BIOLOGIA PLANTARUM 48 (1): 7-11, 2004

# Culture tube closure-type affects potato plantlets growth and chlorophyll contents

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## Abstract

The effect of different hermetic and non-hermetic closure-types (aluminum foil, cotton bung, cotton plug, polypropylene cap and *Steristopper*) on potato (*Solanum tuberosum* L.) plantlets growth and chlorophyll contents was studied in three genotypes belonging to different maturity groups. Plantlets grown in culture tubes closed with aluminum foils and polypropylene caps had higher fresh mass and shoot length, but lower chlorophyll contents, higher senescence index and various morphological abnormalities. Non-hermetic closures like cotton plugs and *Steristoppers* were found optimum for plant growth without any morphological abnormalities. Besides, these plantlets exhibited low senescence index and had higher chlorophyll contents that favour acclimation to *ex vitro* conditions.

*Additional key words*: gas exchange, micropropagation, *Solanum tuberosum*, tissue culture, vessel closure.

## Introduction

In plant tissue culture, vessel closure types have been found to affect plantlets growth and development (Lentini *et al*. 1988, Genoud-Gourichon *et al*. 1993). Culture tubes or vessels tightly sealed with closures have higher relative humidity (Bottcher *et al*. 1988) and water retention strength in semisolid culture medium (Debergh *et al*. 1981). Besides, hermetic closures inhibit the gaseous exchange (McClelland and Smith 1990, Zobayed *et al*. 2001) thereby altering the concentrations of ethylene and carbon dioxide in culture tubes/vessels, which results in abnormalities in plantlets morphology (Lentini *et al*. 1988, Perl *et al*. 1988, Genoud-Gourichon *et al*. 1993).

*In vitro* cultures of potato (*Solanum tuberosum* L.) are especially sensitive to ethylene-induced growth abnormalities (Perl *et al*. 1988, Sarkar *et al*. 1999, Zobayed *et al*. 2001). Various types of phenotypic abnormalities, *e.g.*, flaccidity, vitrification, abnormal swelling of the stem, excessive leaf senescence, aerial rooting, overall growth reduction, *etc*., are frequently observed when potato plantlets are grown in tightly sealed culture tubes and/or vessels (Perl *et* al. 1988, Sarkar *et al*. 1999). It has been shown that the photosynthetic capacity of potato plants cultured *in vitro* are higher in culture tubes with non-sealed closures than that in sealed closures (Kubota and Kozai 1991, Genoud-Gourichon *et al*. 1993). Gas exchange in the culture tubes also affected the chlorophyll (Chl) contents (Haisel *et al.* 1999, Pospíšilová *et al.* 1992, 2000). In the present investigation, the effect of different closure types on potato micropropagation (on semisolid medium) was studied. The present study also examined the relationship between the vessel closure-types and Chl contents (Chl *a*, Chl *b* and total Chl) in different potato cultivars.

*Received* 9 September 2003, *accepted* 6 January 2004.
*Abbreviations*: AL - aluminum foil; CB - cotton bung; Chl - chlorophyll; CP - cotton plug; PC - polypropylene cap; SS - *Steristopper*.
*Acknowledgements*: We thank Dr. S. M. Paul Khurana, Director, Central Potato Research Institute for providing facilities. FA and OH also thank Food and Agricultural Organization (FAO) of the United Nations for providing them short-term training opportunities.
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## Materials and methods

