*Romanian Biotechnological Letters* *Vol. 16, No. 3, 2011*

**ORIGINAL PAPER**

# Shoot Proliferation from Node Explants of Potato (*Solanum tuberosum* cv. Agria). II. Effect of Different Concentrations of $NH_4NO_3$, Hydrolyzed Casein and BAP

Received for publication, November 14, 2010
Accepted, May 18, 2011

**MOTALLEBI-AZAR, A.*, S. KAZEMIANI, F. KIUMARSIE AND N. MOHADDES**
*Department of Horticultural Sciences, Faculty of Agriculture, Tabriz University, Tabriz, Iran.*
**Corresponding author: motallebiazar@gmail.com*
*Tel: 00984113392027, Fax: 00984113356005-00984113345332*

## Abstract

*In order to develop a protocol for rapid shoot proliferation of potato, the node explants were cultured onto Murashige & Skoog (MS) medium supplemented with different concentrations of $NH_4NO_3$ (800, 1900 and 2400 mg $l^{-1}$), hydrolyzed casein (0.0, 100 and 200 mg.$l^{-1}$) and 6–benzyl aminopurine (BAP) (0.0 and 2 mg $l^{-1}$). The most effective concentrations as regards the number of lateral shoots were media supplemented with 2400 mg $l^{-1}$ $NH_4NO_3$, without hydrolyzed casein, or with 800 mg $l^{-1}$ $NH_4NO_3$ and 200 mg $l^{-1}$ hydrolyzed casein, both media containing 2 mg $l^{-1}$ BAP. Maximum percentage of root formation and minimum percentage of callus formation was observed on media without BAP. The maximum number of roots per shoot was recorded at 800 mg $l^{-1}$ and 1900 mg $l^{-1}$ $NH_4NO_3$ in media without BAP. Minimum callus production percentage was observed in culture media containing 1900 mg $l^{-1}$ or 2400 mg $l^{-1}$ $NH_4NO_3$, in the absence of BAP. The present study describes an efficient method for in vitro shoot proliferation of the potato cultivar Agria, which could be also considered for large scale multiplication and propagation in other cultivars of this important vegetable crop.*

**Keywords:** BAP, Hydrolyzed casein, $NH_4NO_3$, shoot proliferation, *Solanum tuberosum.*

## Introduction

The propagation of potato (*Solanum tuberosum* L.) by *in vitro* culture of axillary buds is commonly used in the production of disease-free seed tubers, germplasm exchange, and conservation (Gopal *et al*., 1998). *In vitro*-cultured internodes produce shoots when incubated under suitable conditions. Work on shoot proliferation in potato has mainly focused on the use of growth regulators and there is considerable variation in the results of these studies. The obtained response depended upon a range of factors, including medium composition (salts, vitamins), temperature, light intensity and cultivar (Bajaj, 1987).

In most studies, different types and concentrations of cytokinins were used in proliferation medium. BAP was used to apical dominance removing as well as adventitious shoot induction (Belarmino *et al*., 1994). The highest numbers of potato shoots (80%) were produced on MS medium supplemented by 3 mg $l^{-1}$ of BAP (Uddin, 2002). Roca *et al*., (1978) showed that medium with BAP, $GA_3$ and NAA led to a rapid increase of shoots.

Nitrogen is essential to plant life. It is a constituent of both proteins and nucleic acids and also occurs in chlorophyll. Most animals cannot assimilate inorganic nitrogen or synthesize many of the amino acids unless assisted by bacteria. Both growth and morphogenesis in tissue cultures are considerably influenced by the availability of nitrogen and the form in which it is presented (George *et al*., 2008). Dimassi (2010) reported that inclusion in the substrate of 20 mg $l^{-1}$ $NH_4NO_3$ resulted in the greatest number and length of shoots per explant in olives (*Olea europaea sativa* L.). Villamor (2010) indicated that nitrogen in the form of $KNO_3$ significantly improved proliferation rate of ginger *in vitro*, in

both full and half strength media. Leaf growth was better in media devoid of $NH_4NO_3$. Root formation was significantly better in media without $NH_4NO_3$.

