 0.1, 0.2, 0.4, 0.6, 0.8 and 1 ml of standard glucose solution in different test tubes containing 0, 10, 20, 40, 60, 80 and 100 µg of glucose respectively and made the volume up to 1 ml with distilled water. Then 4 ml of anthrone reagent was added to each test tube and mixed well. All these solutions were treated similarly as described above. The absorbance was measured at 680 nm using the blank containing 1 ml of distilled water and 4.0 ml of anthrone reagent.

The amount of total sugar content was calculated by constructing a calibration curve using glucose as standard. Finally, the percentage of total sugar present in the potato flesh was determined using the formula given below.

Calculation

$$\text{Total sugar in the potato flesh (g per 100 g of potato flesh)} = \frac{\text{Amount of total sugar obtained}}{\text{Weight of potato flesh}} \times 100$$

Determination of reducing sugar

Reducing sugar content of potato flesh was determined by dinitrosalicylic acid method (Miller, 1972).

Regents

a) Dinitrosalicylic acid (DNS) reagent: Simultaneously 1 g of DNS, 200 mg of crystalline phenol and 50 mg of sodium sulphite were placed in a beaker and mixed with 100 ml of 1% NaOH solution by stirring. If it is needed to store the material then sodium sulphite must be added just before the use.

b) 40% solution of Rochelle salt.

Extraction of reducing sugar from potato: Reducing sugar from potato flesh was extracted according to the following procedures:

Procedure

Three ml of the extract was pipetted into test tubes and 3 ml of DNS reagent was added to each of this solution and mixed well. The test tubes were heated for 5 min in a boiling water bath. After development of color, 1 ml of 40% Rochelle salt was added when the contents of the tubes were still warm. The test tubes were then cooled under a running tap water. A reagent blank was prepared by taking 3 ml of double distilled water and 3 ml of DNS reagent in a tube and treated similarly. The absorbance of the solution was measured at 575 nm in a spectrophotometer. The amount of reducing sugar content in potato microtubers were calculated by constructing a calibration curve using glucose as standard.

Calculation

$$\text{Amount of reducing sugar in potato flesh (g per 100 g of potato flesh)} = \frac{\text{Amount of reducing sugar obtained}}{\text{Weight of potato flesh}} \times 100$$

Determination of starch

The starch content of the fresh potato flesh was determined by spectrophotometry anthrone method as described in Laboratory Manual in Biochemistry (Jayaraman, 1981).

Reagents

a) Anthrone reagent: The anthrone reagent was prepared by dissolving 2 g of anthrone in 1 liter of concentrated H_2SO_4.

b) Standard glucose solution: A standard solution of glucose was prepared by dissolving 10 mg of glucose in 100 ml of distilled water.

c) 1 M HCl.

Procedure

Two grams of potato microtuber fleshes were cut into pieces and homogenized well with 20 ml of water. It was then filtered through double layer of muslin cloth. To the filtrate, twice the volume of ethanol was added to precipitate the polysaccharide mainly starch. After kept it overnight in cold the precipitate was collected by centrifugation at 3000 rpm for 15 min. The precipitate was then dried over a steam bath. Then 40 ml of 1 M HCl was added to the dried precipitate and heated to about 70°C. It was then transferred to a volumetric flask and diluted up to 100 ml with 1 M HCl. Then 2 ml of diluted solution was taken in another 100 ml volumetric flask and diluted again up to 100 ml with 1 M HCl. Aliquot of 1 ml of the extract of each treatment was pipetted into test tubes and 4 ml of anthrone reagent was added to the solution of each tube and mixed well. Glass marbles were placed on top of each tube to prevent loss of water by evaporation. The tubes were placed in a boiling water bath for 10 min then removed and cooled. A reagent blank was prepared by taking 1 ml of distilled water and 4 ml of anthrone reagent in a test tube and treated similarly. The absorbance of the blue-green solution was measured at 625 nm in a spectrophotometer. The amount of starch present in the potato flesh was calculated by constructing a calibration curve using glucose as standard.