Three tetraploid (2n=4*x*=48) potato (*Solanum tuberosum* L. ssp. *tuberosum*) genotypes belonging to different maturity groups, Kufri Ashoka (early maturing), Kufri Badshah (medium maturing) and Kufri Sindhuri (late maturing) were used in the present experiment. Three single node cuttings (SNCs) dissected from disease-free stock cultures of these genotypes were cultured per tube (25 × 150 mm) containing 13 $cm^3$ semisolid (6.0 g $dm^{-3}$ agar) Murashige and Skoog (1962; MS) medium supplemented with 30 g $dm^{-3}$ saccharose and growth additives (for details see Sarkar *et al*. 1997). Five different types of culture tube closures, *viz.*, aluminum foils (AL), cotton plugs (CP), cotton bungs (CB), polypropylene caps (PC) (*Himedia*™, Mumbai, India) and *Steristoppers* (SS) (*Heinz Hernez*™, Hamburg, Germany) were used to close the culture tubes. The experiment was carried out in a factorial (5 × 3) completely randomized design (CRD) with five closure-types and three genotypes over two culture periods at 14 d interval. Each treatment comprised five replicate culture tubes. The cultures tubes were incubated under a 16-h photoperiod (irradiance of 60 μmol $m^{-2}$ $s^{-1}$) at temperature of 24 ± 1 °C. Observations of growth parameters such as shoot height, number of green leaves per plant, leaf senescence index (Sarkar *et al.* 2002), fresh mass and Chl contents were recorded after 14- and 28-d incubation. Chlorophyll (Chl *a*, Chl *b* and total Chl) contents were estimated in dimethyl sulfoxide (Barnes *et al.* 1992) by *DU®-64* spectrophotometer (*Beckman Instruments*, Fullerton, USA). The data were analyzed by three-way analyses of variance (*ANOVA*) using the standard procedure (Steel and Torrie 1980), and the means were separated by Least Significant Difference (LSD) test.

## Results and discussion

After 14 d in culture, in early maturing cv. Kufri Ashoka, closure-types did not show any significant ($P \leq 0.05$) effect on shoot height, whereas in medium and late maturing cvs. Kufri Badshah and Kufri Sindhuri shoot height was significantly less when the culture tubes were closed with CB. However, closure-types did have significant ($P \leq 0.05$) effects on shoot height in all three genotypes after 28 d; in cvs. Kufri Ashoka and Kufri Sindhuri maximum shoot height was observed in culture tubes closed with PC, whereas in cv. Kufri Badshah maximum shoot height was in culture tubes closed with CB. A significant increase in shoot height over the culture period occurred in cvs. Kufri Badshah and Kufri Sindhuri when the culture tubes were closed with AL and PC, and in cv. Kufri Ashoka when closed with AL, CB and PC (Table 1).

In cvs. Kufri Badshah and Kufri Sindhuri, closure-types did not have any significant ($P \leq 0.05$) effect on number of green leaves per plant after 14 d. However, a significant increase in number of green leaves was

Table 1. Effects of different culture tube closure-types on potato plantlets growth *in vitro* over two culture periods. Senescence index = $\sqrt{(x + 0.5)} / \sqrt{(y + 0.5)}$, where x = number of senesced plus abscised leaves per plant and y = number of green leaves per plant.

| Cultivar | Closure type | Shoot height [mm] | | Number of green leaves [plant$^{-1}$] | | Senescence index | | Fresh mass [mg plant$^{-1}$] | |
|---|---|---|---|---|---|---|---|---|---|
| | | 14 d | 28 d | 14 d | 28 d | 14 d | 28 d | 14 d | 28 d |
| Kufri | CP | 65.9 | 88.4 | 7.8 | 9.6 | 0.248 | 0.236 | 88.6 | 164.4 |
| Ashoka | SS | 81.4 | 80.2 | 7.7 | 10.3 | 0.247 | 0.216 | 101.1 | 178.0 |
| | PC | 79.5 | 120.3 | 8.2 | 13.2 | 0.379 | 0.250 | 104.2 | 287.1 |
| | AL | 70.9 | 106.3 | 6.8 | 7.9 | 0.353 | 0.335 | 90.8 | 238.4 |
| | CB | 73.1 | 81.1 | 9.7 | 9.7 | 0.223 | 0.260 | 107.9 | 167.6 |
| Kufri | CP | 81.1 | 99.2 | 7.2 | 6.7 | 0.255 | 0.266 | 73.3 | 109.8 |
| Badshah | SS | 83.7 | 96.5 | 7.7 | 7.1 | 0.247 | 0.258 | 72.5 | 93.8 |
| | PC | 76.7 | 97.1 | 8.0 | 6.6 | 0.276 | 0.283 | 74.9 | 119.5 |
| | AL | 79.7 | 105.8 | 7.6 | 6.3 | 0.392 | 0.380 | 80.7 | 142.1 |
| | CB | 63.6 | 68.3 | 6.3 | 6.1 | 0.271 | 0.280 | 62.7 | 86.1 |
| Kufri | CP | 67.9 | 75.1 | 8.9 | 6.6 | 0.232 | 0.311 | 5.3 | 91.5 |
| Sindhuri | SS | 78.3 | 92.2 | 9.2 | 7.3 | 0.228 | 0.317 | 55.8 | 95.1 |
| | PC | 68.2 | 120.3 | 9.8 | 11.1 | 0.221 | 0.221 | 60.4 | 148.9 |
| | AL | 67.0 | 95.4 | 9.3 | 7.3 | 0.227 | 0.382 | 53.9 | 96.3 |
| | CB | 50.2 | 62.0 | 9.0 | 6.9 | 0.231 | 0.265 | 46.2 | 75.0 |
| $LSD_{0.05}$ | | 19.5 | | 1.7 | | 0.089 | | 35.1 | |

