

## GROWTH OF POTATO AXILLARY BUD CULTURES *IN VITRO*

Muhammad Akbar Anjum, Muhammad Amjad<sup>1</sup> & Trevor A. Villiers<sup>1</sup>

University College of Agriculture. B/Z. University, Multan

<sup>1</sup>Horticulture Department, University of Agriculture, Faisalabad

<sup>1</sup>Biological Sciences Department. University of Salford, U.K.

*in vitro* shoot cultures of two commercial cultivars of *Solanum tuberosum*, Desiree and Maris Piper, and of two wild species *S. conunersonii* and *S. acaule*, were established. Growth of axillary buds was assessed on two different media. Espinoza medium proved better for the growth of axillary buds and their subsequent development into plantlets. Among the potato types, the growth from the axillary buds of *S. conunersonii* was the most vigorous followed by the *S. tuberosum* cv. Desiree while the growth from *S. acaule* axillary buds was poor.

**Key words:** axillary bud culture, growth *in vitro*, potato

**Abbreviations:** BAP = 6-Benzylaminopurine; NAA = g-Naphthaleneacetic acid; GA<sub>3</sub> = Gibberellic acid

### INTRODUCTION

Micropropagation is very important for highly heterozygous species such as potato for producing uniform plants. Potatoes can be micropropagated rapidly on a large scale by meristem and shoot-tip culture (Roca *et al.*, 1978; Goodwin *et al.*, 1980), proliferation by axillary shoots developed from *in vitro*-cultured axillary buds (Hussey and Stacey, 1981; Espinoza *et al.*, 1986; AJ-Wareh *et al.*, 1989) and production of adventitious shoots directly on explants or indirectly via a callus phase (Roest and Bokelmann, 1976; Lam, 1977; AJ-Wareh *et al.*, 1989).

Dodds (1985) described two standard methods of potato micropropagation by single node cuttings and shaken shoot cultures. When nodal sections were inoculated onto agar solidified MS culture medium (Murashige and Skoog, 1962) supplemented with GA<sub>3</sub>, Ca-pantothenic acid and sucrose, the number of nodes increased 6- to 7-fold within 3 to 4 weeks. When nodal sections were cultured on a liquid MS medium supplemented with BAP, NAA, GA<sub>3</sub>, Ca-pantothenic acid and sucrose and gently agitated, the number of nodes increased 15- to 20-fold after 2 to 3 weeks (Espinoza *et al.*, 1986). Besides the effect of medium constituents on growth of axillary buds, it also has marked effects on the number of protoplasts recovered and their subsequent planting efficiencies when using the shoot material for protoplast isolation (Shepard and Touen, 1977; Foulger and Jones, 1986). Therefore, in the present work, axillary buds were cultured either on the medium developed by Shahin (1984) or on that developed by Espinoza *et al.* (1986) and their growth was observed.

### MATERIALS AND METHODS

*In Firm* shoot cultures of two commercial cultivars of *Solanum tuberosum*, Desiree and Maris Piper and of two wild species *S. conunersonii* and *S. acaule*, were established from single nodal explants and seedling tissues respectively as described earlier (Anjum, 1994). These cultures were multiplied by subdividing

and subculturing every 4 to 6 weeks. The media used for *in vitro* shoot cultures were as follows:

1. Medium PM of Shahin (1984): MS mineral salts + Nitsch and Nitsch vitamins + 30g l<sup>-1</sup> sucrose + 7g l<sup>-1</sup> agar (pH 5.8).
2. Medium of Espinoza *et al.* (1986): MS mineral salts + MS vitamins + 30g l<sup>-1</sup> sucrose + 8g l<sup>-1</sup> agar + 2 mg l<sup>-1</sup> Ca-pantothenic acid + 0.25 mg l<sup>-1</sup> GA<sub>3</sub> (pH 5.8).

To assess the growth of potato types on different culture media, single nodal explants, each containing an axillary bud and about 5 mm of stem, were prepared from the middle portions of 5 weeks old shoots and transferred either onto the medium of Shahin (1984) or that of Espinoza *et al.* (1986). Only one explant was cultured per culture jar on the surface of solidified medium. The cultures were incubated at 25 ± 1°C in 16 hr photoperiod with a light intensity of approximately 1000 lux and were evaluated over a period of 8 weeks for a) stem height, b) number of nodes, c) number of roots and d) fresh weight of culture. In case of stem height, the height of the dominant shoot was recorded.