Calculation

$$\text{Amount of starch in potato flesh (g per 100 g of potato flesh)} = \frac{\text{Amount of starch obtained}}{\text{Weight of potato flesh}} \times 100$$

Estimation of vitamin C (ascorbic acid)

Ascorbic acid (vitamin C) of potato flesh was determined by the titrimetric method (Bessey and King, 1933).

Reagents

a) Dye solution: 200 mg of 2,6-dichlorophenol indophenol and 210 mg of sodium bicarbonate were dissolved in distilled water and made up to 100 ml. The solution was then filtered.

b) 3% Meta-phosphoric acid reagent: 3 g of meta-phosphoric acid was dissolved in 80 ml of acetic acid and made up to 100 ml with distilled water.

c) Standard ascorbic acid solution (0.1 mg/ml): 10 mg of pure ascorbic acid was dissolved in 3% meta-phosphoric acid and made up to 100 ml with 3% meta-phosphoric acid.

Procedure

10 ml of standard ascorbic acid solution was taken in a conical flask and titrated it with the dye solution.Two grams of potato flesh was cut into small pieces and homogenized well with 3% meta-phosphoric acid (approximately 20 ml) and filtered it through double layer of muslin cloth. The filtrate was centrifuged at 3000 rpm for 10 min and the clear supernatant was titrated with 2,6-dichlorophenol indophenol solution. The amount of ascorbic acid present in potato was determined by comparing with the titration result of standard ascorbic acid solution.

Calculation

$$\text{Amount of ascorbic acid in potato flesh (g per 100 g of potato flesh)} = \frac{\text{Amount of ascorbic acid obtained}}{\text{Weight of potato flesh}} \times 100$$

Data and statistical analysis

The total duration of microtuberization was 16 weeks. The microtubers were harvested and reducing sugar, total sugar and starch were estimated as mentioned in earlier methods. The experiment was laid out in a two factorial (5 × 3) completely randomized design (CRD) involving five cultivars and three levels of JA. For each treatment, four replications were used for the estimation of total sugars, reducing sugar and starch. The data were analyzed by the standard procedure and the means were separated by Least Significant Difference (LSD) test. The data on vitamin C estimation were calculated with mean values of four replicates for each cultivar and storage period.

RESULTS AND DISCUSSION

Determination of reducing, total sugar and starch in potato microtubers

The two tested factors viz. cultivar and JA significantly affected in accumulation of reducing, total sugar and starch contents in microtubers. There was a significant impacts among the JA levels in accumulation of reducing, total and starch contents in microtubers. The 5 μM JA induced microtubers, significantly stimulated the accumulation of reducing, total sugar and starch contents in microtubers. JA at 10 μM was found low contents in reducing, total sugar and starch. No significant

differences were noticed in accumulation of the traits between 0 and 10 µM JA treated microtubers (Fig. 2).

Significant differences were noticed among the cultivars in accumulation of reducing, total sugar and starch in microtubers (Table 1). Among the cultivars, Diamant was the best followed by Shilbilaty at all the accumulation traits. But no significant differences were recorded among the Diamant, All Blue and Shilbilaty in accumulation of starch in microtubers (Fig. 1).

