

Effect of Growth Regulators on *in vitro* Multiplication of Potato

ASMA RABBANI, BEENISH ASKARI, NADEEM AKHTAR ABBASI, MUSSARAT BHATTI†, AZRA QURAISHI†

Department of Horticulture, University of Arid Agriculture, Rawalpindi-Pakistan

†Agriculture Biotechnology Institute, National Agricultural Research Centre, Islamabad-Pakistan

ABSTRACT

Effects of different concentrations of GA₃ and BAP (Benzylamino purine) on *in vitro* multiplication of potato variety "Desiree" were studied. For rapid multiplication, different concentrations of GA₃ were used. Maximum shoot length i.e. 8.96 cm was obtained when 4 mg L⁻¹ GA₃ was applied. Number of nodes was not significantly affected by any of the GA₃ concentrations (1, 2, 3, 4, 5 mg L⁻¹) used in this study. Maximum number of shoots (14) was obtained when 2 mg L⁻¹ BAP was applied.

Key Words: Growth regulators; Potato

INTRODUCTION

Potato is a crop of worldwide importance. It supplies at least 12 essential vitamins, minerals, proteins, carbohydrates and iron (Gray & Hughes, 1978; Thornton & Siczka, 1980). Conventionally, potato is propagated through tubers. This propagation is characterized by low ratio of multiplication that ranges from 1:4 to 1:15. Tissue culture offers an excellent technique for rapid multiplication of potato plant (Tovar and Dodds, 1986). Objective of rapid multiplication is to obtain large number of clonal plants while multiple shoot induction leads to production of disease free mother plants and seed tubers in large number. In order to achieve rapid multiplication, addition of GA₃ to the culture media has been reported to improve growth and development of shoots (Roest & Bokelmann, 1976; Muller & Lipschutz, 1984). Ahmed *et al.* (1993) studied the nodal fragments from *in vitro* grown potato plants and reported that culture media with 4.5 mg L⁻¹ GA₃ gave better results. Likewise, BAP also improves growth of potato plants (Lam, 1975; Jacobsen, 1977; Mumtaz & Quraishi, 1989). Keeping in view the results of previous investigations, present study was aimed to obtain optimum concentration of GA₃ and BAP for *in vitro* rapid multiplication and multiple shoot induction of potato variety "Desiree", which ultimately leads to mass multiplication of healthy stock and successful *in vitro* seed tuber production.

MATERIALS AND METHODS

Experiments were carried out during 1999-2000 at Agriculture Biotechnology Institute (ABI), National Agriculture Research Center (NARC), Islamabad. Potato variety "Desiree" was used, which is a red potato seed variety and is grown on 78% of potato growing areas of Pakistan. Nodal fragments from *in vitro* raised plants were used for rapid multiplication while stem segments with 2-3 axillary buds along their length were taken for multiple

shoot induction. Culture medium used for *in vitro* rapid multiplication was Murashige and Skoog (MS) medium as recommended by Mellor and Stacesmith (1987). Two experiments were conducted one each for rapid multiplication and multiple shoot induction with the following combinations of media:

Experiment # 1 Media combinations for rapid multiplication	Experiment # 2 Media combinations for multiple shoot induction
T ₁ = MS (control)	T ₁ = MS (control)
T ₂ = MS+1 mgL ⁻¹ GA ₃	T ₂ = MS+0.5 mgL ⁻¹ BAP
T ₃ = MS+2 mgL ⁻¹ GA ₃	T ₃ = MS+1.0 mgL ⁻¹ BAP
T ₄ = MS+3 mgL ⁻¹ GA ₃	T ₄ = MS+1.5 mgL ⁻¹ BAP
T ₅ = MS+4 mgL ⁻¹ GA ₃	T ₅ = MS+2.0 mgL ⁻¹ BAP
T ₆ = MS+5 mgL ⁻¹ GA ₃	T ₆ = MS+2.5 mgL ⁻¹ BAP

The pH of media was adjusted to 5.8. For rapid multiplication agar was added to the media @ 5 mg L⁻¹, as solid medium was required while in case of multiple shoot induction liquid medium was used. Then media was autoclaved at 121°C for 25 minutes for sterilization. Cultures were inoculated in the laminar flow bench and incubated at 25±1°C under 16 hours of light. For multiple shoot induction, liquid medium was shaken on horizontal electric shaker at 60 rpm for 12 hours per day for 21 days. Data were recorded on the following parameters:

Experiment 1: Shoot length (cm) and Number of nodes: Length of the largest shoot in each test tube after eight to ten days in the growth chamber and the number of nodes of largest shoot sprouted.

Experiment 2: Number of shoots raised from each stem segment in each flask after three weeks in the growth chamber.

In both the experiments, each treatment was replicated five times. In experiment 1, there were five test tubes per treatment per replication, while in experiment 2, there were five flasks (25 mL) per treatment per replication. Treatments were laid out according to completely randomized design (CRD).

RESULTS AND DISCUSSION

Results were obtained during 8-10 days in case of rapid multiplication while in three weeks in case of multiple shoot induction.

Experiment 1:

Shoot length. Statistical analysis of data showed significant difference among treatments (Table I).