Table 2. Effects of different culture tube closure-types on chlorophyll (Chl) contents in potato plantlets *in vitro*.

| Cultivar | Closure type | Chl *a* [mg $g^{-1}$ (f.m.)] | | Chl *b* [mg $g^{-1}$ (f.m.)] | | Total Chl [mg $g^{-1}$ (f.m.)] | |
|---|---|---|---|---|---|---|---|
| | | 14 d | 28 d | 14 d | 28 d | 14 d | 28 d |
| Kufri | CP | 0.42 | 0.53 | 0.25 | 0.30 | 0.68 | 0.83 |
| Ashoka | SS | 0.37 | 0.66 | 0.20 | 0.37 | 0.58 | 1.02 |
| | PC | 0.25 | 0.23 | 0.15 | 0.13 | 0.41 | 0.36 |
| | AL | 0.13 | 0.13 | 0.09 | 0.08 | 0.22 | 0.22 |
| | CB | 0.31 | 0.51 | 0.19 | 0.30 | 0.51 | 0.80 |
| Kufri | CP | 0.29 | 0.37 | 0.18 | 0.23 | 0.49 | 0.60 |
| Badshah | SS | 0.28 | 0.43 | 0.18 | 0.25 | 0.48 | 0.68 |
| | PC | 0.27 | 0.38 | 0.16 | 0.22 | 0.44 | 0.60 |
| | AL | 0.20 | 0.21 | 0.12 | 0.13 | 0.33 | 0.34 |
| | CB | 0.26 | 0.33 | 0.17 | 0.19 | 0.44 | 0.53 |
| Kufri | CP | 0.38 | 0.57 | 0.24 | 0.32 | 0.64 | 0.89 |
| Sindhuri | SS | 0.44 | 0.56 | 0.26 | 0.31 | 0.72 | 0.87 |
| | PC | 0.37 | 0.54 | 0.22 | 0.30 | 0.60 | 0.85 |
| | AL | 0.27 | 0.29 | 0.17 | 0.17 | 0.45 | 0.47 |
| | CB | 0.49 | 0.66 | 0.30 | 0.37 | 0.81 | 1.03 |
| $LSD_{0.05}$ | | 0.09 | | 0.06 | | 0.14 | |

Table 3. Analyses of variance for potato plantlets growth parameters and chlorophyll (Chl) contents in closure-type experiment. ** and * - significant at $P \leq 0.01$ and $P \leq 0.05$, respectively.

| Source | d. f. | Mean squares | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Shoot height | Green leaves | Senescence index | Fresh mass | Chl *a* | Chl *b* | Total Chl |
| Culture period (C.p.) | 1 | 14520.30** | 0.04 | 0.01 | 155234.40** | 0.47** | 0.10** | 0.86** |
| Genotype (G) | 2 | 749.91* | 60.22** | 0.01 | 79705.01** | 0.31** | 0.09** | 0.76** |
| C.p. × G | 2 | 98.98 | 42.72** | 0.03** | 18637.32** | 0.01 | 0.002 | 0.01 |
| Closure-type (C.t.) | 4 | 2965.97** | 17.39** | 0.05** | 8733.63** | 0.31** | 0.10** | 0.77** |
| C.t. × C.p. | 4 | 1416.31** | 7.03** | 0.01 | 6284.80** | 0.03** | 0.01** | 0.07** |
| G × C.t. | 8 | 493.44* | 6.03** | 0.01 | 1771.69* | 0.05** | 0.01** | 0.12** |
| C.p. × G × C.t. | 8 | 235.90 | 3.83* | 0.01 | 1500.08 | 0.01** | 0.005** | 0.04** |
| Error | 120 | 243.48 | 1.74 | 0.01 | 784.02 | 0.005 | 0.002 | 0.01 |