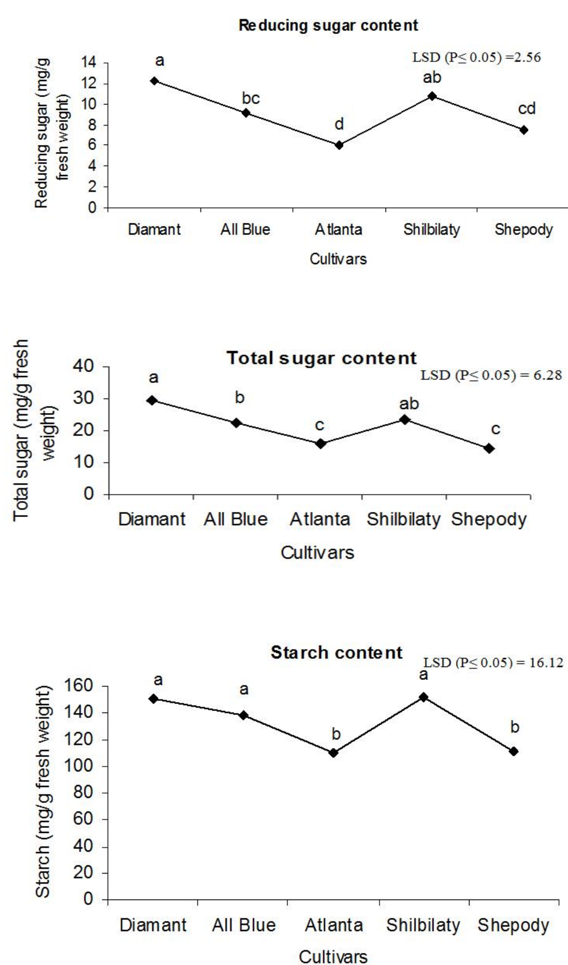

Fig. 1. Performances of cultivars in accumulation of reducing sugar, total sugar and starch in freshly harvested microtubers. Each value is an average of 3 treatments ×4 replications.

Several reports have been published concerning the effect of JA on potato tuberization (Koda *et al.,* 1991; Pelacho and Mingo-Castel, 1991a; Ravnikar *et al*., 1992; Helder *et al.,* 1993; Jackson and Willmitzer 1994; Castro *et al.,* 2000; Pruski *et al.,* 2001, 2002). It was surprising that 5 µM JA induced microtubers resulted with increased reducing sugar, total sugar and starch accumulation. However, JA and cultivars interacted between themselves to regulate the levels of reducing sugars, total sugars and starch in tubers. This invariably indicates that this JA level (5 µM) affected endogenous biochemical triggers in accumulation of reducing sugar, total sugar and starch depending on cultivars. A time course estimation of reducing sugar, total sugars and starch during various stages of tuber development from single-node cuttings under varying treatments of JA will be needed to examine the specific roles of JA in accumulation characteristics. Reducing sugar levels can be an estimate of the maturity of tubers grown in vitro (Xiong *et al*., 2002; Sharma *et al.,* 2004). Since JA caused an increase in reducing sugars in tubers, their yet another role in tuberization can be attributed to tuber maturity, possibly through an endogenous regulation of GA: ABA balance.

Table 1. The accumulation of reducing sugar, total sugar and starch in freshly harvested microtubers grown in three levels of jasmonic acid (JA) treatments. Each value is an average of 4 replicates.

Cultivars	Microtubers grown in JA (µM)	Reducing sugar mg/g fresh weight (Mean ± SE)	Total sugar mg/ g fresh weight (Mean ± SE)	Starch mg/g fresh weight (Mean ± SE)
Diamant	0	10.00±0.81	26.25±2.52	153.75± 2.39
	5	18.25± 0.35	42.0± 3.13	171.25±5.15
	10	8.50 ±0.04	20.5 ±2.46	126.25±3.75
All Blue	0	6.25 ±0.47	16.25 ±1.75	127.25± 4.64
	5	11.75±0.25	36.75 ±0.85	145.0±8.66
	10	9.50 ±0.64	14.75± 1.54	141.75± 8.12
Atlanta	0	3.50 ±0.28	10.75 ±0.47	90.75±2.12
	5	8.25 ±0.47	20.25±1.03	121.0±2.73
	10	6.25± 0.47	16.50± 2.5	117.5±2.70
Shilbilaty	0	9.00 ±0.40	23.25 ±2.28	141.25±6.57
	5	15.25±0.62	24.5 ±2.06	177.5±5.20
	10	8.00 ±0.70	23.0 ±2.12	136.25 ±1.25
Shepody	0	3.75± 0.75	12.25± 1.03	101.25 ±5.15
	5	11.75± 1.54	20.5 ±1.65	132.50 ±2.50
	10	7.25 ±1.43	11.50 ±0.95	98.75± 5.15