### RESULTS

Axillary buds started to grow after 3 to 5 days of culture on nutrient medium in *S. conunersonii* and 5 to 7 days in Desiree, Maris Piper and *S. acaule*. In most cases root development from the nodes was also observed after 5 to 6 days. In two to three weeks time, roots were well branched and often extended several times around the base of the culture jar. The roots also developed at nodes well above the medium surface and this was observed frequently in *S. conunersonii*.

The medium of Espinoza *et al.* (1986) proved better for the growth of axillary buds and their subsequent development into

Table I. Growth of potato plantlets after 8 weeks of culturing axillary buds on different media

Potato type	Medium*	Shoot height (cm)	No. of nodes	No. of roots	Fresh weight (mg)
Desiree	A	09.80±1.19**	16.50±2.29	4.50±0.50	197.0±37.3
	B	10.15±2.18	18.75 ±2.68	6.25±0.83	322.5±98.3
Maris Piper	A	6.15±1.16	10.25 ± 1.78	3.25±0.83	88.0±08.60
	B	8.45±0.97	11.75 ± 1.48	6.00±0.71	123.5±16.4
S. acaule	A	5.85±0.71	12.75±0.83	4.75±0.83	86.5±33.5
	B	8.38±1.15	17.00±2.55	5.00±0.71	91.5±21.9
S. conunersonii	A	12.50± 1.76	18.50± 1.12	6.50 ±0.50	337.5 ±9 .1.6
	B	13.88±1.33	17.50±1.12	6.25±0.83	357.2±46.1

\*Medium; A = Shahin (1984); B = Espinoza *et al.* (1986) \*\*Data represent means ± SO of four replications.

plantlets than the medium of Shahin (1984). Among the types of potato used, growth from the axillary buds of *S. commersonii* was the most vigorous followed by the *S. tuberosum* cultivars, while the growth from *S. acaule* axillary buds was poor. Of the two *S. tuberosum* cultivars, the growth from Desiree axillary buds was better than from those of Maris Piper (Table I). There were some exceptions where the growth of the dominant shoot became limited by the height of the culture jar and this appeared to break apical dominance, possibly due, at least in part, to the shading effect of the opaque jar lids.

The axillary buds developed into complete plantlets within 3 to 4 weeks of culture. Depending on the number of nodes per shoot, 5 to 10 plantlets could be regenerated from a single shoot. Cultures were multiplied by subculture of axillary buds at 4 to 6 week intervals depending upon the rates of growth. During shoot multiplication, if culture jars were tightly closed, shoot morphology became stoloniferous-like, with small leaves, or shoots with numerous partially developed leaves and with adventitious roots. In some cultures of cv. Desiree and Maris Piper, 1 to 2 microtubers were also produced after 8 to 10 weeks of culture.

## DISCUSSION

During vegetative growth, the potato produces a succession of nodes each with a leaf and its associated axillary bud. If a nodal cutting, including an axillary bud and about 5 mm of stem, is grown on a synthetic medium, the axillary bud will develop into a complete plantlet. This technique of single node culture is mainly used for rapid *in vitro* shoot multiplication. Nodal cuttings can be obtained from *in vitro*-derived shoots, greenhouse grown plants or tuber sprouts. The culture medium could be either growth regulator free (Hussey and Stacey, 1981; Tavazza and Ancora, 1986) or containing GA, (Roca *et al.*, 1978; Espinoza *et al.*, 1986). In the present work, axillary buds were cultured on two different media, namely that

developed by Shahin (1984) which does not contain any growth regulator, or the one developed by Espinoza *et al.* (1986), containing 0.25 mg l<sup>-1</sup> GA<sub>3</sub>. Growth was more rapid on the medium of Espinoza *et al.* (1986) compared with the medium of Shahin (1984), probably due to the presence of GA<sub>3</sub> in the former medium which stimulates cell division and/or cell enlargement.

The potato types differed for all the growth parameters measured (Table I). These differences were probably due to specific nutritional and hormonal requirements in the medium. Variability in the growth of axillary buds has already been reported among potato genotypes (Al-Wareh *et al.*, 1989; Caligari and Powell, 1989; Colernan *et al.*, 1990).