Hydrolyzed casein is a convenient source of substances which may promote plant growth, but they are by nature relatively undefined supplements. The proportion of individual amino acids in different hydrolyzed caseins depends on the nature of the source protein and the method by which the product has been prepared. Hydrolyzed casein produced an improvement in the growth of *Cardamine pratensis* and *Silene alba* suspensions, only if the medium is deficient in phosphorus (George *et al*., 2008). *Nicotiana tabacum* callus grew better on a nitrogen free MS medium when a mixture of the amino acids: L-glutamine (6 mM), L-aspartic acid (2 mM), L-arginine (1 mM) and glycine (0.1 mM) were added, rather than with 2 g $l^{-1}$ hydrolyzed casein (which would have provided about 2 mM glutamine, 0.6 mM aspartic acid, 0.2 mM arginine and 0.3 mM glycine) (Muller and Grafe, 1978). The growth rate of cell suspensions is frequently increased by the addition of hydrolyzed casein or one or more amino acids (particularly glutamine) to media containing both nitrate and ammonium ions. An observation by Murashige and Skoog (1962) that the presence of hydrolyzed casein allowed vigorous organ development over a broader range of IAA and kinetin levels may be of significance (George *et al*., 2008).

Here, we report the effects of different concentrations of $NH_4NO_3$, hydrolyzed casein and BAP on *in vitro* shoot proliferation in potato cv. Agria, for improving the micropropagation procedure.

## Materials and Methods

This research was conducted at Tissue Culture Laboratory of Horticultural Sciences, Tabriz University. In order to regenerate shoots, the nodes were cut into pieces of 0.3 – 0.5 cm, containing one axillary bud in each explant and were cultured on MS media containing three concentrations of $NH_4NO_3$ (800, 1900 and 2400 mg $l^{-1}$) and three concentrations of hydrolyzed casein (0.0, 100 and 200 mg.$l^{-1}$), 3% sucrose, 0.8% agar and supplemented with two concentrations of BAP (0.0 and 2 mg.$l^{-1}$) for growth of lateral shoots. The cultures were incubated at 25±2$^{o}$C, under 16h of light photoperiod. Four replications were tested for each treatment and after four weeks data regarding the number of lateral shoots/explant, number of roots per explant, main and lateral shoot length, root length, number of nodes per shoot and callus formation percentage were recorded. This study was carried out in a factorial experiment, based on a completely randomized design, with four replications and each culture jar containing six single-node explants. Data obtained from this study were analyzed using SPSS software Ver.16. The means comparison was carried out by Duncan's New Multiple Range.

## Results and Discussion

Formation and development of shoots were observed in all media supplemented with or without BAP. Therefore analysis of variance and mean comparison were ignored for shoot production percentage. Uddin (2002) reported that the highest (80%) number of potato shoots was produced on MS medium supplemented by BAP (3 mg.$l^{-1}$). Data analysis showed that the number of lateral shoots was not influenced by different concentrations of $NH_4NO_3$, hydrolyzed casein and their interactions. The number of lateral shoots was significantly affected by different concentrations of BAP and Hydrolyzed casein × $NH_4NO_3$ × BAP interactions ($p<0.05$). Addition of BAP to all culture media increased the number of lateral shoots compared to the media without BAP. Among all concentrations and combinations,

