PERFORMANCE OF DIFFERENT PROTOCOLS ON IN VITRO TUBERIZATION IN POTATO (Solanum tuberosum)

M. ZAKARIA¹, M. M. HOSSAIN²
M. A. KHALEQUE MIAN³ AND T. HOSSAIN⁴

Abstract

Five protocols of micro tuberization were used to induce large size microtuber in three recommended potato varieties, namely Cardinal, Diamant, and Heera under complete dark condition. Tuberization was the earliest (11.8 days) in the protocol P₂ (MS + 5 mg/l BAP + 500 mg/l CCC + 8% sucrose), which was closely followed by that in P₁ (12.7 days) (MS + 5 mg/l BAP + 50 mg/l coumarin + 8% sucrose). Maximum number of microtubers/flask (12.8) was obtained from the protocol P₁ followed by that of P₂ (11.6) that contained growth retardant; but higher average weight of microtuber was obtained in the protocols P₅ (30 days old plantlet + MS media containing 40 meq K + 10 mg/l BA + 9% sucrose), P₄ (MS + 10 mg/l BA + 8% sucrose), and P₃ (MS + 5.0 mg/l BAP + 6% sucrose) which contained BA in absence of growth retardant. The average weight of microtuber was the highest (329.0 mg) in protocol P₅, followed by that in P₄ (280.7 mg), while it was the lowest in protocol P₁. The variety Diamant produced maximum average weight of microtuber (246.3 mg), while Heera produced minimum (226.1 mg), which was statistically similar to Cardinal (228.1 mg). The highest percentage (52.2) of >300 mg size and lowest percentage (19.3) of <150 mg size microtuber was produced in P₅ protocol in the variety Diamant. On overall consideration, all the varieties performed best with the protocol P₅.

Keywords: Protocol, microtuber, potato.

Introduction

Micropropagation systems through node and shoot culture and in vitro tuberization have enabled maintenance and propagation of disease-free planting materials in the laboratory for the potato breeder and farmer. Both shoot and in vitro tuberization systems are advantageous because of maintenance of disease-free materials. Moreover, in vitro tuberization of potato in the laboratory helps produce planting materials which are convenient to handle in transport, storage, and field planting. There are several published protocols for in vitro tuberization in potato (Kim, 1982; Wang and Hu, 1982; Hussey and Stacey, 1984; Tovar et al., 1985). In general, there are two basic stages in all protocols. The first stage

¹Associate Professor, Department of Horticulture, Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Gazipur. ²Professor, Department of Horticulture, BSMRAU, Gazipur. ³Professor, Department of Genetics and Plant Breeding, BSMRAU, Gazipur. ⁴Professor, Department of Crop Botany, BSMRAU, Gazipur, Bangladesh.
ZAKARIA et al.

Aims to produce vegetative growth and the second stage to induce tuberization and allow enlargement of the tuberlets. Different authors recommended different media for vegetative multiplication and tuberization. Tovar et al. (1985) recommended MS + 0.5 mg/l BAP + 0.4 mg/l GA + 0.01 mg/l NAA + 2% sucrose for the propagation phase and MS + 5.0 mg/l BAP + 500 mg/l CCC + 8% sucrose for the tuberization phase, which was useful for over 50 different genotypes covering a wide genetic base and ploidy levels and found 10 microtubers per flask having a weight of 49.8 to 143.5 mg. Wang and Hu (1982) obtained microtubers having an average weight of 250 mg in MS + 10.0 mg/l BAP + 8.0% sucrose while Hussey and Stacey (1984) found microtubers having a weight of 60 to 120 mg by using MS + 2.0 mg/l BAP + 6.0% sucrose. Kim (1982) induced 1-2 microtubers per node in MS + 5.0 mg/l BAP + 6% sucrose. It is clear that microtuber number and average weight varied among protocols. The discrepancies between these findings are probably due to the use of different genotypes (Coleman and Coleman, 2000; Zakaria et al., 2008a) different mixtures of nutrient salts, and/or different concentrations of the growth regulators added (Zakaria et al., 2008b), together with possible variation in the size of vessel, light intensity and temperature. Therefore, the present study was undertaken to evaluate the performance of different protocols on in vitro tuberization in different potato varieties and to find out variety specific protocols.