Mean square values from ANOVA

Source of variations	Degrees of freedom (df)	Mean square values		
Cultivar (C)	4	73.64*	444.76*	5114.85*
JA dose (J)	2	237.95*	852.51*	4505.81*
C × J	8	12.92*	110.32*	611.67*
Error	45	2.43	14.60	96.05

*, Significant at 5 , 1 and 0.1 % levels respectively.

The present experiment showed that the microtubers raised from 5 µM JA, resulted better starch accumulation than the control and 10 µM JA treated microtubers. The microtubers raised from 5 µM media produced larger microtubers (data not shown) may have relation with maximum starch accumulation. Tsafack *et al*. (2009) described that tuberization and subsequent tuber growth are characterized by massive accumulation of starch which has directly related with tuber size. In this experiment, 5 µM JA-grown microtubers may have contribution to accumulate more starch than the other two (control and 10 µM JA) sources. However, further investigation is needed to study the effect of JA in relation to accumulation characteristics.

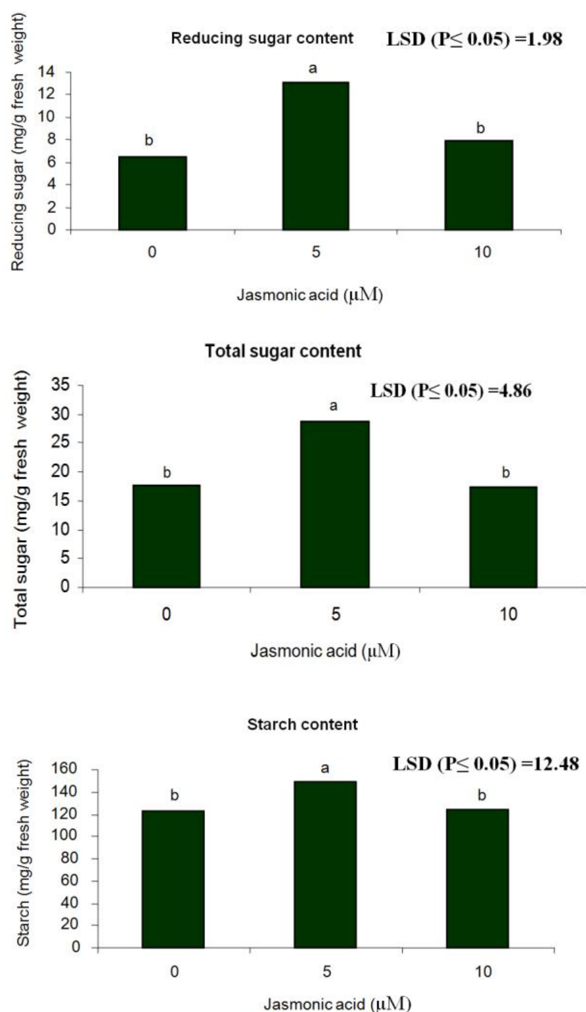

Fig. 2. Performances of JA in accumulation of reducing sugar, total sugar and starch in microtubers. Each value is an average of 5 cultivars × 4 replicates.

Estimation of vitamin C contents in potato microtubers

Duration of storage periods appears to have the greatest impact on vitamin C contents in microtubers. It was found that freshly harvested microtubers showed high vitamin C content but gradually it declined in the extended storage periods. Over a period of 8 weeks in storage, microtubers decreased in vitamin C content (from 18.7 to 10.82 mg/ 100 g) in different cultivars. Just after harvest (0 week), the vitamin C content of Diamant (24.4 mg/100 g) was high which gradually decreased as 16.2 mg/100 g at 8-week storage (Fig. 3). This declining trend was consistent for all the cultivars. The finding is in agreement with Perkins (1993) who showed rapid decline in conventional potato tubers stored for the first 10 weeks after which the decline rate continued but slowly. Shekhar *et al*. (1978) found that vitamin C is very low in newly developed tubers that it increased throughout the active growing phase of tubers and reached its highest level during the maturity of tubers. From that point, potatoes experienced a continuous decline in vitamin C for as long as they remained in storage.