2400 mg $l^{-1}$ $NH_4NO_3$, in media without hydrolyzed casein, as well as 800 mg $l^{-1}$ $NH_4NO_3$ in medium containing 200 mg $l^{-1}$ hydrolyzed casein, both in the presence of 2 mg $l^{-1}$ BAP, were the most effective concentrations for number of lateral shoots. Therefore, increasing concentration of hydrolyzed casein had a positive effect on shoot growth from node and ultimately on number of lateral shoots, when was associated with reduction of $NH_4NO_3$ concentrations (800 mg $l^{-1}$), in media containing 2 mg $l^{-1}$ BAP. The minimum number of lateral shoots was found in MS medium containing 2400 mg $l^{-1}$ $NH_4NO_3$, without hydrolyzed casein and on 800 mg $l^{-1}$ of $NH_4NO_3$ in culture medium containing 200 mg $l^{-1}$ of hydrolyzed casein, both in the absence of BAP. So if the medium is free of hydrolyzed casein, the maximum number of lateral shoot was observed in media containing 2400 mg $l^{-1}$ $NH_4NO_3$, and by increasing hydrolyzed casein concentration (200 mg $l^{-1}$), maximum number of lateral shoot were observed in media containing 800 mg $l^{-1}$ $NH_4NO_3$. Therefore, hydrolyzed casein concentration was critical in shoot production (Figure 1). Dimassi (2010) reported that inclusion in the substrate of 20 mg $l^{-1}$ $NH_4NO_3$ resulted in the greatest number of shoots per explant in olives. Villamor (2010) reported that the results indicated that nitrogen in the form of $KNO_3$ significantly improved proliferation rate of ginger *in vitro*.

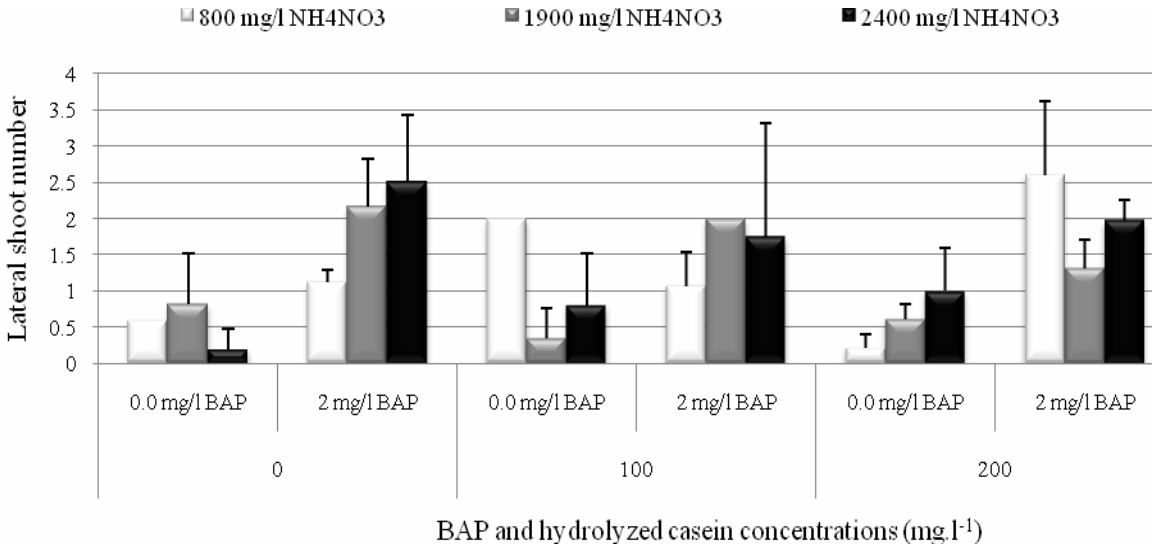


**Figure 1.** Mean number of lateral shoots in different concentrations of BAP, hydrolyzed casein and $NH_4NO_3$

Main shoots length was ranged between 4-13 cm. Main shoot length was not influenced by different concentrations of hydrolyzed casein but was significantly affected by different concentrations of $NH_4NO_3$, BAP, $NH_4NO_3$ × BAP and hydrolyzed casein × $NH_4NO_3$ × BAP interactions ($p<0.05$). Among all concentrations and combinations, 800 mg $l^{-1}$ of $NH_4NO_3$, in media without hydrolyzed casein and BAP were found to be the most effective concentration for improving main shoot length and other treatments have not significantly promoted main shoots length (Fig. 2). Dimassi (2010) reported that inclusion in the substrate of 20 mg $l^{-1}$ $NH_4NO_3$ resulted in the greatest length of shoots per explant in olives. Hydrolyzed casein produced an improvement in the growth of *Cardamine pratensis* suspensions, only if the medium was deficient in phosphorus (George *et al*., 2008).