Materials and Method

In vitro grown microplants of three recommended potato cultivars viz., Cardinal, Diamant and Heera were used in the study. The microplants were multiplied through subculture of single stem nodes at every three weeks interval in Tissue culture laboratory of Horticulture Department, Bangabandhu Sheikh Mujibur Rahman Agricultural University. The experiment was carried out during the period from July to September 2008. The propagation media used for different protocols are mentioned in the Table 1. All the propagation media were solidified with 0.8% agar. Temperature of the growth chamber was maintained at 23 ± 1°C with 16 hours photoperiod at 3000 lux intensity from fluorescent tubes. At first phase, microplants were multiplied in solid media and then these were transferred into liquid media for microtuberization. Eight stem segments (Each with 3 nodes) of 30 days old in vitro microplants of Cardinal, Diamant, and Heera were cultured in 250 ml Erlenmeyer flasks containing 40 ml microtuber induction liquid medium, which was based on MS medium supplemented as per different protocols as shown in Table 2. The cultures were incubated in the dark at 20°C in continuous dark. The experiment included five protocols and three recommended potato varieties which in combination made 15 treatment combinations. The experiment was laid out in a factorial Completely Randomized Design (CRD) with three replications. The induced microtubers were harvested aseptically after 70 days of incubation. The collected data were
Performance of different protocols was analyzed with the help of computer using MSTAT-C programme and the mean separation was done by Duncan’s New Multiple Range Test.

### Table 1. Propagation media for in vitro multiplication of plantlet in potato.

<table>
<thead>
<tr>
<th>Media code</th>
<th>Media composition</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>DOD</td>
<td>MS (Murashige and Skoog, 1962) + GA 0.4 mg/l + BAP 0.5 mg/l + NAA 0.01 mg/l + 2.5% sucrose</td>
<td>Dodds, et al., 1988</td>
</tr>
<tr>
<td>CIP</td>
<td>MS + GA 0.4 mg/l + BAP 0.5 mg/l + NAA 0.01 mg/l + 2% sucrose</td>
<td>Tovar, et al., 1985</td>
</tr>
<tr>
<td>KIM</td>
<td>MS + 0.1 mg/l GA + 0.5 mg/l Kinetin + 2% sucrose</td>
<td>Kim, 1982</td>
</tr>
<tr>
<td>SAR</td>
<td>MS + 0.1 mg/l GA + 0.01 mg/l NAA + 4 mg/l Calcium pantathionate + 3% sucrose</td>
<td>Sarkar, et al., 1997</td>
</tr>
</tbody>
</table>

### Table 2. Different tuberization protocols of potato.

<table>
<thead>
<tr>
<th>Protocol code</th>
<th>Propagation medium code</th>
<th>Tuberization media</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>DOD</td>
<td>MS + 5 mg/l BAP + 50 mg/l coumarin + 8% sucrose (Dodds et al., 1988).</td>
</tr>
<tr>
<td>P2</td>
<td>CIP</td>
<td>MS + 5 mg/l BAP + 500 mg/l CCC + 8% sucrose (Tovar et al., 1985).</td>
</tr>
<tr>
<td>P3</td>
<td>KIM</td>
<td>MS + 5.0 mg/l BAP + 6% sucrose, (Kim, 1982).</td>
</tr>
<tr>
<td>P4</td>
<td>SAR</td>
<td>MS + 10 mg/l BA + 8% sucrose (Naik et al., 1998)</td>
</tr>
<tr>
<td>P5</td>
<td>SAR</td>
<td>MS with 60 meq N &amp; 40 meq K + 10 mg/l BA + 9% sucrose (Zakaria, 2003)</td>
</tr>
</tbody>
</table>