Table I. Effect of GA₃ on shoot length and number of nodes during *in vitro* rapid multiplication

Treatments	Mean Shoot Length	Mean No. of Nodes
T ₁ : Control	6.50c	5.20 NS
T ₂ : MS+1 mgL ⁻¹ GA ₃	5.54c	4.80 NS
T ₃ : MS+2 mgL ⁻¹ GA ₃	5.50c	4.80 NS
T ₄ : MS+3 mgL ⁻¹ GA ₃	7.64a	5.80 NS
T ₅ : MS+4 mgL ⁻¹ GA ₃	8.96a	6.20 NS
T ₆ : MS+5 mgL ⁻¹ GA ₃	6.68c	5.20 NS

Means followed by same letter in a column are not significantly different at P=0.05; NS = non-significant; Means are separated according to Duncan Multiple Range test.

The treatment T₅ gave maximum (8.96 cm) shoot length. T₅ was followed by T₄ which gave 7.64 cm of shoot length. Rest of the treatments i.e. T₁, T₂, T₃ and T₆ were statistically non-significant. Results of this study are in agreement with Ahmed *et al.* (1993) who obtained maximum shoot length at 4-5 mg L⁻¹ GA₃ along with MS medium. In plants, GA₃ is involved physiologically in cell elongation (Levitt, 1974). Therefore, this treatment resulted in increased shoot length.

Number of nodes. In case of number of nodes, effect of different concentrations of GA₃ along with MS medium was non-significant (Table I). The reason might be that the concentration of GA₃ used in this experiment was higher, which did not affect number of nodes; whereas, in a previous study it was observed that GA₃ at 0.01 mg L⁻¹ increased node production (Firman, 1984). Higher concentration of GA₃ along with combination of NAA (1.0 mg L⁻¹) supplemented with vitamins has also been reported to increase number of nodes (Miller *et al.*, 1985). This shows that higher concentration of GA₃ should be supplemented with other phytohormones (like BAP and NAA) and vitamins (Hassan *et al.*, 1990) to increase node production.

Experiment 2:

Number of shoots. Statistical analysis showed that T₅ was the best combination for *in vitro* multiple shoot induction (Table II) as it gave maximum number (14) of shoots. The second best combination found was T₄ followed by T₃. T₁, T₂ and T₆ showing non-significant results.

CONCLUSION

GA₃ and BAP significantly affect *in vitro* multiplication of potato. GA₃ can significantly increase the shoot length during *in vitro* rapid multiplication at higher concentrations. BAP can bring significant improvement in *in vitro* multiple shoot induction by increasing the number of shoots when used at moderate concentrations.

Table II. Effect of BAP on *in vitro* multiple shoot induction

Treatments	No. of Shoots
T ₁ : Control	9.8c
T ₂ : MS+1 mgL ⁻¹ GA ₃	10.4c
T ₃ : MS+2 mgL ⁻¹ GA ₃	11.1bc
T ₄ : MS+3 mgL ⁻¹ GA ₃	12.0b
T ₅ : MS+4 mgL ⁻¹ GA ₃	13.2a
T ₆ : MS+5 mgL ⁻¹ GA ₃	10.3c

Means followed by same letter in a column are not significantly different at P=0.05; Means were separated according to Duncan Multiple Range test.

REFERENCES

- Ahmed, S., M. Bhatti, Hidayatullah and A. Qurashi, 1993. An improved method for *in vitro* multiplication of potato. *Advances in Plant Tissue Culture, Pakistan*, 61-5.
- Firman, D.M., 1984. Gibberellic acid as a media additive for *in vitro* propagation of potato. *J. Agric. Sci.*, 103: 703-4.
- Gray, D. and J.C. Hughes, 1978. Tuber Quality. *In: The Potato Crop*, P.M.Harris, p. 511. Halsted press, New York.
- Hassan, S., M.J. Turangzai and I. Khan, 1990. Production of virus free seed potato through tissue culture techniques. *Sarhad J. Agric.*, 6: 365-9.
- Jacobsen, E., 1977. Doubling dihaploid potato clones via leaf tissue culture. *Z. Pflanzenzucht*, 80: 80-2.
- Lam, S.L., 1975. Shoot formation from potato tuber discs *in vitro*. *American Pot. J.*, 54: 465-7.
- Levitt, J., 1974. *Introduction to Plant Physiology*. pp. 318. The C.V. Mosby Company, Saint Louis.
- Mellor, F.C. and K. Stacesmith, 1987. Virus free potatoes through meristem culture. *In: Biotechnology in Agriculture and Forestry*, Baijg., Y.P.S. (ed.), pp. 230-41.
- Miller, P.R., L. Amirourche, T. Stuchbury and S. Matthews, 1985. The use of plant growth regulators in micropropagation of slow growing potato cultivars. *Potato Res.*, 28: 479-86.
- Muller, S.A. and L. Lipschutz, 1984. Potato. *In: Handbook of Plant Cell Culture (Vol.3)*, P.V. Ammirato, D.A. Evans, W.R. Sharp and Y. Yamada (eds.), pp. 295. Collier Mcmillan Publishers, London.
- Mumtaz, N. and A. Quraishi, 1989. *In vitro* performance of selected potato cultivars. *Sarhad J. Agric.*, 5: 363-7.
- Roest, S and G.S. Bokelmann, 1976. Vegetative propagation of *Solanum tuberosum* L. *in vitro*. *Potato Res.*, 19: 173-8.
- Thornton, R.E. and J.B. Sieczka, 1980. Commercial Potato Production in North America. *American Pot. J.*, 57: 534-6.
- Tovar, P. and J.H. Dodds, 1986. *Tissue Culture Propagation of Potato*. CIP slide Training series 1-5 Int. Potato center, Dept. of training and communications, P. O. Box. 5659, Lima, Peru.

(Received 06 November 2000; Accepted 20 March 2001)