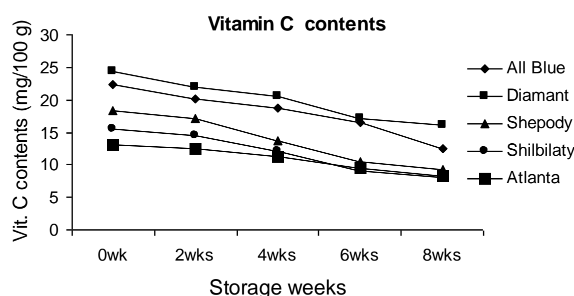

Fig. 3. Vitamin C content in potato microtubers after immediate harvest and extended storage periods.

To improve potatoes as a source of vitamin C, further work could be done thorough microtubers involving *in vitro* cultural and genetic manipulation and their storage mechanisms. Potato requires long-term storage for up to 10 months or more, modification of storage management factors may be necessary to minimize losses of vitamin C in potato tubers. It also brings to light the need to improve the potato as a source of vitamin C.

REFERENCES

Abdala, G., G. Castro, M. Guinazu, R. Tizio and O. Miersch. 1996. Occurrence of jasmonic acid in organs of *Solanum tuberosum* L. and its effect on tuberization. Plant Growth Regul. 19:139–143.

Bessy, O.A and C.G. King. 1933. The distribution of vitamin C in plant and animals tissues and its determination. J. Biol. Chem. 103: 687-698.

Castro, G., G. Abdala, C. Aguero and R. Tizio. 2000. Interaction between jasmonic and gibberellic acids on in vitro tuberization of potato plantlets. Potato Research. 43: 83-88.

Coleman, W.K., D.J. Donnelley and S.E. Coleman. 2001. Potato microtubers as research tools: a review. American Journal of Potato Res . 78: 47-55.

FAO. 2002. Vitamin C. Chapter 6 In: Human Nutrition and Mineral Requirements. Report of a joint FAO/WHO expert consultation, Bangkok, Thailand.

Helder, H., O. Miersch, D. Vreugdenhil and G. Sembdner. 1993. Occurrence of hydroxylated jasmonic acids in leaflets of *Solanum demissum* plants grown under long and short day conditions. Plant. Physiol. 88: 647–653.

Jackson, S.D. 1999. Multiple signaling pathways control tuber induction in potato. Plant. Physiol. 119: 1-8.

Jackson, S.D. and L. Willmitzer. 1994. Jasmonic acid spraying does not induce tuberization in short day requiring potato species kept in non-inducing conditions. Planta. 194:155–159.

Jayaraman, J. 1981. Laboratory Manual in Biochemistry. New Age International Ltd. New Delhi. pp. 180.

Koda, Y., Y. Kikuta, H. Takazi, Y. Tsujino, S. Sakamura and T. Yoshihara. 1991. Potato tuber-inducing activities of jasmonic acid and related compounds. Phytochemistry. 30:1435–1438.

Koda, Y. 1997. Possible involvement of jasmonates in various morphogenic events. Plant. Physiol. 98: 407-412.

Koda, Y. and Y. Okazawa. 1988. Detection of potato tuber inducing activity in potato leaves and old tubers. Plant Cell Physiol. 29: 969-974.

Loomis, W.E. and Shull, A.C. 1937. Methods in Plant Physiology: a laboratory manual and research handbook. Mc Graw Hill Book Company, Inc. NewYork and London.

Mosure, J. 2004. Vitamin C (Ascorbic Acid). Fact Sheet No. HYG-5552-05. Ohio State University , Columbus, Ohio.