### Results and Discussion

**Performance of protocols:** Tuberization was the earliest (11.8 days) with P2 (CIP) protocol closely followed by P1 (Table 3). Tuberization was most delayed (15.9 days) with P3 protocol. Rapid tuberization with P2 protocol might be due to the presence of optimum concentrations of BA and CCC in the medium. Microtuberization promoted in presence of CCC (Hossain and Sultana, 1998; Zakaria et al., 2008b) and BA (Islam, 1995 and Zakaria et al., 2008b). Protocol P5 showed earlier tuberization than P3 which may be due to the presence of high concentration of BA and sucrose. Such a result was also reported by Palmer and Smith (1969) and Wang and Hu (1982). The maximum number of microtubers/flask (12.8) was with protocol P1 closely followed by P2 (11.6) The minimum number of tubers/flask was with P3 (9.9) The protocols P1 and P2 showed higher number of microtubers per flask because of the presence of 50 mg/l coumarin and 500 mg/l CCC, respectively. More number of microtubers was reported with 50 mg/l coumarin (Dodds et al., 1988) and with 500 mg/l CCC.
(Hossain and Sultana, 1998). Coumarin at the rate of 50 mg/l produced more number of microtubers than 500 mg/l CCC (Dodds et al., 1988) which corroborate with the present findings. The average weight of microtuber was the highest (329.0 mg) with P₅ protocol, which was closely followed by P₄ (280.7 mg), while it was the lowest with P₁ protocol. The protocol P₅ produced the highest average weight of microtuber might be due to the presence of high concentrated BA (10 mg/l) and sucrose (9%). High concentration of BA in presence of high concentration of sucrose showed best performance in microtuberization (Teixeira and Pinto, 1991 and Naik et al., 1998). The heaviest microtuber with 10 mg/l BA (Teixeira and Pinto, 1991) and with 9% sucrose was reported by Jeoung-Lai et al. (1996). Production of lower weight of microtubers by protocols P₁ and P₂ might be due to presence of growth retardant (Dodds et al., 1988; Harvey et al., 1991 and Lian-Yong et al., 1996). The highest percentage (44.6) of large size (>300 mg) microtuber was produced with protocol P₅ followed by P₄ (29.3%), while in P₁, no large sized microtubers were produced. The maximum percentage (45.4) of <150 mg size microtubers was found with P₁. Dodds et al. (1988) found very small (<100 mg) microtubers with P₁ protocol. Tovar et al. (1985) also reported small microtubers (<150 mg) with P₂ protocol. Wang and Hu (1982) found the highest microtuber by using 10 mg/l BA in combination with 8% sucrose.

Table 3. Effect of protocols on induction and development of potato microtuber.

<table>
<thead>
<tr>
<th>Protocols</th>
<th>Days to tuber initiation</th>
<th>No. of microtubers/flask</th>
<th>Av. wt of microtuber (mg)</th>
<th>Grade of microtubers by number (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt;150 mg</td>
</tr>
<tr>
<td>P₁</td>
<td>12.7 c</td>
<td>12.8 a</td>
<td>157.4 e</td>
<td>45.4</td>
</tr>
<tr>
<td>P₂</td>
<td>11.8 c</td>
<td>11.6 b</td>
<td>177.5 d</td>
<td>44.3</td>
</tr>
<tr>
<td>P₃</td>
<td>15.9 a</td>
<td>9.9 d</td>
<td>223.9 c</td>
<td>32.4</td>
</tr>
<tr>
<td>P₄</td>
<td>14.9 ab</td>
<td>10.4 c</td>
<td>280.7 b</td>
<td>27.0</td>
</tr>
<tr>
<td>P₅</td>
<td>14.2 b</td>
<td>10.9 c</td>
<td>329.0 a</td>
<td>21.3</td>
</tr>
</tbody>
</table>