Lack of ventilation during shoot growth in closed vessels can lead to dramatic modifications of gaseous composition (Coumac *et al.*, 1991). Volatile products such as ethylene can accumulate and inhibit growth. If the culture jars were tightly closed, shoot morphology became altered and growth was also reduced which is most likely to be the result of ethylene accumulation (Hussey and Stacey, 1981; Wang and Hu, 1985). The effect of ethylene has implications for the choice of culture vessel used both for experimental studies, and for commercial micropropagation systems. Therefore, culture vessels should be used with covers which allow the maximum gaseous exchange, at the same time preserving aseptic conditions.

## REFERENCES

- Al-Wareh, H., N.L., Trolinder and J.R. Goodin. 1989. Callus initiation, shoot regeneration and micropropagation of three potato cultivars. Hort. Sci. 24: 680-682.
- Anjum, M.A. 1994. Tissue culture and frost tolerance studies in *Solanum*. Ph.D. Thesis, University of Salford, Salford (UK).
- Caligari, P.D.S. and W. Powell. 1989. Variability in response of potato cultivars to micropropagation. 1. *In vitro* performance. Ann. App. Biol. 115: 115-121.

## Potato axillary bud cultures

- Colernn. M., R. Waugh and W. Powell. 1990. Genetic analysis of *in vitro* cell and tissue culture response in potato. *PI. Cell. Tissue and Organ Cult.* 23: 181-186.
- Cournac, L., B. Dimon, P. Carrier, A. Lohou and P. Chagvardieff. 1991. Growth and photosynthetic characteristics of *Solanum tuberosum* plantlets cultivated *in vitro* in different conditions of aeration, sucrose supply and CO<sub>2</sub> enrichment. *PI. Physiol.* 97: 112-117.
- Dodds, J.H. 1985. Tissue culture propagation of potatoes: Advantages and disadvantages. In *Innovative Methods for Propagating Potatoes*, pp. 295-303. Report of the 28th Planning Conference. December 10-14, 1984, International Potato Centre, Lima, Peru.
- Espinoza, N., R. Estrada, P. Tovar, J. Bryan and J.H. Dodds. 1986. Tissue culture micropropagation, conservation and export of potato germplasm. *Specialized Technology Document I*, pp. 1-20. International Potato Centre, Lima, Peru.
- Foulger, D. and M.G.K. Jones. 1986. Improved efficiency of genotype dependent regeneration from protoplasts of important potato cultivars. *PI. Cell Rep.* 5: 72-76.
- Goodwin, P.B., Y.C. Kimm and T. Adisarwanto. 1980. Propagation of potato by shoot-tip culture. I. Shoot multiplication. *Potato Res.* 23: 9-18.
- Hussey, G. and N.J. Sraacey. 1981. *In vitro* propagation of potato (*Solanum tuberosum* L.). *Ann. Bot.* 48: 787-796.
- Lam, S.E. 1977. Plantlet formation from potato tuber discs *in vitro*. *Amer. Potato J.* 54: 465-468.
- Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant.* 15: 473-479.
- Roca, W.M., N.O. Espinoza, M.R. Roca and J.E. Bryan. 1978. A tissue culture method for rapid propagation of potatoes. *Amer. Potato J.* 55: 249-267.
- Roest, S. and G.S. Bokelmann. 1976. Vegetative propagation of *Solanum tuberosum* L. *in vitro*. *Potato Res.* 19: 173-178.
- Shahin, E.A. 1984. Isolation, culture and regeneration of potato leaf protoplasts from plants preconditioned *in vitro*. In *Cell Culture and Somatic Cell Genetics of Plants* (Ed. I.K. Vasil), pp. 381-390. Academic Press, Inc., Orlando.
- Shepard, J.F. and R.E. Totten. 1977. Mesophyll cell protoplasts of potato. *PI. Physiol.* 60: 313-316.
- Tavazza, R. and G. Ancora. 1986. Plant regeneration from mesophyll protoplasts in commercial potato cultivars (Primura, Kennebec, Spunta, Desiree). *PI. Cell Rep.* 5: 243-246.
- Wang, P.J. and C.Y. Hu. 1985. Potato tissue culture and its applications in agriculture. In *Potato Physiology* (Ed. P.H. Li), pp. 503-577. Academic Press, Inc., London.