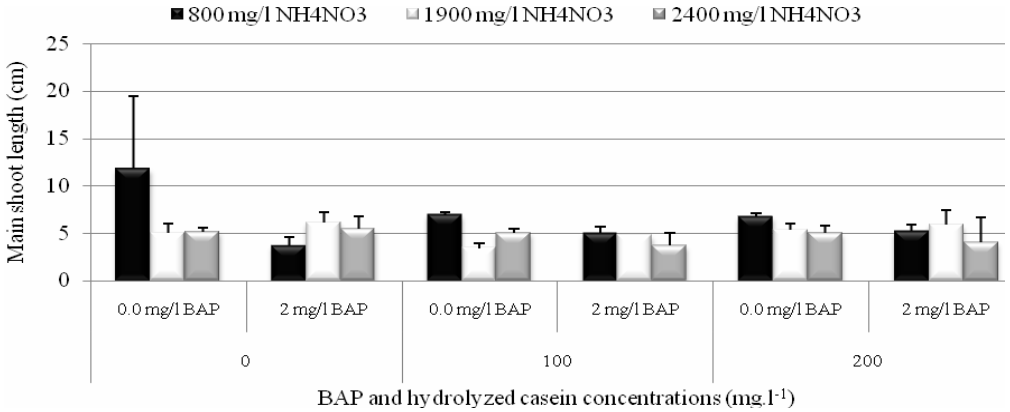


**Figure 2.** Mean main shoot length in different concentrations of BAP, hydrolyzed casein and $NH_4NO_3$

Lateral shoot length in different concentrations of hydrolyzed casein, $NH_4NO_3$ and BAP was ranged between 1-8 cm, but it was not influenced by different concentrations of hydrolyzed casein, $NH_4NO_3$, BAP and their interactions ($p< 0.05$).

Number of main shoot nodes was ranged between 4-12 in different concentrations of hydrolyzed casein, $NH_4NO_3$ and BAP but it was not affected by treatments. However, number of lateral shoot nodes was significantly affected by different concentrations of BAP, hydrolyzed casein × BAP and hydrolyzed casein × $NH_4NO_3$ × BAP interactions ($p<0.05$). Maximum node number was observed in media without hydrolyzed casein and containing 2 mg $l^{-1}$BAP, 800 mg $l^{-1}$ $NH_4NO_3$ and minimum node number was observed in media with 800 mg $l^{-1}$ $NH_4NO_3$, without BAP and hydrolyzed casein. However, non significant difference was observed with using other treatments. So we can conclude that BAP concentrations had a main role for promoting the number of lateral shoot nodes (Fig. 3). Villamor (2010) reported that his results indicated that in *Zingiber officinale* leaf growth was better in media devoid of $NH_4NO_3$.