Miller, G.L. 1972. Use of di-nitrosalicyclic acid reagent for determination of reducing sugar. Anal. Chem. 31(3): 426-428.

Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bioassay with tobacco tissue cultures. Plant. Physiol. 15 : 473-497.

Perkins, L.B. 1993. Comparisons of sugars, glycoalkaloids, vitamin C and organic acids in six potato cultivars from tuber formation throughout storage. Master's Thesis, University of Maine, Orono, Maine.

Pelacho, A.M. and A.M. Mingo-Castel. 1991a. Jasmonic acid induces tuberization of potato stolon cultured in vitro. Plant Physiology. 97: 1253-1255.

Pelacho, A.M. and A.M. Mingo-Castel. 1991b. Effects of photoperiod on kinetin-induced tuberization of isolated potato stolons cultured in vitro. Amer. Pot. J. 68: 533-541.

Pruski, K., P. Duplessis, T. Lewis, T. Astatkie, J .Nowak and P.C. Struik. 2001. Jasmonate effect on in vitro tuberization of potato (*Solanum tuberosum* L.) cultivars under light and dark conditions. Potato Res. 44: 315-325.

Pruski, K., T. Astatkie and J. Nowak. 2002. Jasmonate effect on in vitro tuberization and tuber bulking in two potato cultivars (*Solanum tuberosum* L.) under different media and photoperiod conditions. In Vitro Cell Develop Biol Plant. 38: 203-209.

Ravnikar. M., B. Vilhar and N. Gogala. 1992. Stimulatory effects of jasmonic acid on potato node and protoplast culture. J. Plant Growth Regul. 11: 29-33.

Rahman, M.H., S. A. Haider, M . Hossain and R. Islam, 2013. Involvement of Jasmonic acid and light periods on potato microtuber growth response. Int. J. Biosci. 3(5): 87-94.

Rosell, G., F.G. de Bertoldi and R. Tizio. 1987. In vitro mass tuberization as a contribution to potato micropropagation. Potato Research. 30: 111-116.

Sharma, S., A. Chanemougasoundharam, D. Sarkar and S.K. Pandey. 2004. Carboxylic acids affect induction, development and quality of potato (*Solanum tuberosum* L.) microtubers grown in vitro from single-node explants. Plant Growth Regul. 44: 219–229.

Shekhar, V.C., W.M. Iritani and R. Arteca. 1978. Changes in ascorbic acid content during growth and short-term storage of potato tubers (*Solanum tuberosum* L.). American Potato Journal. 55: 663–670.

Tovar, P., L. Schilde-Rentschler and J.H. Dodds. 1985. Induction and use of in vitro potato tubers. CIP Circular International Potato Center, Lima, Peru. 13: 1-5.

Tsafack, T.J.J., G.P. Charles, A. Hourmant, N.D. Omokolo and M. Branchard. 2009. Effect of photoperiod and thermoperiod on microtuberization and carbohydrate levels in Cocoyam (*Xanthosoma sagittifolium* L. Schott). Plant Cell Tissue and Organ Cult. 96: 151-159.

Van den Berg, J.H. and E.E. Ewing.1991. Jasmonates and their role in plant growth and development with special reference to the control of potato tuberization : a review . Amer. Pot. J. 68: 781- 794.

Wang, P.J. and C.Y. Hu.1982. *In vitro* mass tuberization and virus-free seed potato production in Taiwan. Amer. Pot. J. 59: 33–39.

Xiong, X., GCC Tai and JEA Seabrook . 2002. Effectiveness of selection for quality traits during the early stage in potato breeding population. Plant Breed. 121: 441–444.

Yoshihara, T., E. Omer, H. Koshino, S. Sakamura , Y. Kikuta and Y. Koda. 1989. Structure of a tuber inducing stimulus from potato leaves (*Solanum tuberosum* L.). Agr. Biol. Chem. 53: 2835–2837.