recorded after 14 d in cv. Kufri Ashoka when grown in culture tubes closed with CB as compared to that with either AL or CP or SS. After 28 d, maximum number of green leaves developed in cvs. Kufri Ashoka (13.2) and Kufri Sindhuri (11.1) when the plants were grown in culture tubes closed with PC. However, closure-types did not show any significant ($P \leq 0.05$) effect on number of green leaves per plant in cv. Kufri Badshah. In cv. Kufri Badshah, no significant ($P \leq 0.05$) increase in number of green leaves occurred over the culture period. Number of green leaves increased over the culture period in cv. Kufri Ashoka in plants grown in culture tubes closed with CP, PC or SS. However, a decline in number of green leaves occurred in late maturing cv. Kufri Sindhuri after 28 d when the culture tubes were closed with AL, CB, CP or SS (Table 1).

Senescence index is a measure of the ratio of senesced plus abscised leaves to green leaves. In late maturing cv. Kufri Sindhuri, no significant ($P \leq 0.05$) difference in senescence index due to closure-type effect was observed after 14 d. A significant ($P \leq 0.05$) increase in senescence index was, however, observed in cv. Kufri Ashoka with AL or PC, while in cv. Kufri Badshah with AL only. After 28 d, maximum senescence index was recorded in culture tubes closed with AL in all the genotypes. Except for AL, other closure-types did not have any significant ($P \leq 0.05$) effect on senescence index. Senescence index increased significantly ($P \leq 0.05$) over the culture period in cvs. Kufri Ashoka and Kufri Sindhuri when the microplants were grown in culture tubes closed with PC and AL, respectively.

Closure-types did not have any significant ($P \leq 0.05$) effect on fresh mass in all the genotypes after 14 d. In general, except in culture tubes closed with CB, a significant ($P \leq 0.05$) increase in fresh mass occurred with all closure-types with increasing culture period. After 28 d, in cv. Kufri Ashoka, maximum fresh mass was obtained in cultures closed with PC (287.1 mg)

followed by AL (238.4 mg). In contrast, maximum microplant fresh mass in cvs. Kufri Badshah and Kufri Sindhuri was observed when the culture tubes were closed with AL and PC (Table 1).

After 14 d, in early maturing cv. Kufri Ashoka, Chl *a*, Chl *b* and total Chl contents were highest in culture tubes closed with either CP or SS, and in all genotypes were least when closed with AL (Table 2). However, after 28 d, Chl *a*, Chl *b* and total Chl contents were significantly ($P \leq 0.05$) maximum in cvs Kufri Ashoka and Kufri Badshah when the microplants were grown in culture tubes closed with SS. In cv. Kufri Sindhuri, maximum Chl (*a*, *b* and total) contents were observed in culture tubes closed with CB. However, Chl contents were invariably less in culture tubes closed with AL or PC.

The analyses of variance showed that closure-type had significant ($P \leq 0.01$) main effects on growth parameters and Chl contents (Table 3). Variation due to culture period was significant ($P \leq 0.01$) for shoot height, fresh mass and Chl contents. Two-way genotype × closure-type interactions were significant ($P \leq 0.01$ - 0.05) for all characters except for leaf senescence index. There were also strong closure-type × culture period interactions ($P \leq 0.01$ - 0.05) for shoot height, number of green leaves, fresh mass and Chl contents. This indicated that the closure-type effects on plant growth and Chl contents were not uniform over the culture period. Three-way interactions involving closure-type × genotype × culture period were significant ($P \leq 0.05$) only for number of green leaves and Chl contents, suggesting that the combined effect of closure-type and genotype was not uniform over the culture period.