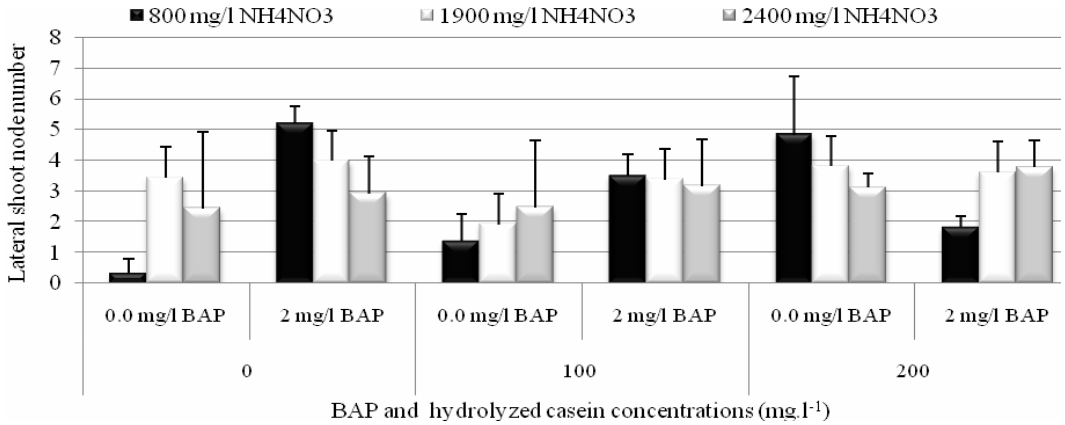


**Figure 3.** Mean lateral shoot node number in different concentrations of BAP, hydrolyzed casein and $NH_4NO_3$

The roots developed on plantlets had approximately 8-28 cm in length. Root number was not influenced by different concentrations of $NH_4NO_3$, hydrolyzed casein and their interactions. Root number was significantly influenced by concentrations of BAP and by $NH_4NO_3$× BAP interactions ($p<0.05$). Addition of BAP to all media decreased the root number, compared to the media without BAP. Maximum number of roots was found on 800 mg $l^{-1}$ and on 1900 mg $l^{-1}$ of $NH_4NO_3$, in media without BAP. Minimum number of roots was observed in medium containing 2 mg $l^{-1}$BAP and 800 mg $l^{-1}$ $NH_4NO_3$. Non significant

difference was observed with using other treatments (Fig. 4). Thus BAP had a negative effect on the root number, since BAP is a shoot induction hormone. That is probably due to nitrogen application in medium. Villamor (2010) reported that the results indicated that in *Zingiber officinale* root formation was significantly better in media without $NH_4NO_3$.

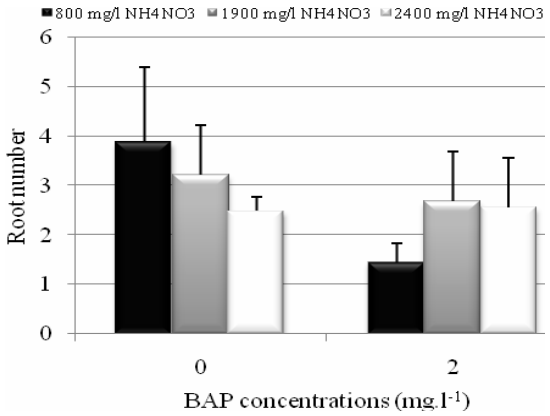


**Figure 4.** Mean number of roots in different concentrations of BAP and $NH_4NO_3$

Calli produced on the base of shoots were soft, dark green to brownish green color and had ranged between 3-8 mm in diameter. Percentage of callus formation was significantly affected by $NH_4NO_3$, BAP and $NH_4NO_3$× BAP interactions ($p<0.05$). Callus formation in the proliferation stage is an undesirable characteristic, because the callus and the shoot cells compete in nutrient absorption and supply. Addition of BAP to all culture media increased the percentage of callus formation, compared to the culture media without BAP. Razdan and Mattoo (2004) reported that usually both an auxin and a cytokinin are used for callus development, but however, cytokinin is occasionally omitted, depending on the purpose for which the callus development is necessary. Callus production was not observed at 800 mg $l^{-1}$ of $NH_4NO_3$ in the culture medium without BAP. Minimum percentage of calli occurred in media containing 1900 mg $l^{-1}$ and 2400 mg $l^{-1}$ $NH_4NO_3$ and without BAP. Maximum percentage of callus formation was observed on culture media supplemented by 2 mg $l^{-1}$ of BAP, regardless of the $NH_4NO_3$ concentrations (Fig. 5). No similar results were reported in earlier experiments, because despite of the fact that Agria is an important cultivar all over the world, this is the first *in vitro* work with cv. Agria.