The results clearly documented that closure-types with varying degrees of permeability as used in this experiment had significant effects on the growth, morphology and Chl contents of the potato plants. AL and PC, which are least permeable to gas exchange, surprisingly sustained higher shoot height and fresh mass. This is in contrast to reported observations that higher shoot height and fresh mass were obtained in non-hermetically closed cultures (Genoud-Gourichon *et al*. 1993, Lucchesini and Sodi 2000). Higher fresh mass found in sealed vessels might be due to ethylene-induced swelling of the potato shoots (Zobayed *et al*. 2001). Morphological changes, viz., small folded leaves, hooked stem apices, stoloniferous shoots, callusing in shoot bases and higher senescence index were associated with AL and PC closures. Ethylene accumulation in culture tubes due to these closure-types might be responsible for inducing these types of growth abnormalities (Hussey and Stacey 1984, Jackson *et al*. 1991). As compared to PC closures, AL closures fostered lesser number of green leaves but higher senescence index. This could be explained by the fact that AL closures would perhaps have completely inhibited gas exchange thereby increasing the ethylene concentration in the culture tube head space.

The lesser relative humidity and higher gas exchange in culture tubes closed with non-hermetic closures like CB, CP and SS would have increased the Chl contents. Smith *et al*. (1990) reported that reduced relative humidity of the culture vessel was associated with higher Chl contents in the leaves. Pospíšilová *et al*. (2000) obtained higher contents of Chl *a*, Chl *b* and high Chl *a/b* ratio in leaves of plantlets grown in Magenta boxes than those in glass vessels. Chlorophyllous plantlets have been found to have sufficient photosynthetic ability which is restricted by low concentration of carbon dioxide in the vessel during the photoperiod (Solarová 1989, Kozai 1991, Kubota and Kozai 1991, Serret *et al*. 1997). Hence, non-hermetic closure-types would result in higher content of carbon dioxide during photoperiod leading to photoautotrophy of plantlets (During and Harst 1996, Serret *et al*. 1997). Photosynthetic ability of *in vitro*-grown microplants favours further acclimation to conditions *ex vitro* (Kozai 1991, Serret *et al*. 1997). It has been reported that the use of cotton plugs reduced vitrification of shoots and increased the percentage of plantlets that successfully acclimatized compared to the use of glass caps (Lucchesini and Sodi 2000).

In conclusion, it is apparent that culture tubes closed with non-hermetic closures like CP or SS fostered optimum microplant growth without any morphological abnormalities, and induced higher chlorophyll contents. Hence CP and SS are better suited than other closure-types for efficient potato micropropagation and successful acclimation of micropropagated plants to *ex vitro* conditions.

## References

Barnes, J.D., Balaguer, L., Manrique, E., Elvira, S., Davison, A.W.: A reappraisal of the use of DMSO for the extraction and determination of chlorophylls *a*, and *b* in lichens and higher plants. - Environ. exp. Bot. **32**: 85-100, 1992.

Bottcher, I., Zoglauer, K., Goring, H.: Induction and reversion of vitrification of plants cultured *in vitro*. - Physiol. Plant. **72**: 560-564, 1988.

Debergh. P., Harbaoui, Y., Lemeu, R.R.: Mass propagation of globe artichoke (*Cynara scolymus*). Evaluation of different hypotheses to overcome vitrification with special reference to water potential. - Physiol. Plant. **53**: 181-187, 1981.

During, H, Harst, M.: Stomatal behaviour, photosynthesis and photorespiration of *in vitro*-grown grapevines: effects of light and $CO_2$. - Vitis **35**: 163-167, 1996.

Genoud-Gourichon, C., Veisseire, P., Coudret, A.: Effects of pre-treatment and cap closure on photosynthesis

potentialities of potato cultivated *in vitro*. - Photosynthetica **29**: 73-79, 1993.

Haisel, D., Pospíšilová, J., Synková, H., Čatský, J., Wilhelmová, N., Plzáková, Š.: Photosynthetic pigments and gas exchange of *in vitro* grown tobacco plants as affected by $CO_2$ supply. - Biol. Plant. **42**: 463-468, 1999.

Hussey, G., Stacey, N. J.: Factors affecting the formation of *in vitro* tubers in potato (*Solanum tuberosum* L.). - Ann. Bot. **53**: 565-578, 1984.