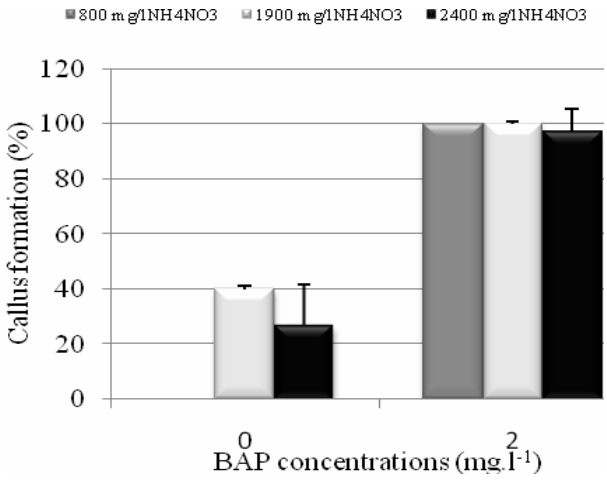


**Figure 5.** Mean callus formation percentage in different concentrations of BAP and $NH_4NO_3$

## Conclusion

Shoot proliferation in potato is influenced by many factors including carbohydrate supply, nitrogen status, carbon to nitrogen ratio, day length, temperature, genotype, amino acids content and endogenous and exogenous balance of growth regulators. Among these, $NH_4NO_3$ and growth regulators have been suggested to play a prominent role. In our experiments, the combination of hydrolyzed casein and of $NH_4NO_3$ proved to have a more positive influence on shoot proliferation, compared to the using alone of hydrolyzed casein and of $NH_4NO_3$. Our results showed that exogenous supply of BAP increased shoot production. The present study showed that high hydrolyzed casein and $NH_4NO_3$ concentrations also increased shoot diameter and leaf area. Maximum percentage of root formation and minimum percentage of callus formation was observed in media without BAP. Maximum number of roots per shoot was found at 800 mg $l^{-1}$ and at 1900 mg $l^{-1}$ $NH_4NO_3$ , in media without BAP. Minimum callus production percentage was observed in medium containing 1900 mg $l^{-1}$ or 2400 mg $l^{-1}$ of $NH_4NO_3$ , in the absence of BAP. The results showed that hydrolyzed casein, $NH_4NO_3$ and BAP played a significant role for *in vitro* micropropagation of the potato cv. Agria, a well-known potato commercial cultivar all over the world, but to our knowledge this is the first work in this respect. So there was no scope to compare the findings of the present study with previous ones. We consider that the findings of this study will help the researchers involved in further *in vitro* experiments with potato, cv. Agria, as well as with other cultivars of this important vegetable crop.

## References

1. Y. P. S. BAJAJ. *Biotechnology in agriculture and forestry*. Vol. 3: Potato. Published by Springer- Verlag (1987).
2. K. DIMASSI. *Micropropagation studies of the cv. Kalamon olives (Olea europaea sativa l.).* International Society for Horticultural Science (2010).
3. E. F. GEORGE, M. A. HALL, G. DE KLERK. *Plant Propagation by Tissue Culture 3rd Edition* Volume 1. The Background. Published by Springer (2008).
4. J. GOPAL, J. L. MINOCHA, H. S. DHALIWAL. *Microtuberization in potato (Solanum tuberosum L.).* Plant Cell Reports 16: 794-798 (1998).
5. A. J. MULLER, R. GRAFE. *Isolation and characterization of cell lines of Nicotiana tabacum lacking nitrate reductase.* Mol. Gen. Genet. 161: 67-76 (1978).
6. M. K. Razdan, A. K. Mattoo. *Genetic Improvement of Solanaceous Crops Volume I: Potato.* Science Publishers, Inc (2004).
7. W. M. ROCA, N. O. ESPINOZA, M. R. ROCA, J. E. BRYAN. *A tissue culture method for the rapid propagation of potatoes. The International Potato Center (CIP)* (1978).
8. S. N. UDDIN. *In vitro propagation of Elite indigenous potato (Solanum tuberosum L. var Indurkani) of Bangladesh*. Journal of Plant Sciencesi 3: 212-216 (2006).
9. C. C. VILLAMOR. *Influence of media strength and sources of nitrogen on micropropagation of ginger, Zingiber officinale rosc*. E-International Scientific Research Journal 2: 2094-1749 (2010).