Jackson, M.B., Abbott, A.J., Belcher, A.R., Hall, K.C., Butler, R., Cameron, J.: Ventilation in plant tissue culture and effects of poor aeration on ethylene and carbon dioxide accumulation, oxygen depletion and explant development. - Ann. Bot. **67**: 229-237, 1991.

Kozai, T.: Photoautotrophic micropropagation. - *In Vitro* cell. dev. Biol. Plant. **27**: 47-51, 1991.

Kubota, C., Kozai, T.: Growth and net photosynthetic rate of *Solanum tuberosum in vitro* under forced and natural ventilation. - HortScience **27**: 1312-1314, 1991.

Lentini, Z., Mussell, H., Mutschler, M.A., Earle, E.D.: Ethylene generation and reversal of ethylene effects during development *in vitro* of rapid-cycling *Brassica campestris* L. - Plant Sci. **54**: 75-81, 1988.

Lucchesini, M., Sodi, A. M.: Effects of vessel permeability to gas exchanges on the *in vitro* plantlets of *Myrtus communis* L. - Agr. mediter. **130**: 78-84, 2000.

McClelland, M.T., Smith, M.A.L.: Vessel type, closure, and explant orientation influence *in vitro* performance of five woody species. - HortScience **25**: 797-800, 1990.

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

Perl, A.D., Aviv, D., Galun, E.: Ethylene and in vitro culture of potato: Suppression of ethylene generation vastly improves protoplast yield, plating efficiency and transient expression of an alien gene. - Plant Cell Rep. **7**: 403-406, 1988.

Pospíšilová, J., Haisel, D., Synková, H., Čatský, J., Wilhelmová, N., Plzáková, Š., Procházková, D., Šrámek, F.: Photosynthetic pigments and gas exchange *during ex vitro* acclimation of tobacco plants as affected by $CO_2$ supply and abscisic acid. - Plant Cell Tissue Organ Cult. **61**: 125-133, 2000.

Pospíšilová, J., Solárová, J., Čatský, J.: Photosynthetic responses to stresses during *in vitro* cultivation. - Photosynthetica **26**: 3-18, 1992.

Sarkar, D., Naik, P.S., Chandra, R.: Effect of inoculation density on potato micropropagation. - Plant Cell Tissue Organ Cult. **48**: 63-66, 1997.

Sarkar, D., Kaushik, S.K., Naik, P.S.: Minimal growth conservation of potato microplants: silver thiosulfate reduces ethylene-induced growth abnormalities during prolonged storage *in vitro*. - Plant Cell Rep. **18**: 897-903, 1999.

Sarkar, D., Sood, K.C., Chakrabarti, S.K., Naik, P.S.: Growing of potato microplants in the presence of alginate silverthiosulfate capsules reduces ethylene-induced culture abnormalities during minimal growth conservation *in vitro*. - Plant Cell Tissue Organ Cult. **68**: 79-89, 2002.

Serret, M.D., Trillas, M.I., Matas, J., Aruas, J.L.: The effect of different closure types, light, and sucrose concentrations on carbon isotope composition and growth of *Gardenia jasminoides* plantlets during micropropagation and subsequent *acclimation ex vitro*. - Plant Cell Tissue Organ. Cult. **47**: 217-230, 1997.

Smith, E.F., Roberts, A.V., Mottley, J.: The preparation *in vitro* of chrysanthemum for transplantation to soil. 3. Improved resistance to desiccation conferred by reduced humidity. - Plant Cell Tissue Organ Cult. **21**: 141-145, 1990.

Solárová, J.: Photosynthesis of plant regenerants. Diurnal variation in $CO_2$ concentration in cultivation vessels resulting from plantlets photosynthetic activity. - Photosynthetica **23**: 100-107, 1989.

Steel, R.G.D., Torrie, J.H.: Principles and Procedures of Statistics: A Biometrical Approach. - McGraw Hill, New York 1980.

Zobayed, S.M.A., Amstrong, J., Amstrong, W.: Micro-propagation of potato: Evaluation of closed, diffusive and forced ventilation on growth and tuberization. - Ann. Bot. **87**: 53-59, 2001.