Level of significance ** ** ** NA NA NA

CV (%) 3.57 3.14 2.16 - - -

Means bearing same letter (s) in the same column do not differ significantly at 1% level of probability. NA- Not analyzed

Performance of potato variety: Microtuber initiation was most delayed (15.1 days) in the variety Diamant, while it was most rapid (12.3 days) in Heera (Table 4). This finding is consistent with the normal behaviour of potato cultivar in the field condition where tuberization occurs earlier in Heera than in Cardinal and Diamant (Rashid, 1999). Islam (1995) reported that microtuber initiation time
varied with genotype. He found earlier microtuber initiation with exotic varieties (Cardinal, Kufri Shindhuri) compared to local varieties of potato. Statistically similar numbers of microtuber per flask were observed with Cardinal and Diamant. The minimum number of microtubers was found with Heera. Varietal differences on the number of microtubers were found by Islam (1995) who reported that the local varieties of potato produced poor number of microtubers compared to exotic varieties. Hussain and Sultana (1998) also found that the variety Chamak produced very poor number of microtubers compared to Patrones. Average weight of microtuber was the highest (246.3 mg) in Diamant (Table 4). The minimum average weight of microtuber was shown by Heera, which was statistically similar to Cardinal. Genotypic variation on average weight of microtuber was observed by Islam (1995) who reported that the exotic varieties of potato produced higher average weight of microtuber than local varieties. Hussain and Sultana (1998) found that the variety Patrones produced significantly heavier microtuber compared to Lalsheel and Chamak. The highest percentage (22.0) of >300 mg size microtuber was produced by Diamant, while it was minimum in Heera (17.1%). Cardinal produced maximum percentage (35.7) of <150 mg size microtuber followed by Heera (34.2%) and Diamant (32.4%).

<table>
<thead>
<tr>
<th>Variety</th>
<th>Days to tuber initiation</th>
<th>No. of microtubers/flask</th>
<th>Av. wt of microtuber (mg)</th>
<th>Grade of microtubers by number (%)</th>
<th>&lt;150mg</th>
<th>150-300 mg</th>
<th>&gt;300mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardinal</td>
<td>14.3 b</td>
<td>11.5 a</td>
<td>228.7 b</td>
<td>35.7</td>
<td>45.2</td>
<td>19.1</td>
<td></td>
</tr>
<tr>
<td>Diamant</td>
<td>15.1 a</td>
<td>11.9 a</td>
<td>246.3 a</td>
<td>32.4</td>
<td>45.6</td>
<td>22.0</td>
<td></td>
</tr>
<tr>
<td>Heera</td>
<td>12.3 c</td>
<td>9.9 b</td>
<td>226.1 b</td>
<td>34.2</td>
<td>48.7</td>
<td>17.1</td>
<td></td>
</tr>
<tr>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>CV (%)</td>
<td>3.57</td>
<td>3.14</td>
<td>2.16</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

Means bearing same letter (s) in the same column do not differ significantly at 1% level of probability. NA- Not analyzed.

**Interaction effect of protocols and variety:** The protocol P₂ followed by P₁ enhanced tuber initiation in all the varieties (Table 5). Tuber initiation was most delayed (17.3 days) with protocol P₃ in Diamant. Hussain and Sultana (1998) found that the tuberization was earlier with 5 mg/l BA + 500 mg/l CCC irrespective of genotypes which corroborates the present findings. The highest number of microtubers (13.7) was produced with P₁ protocol in Diamant. The lowest number of microtubers per flask was produced by P₃ protocol in case of all varieties. Hussain and Sultana (1998) found maximum number of microtubers per plant with 5 mg/l BAP + 500 mg/l CCC, which was similar to the
Table 5. Interaction effect of protocols and potato varieties on induction and development of microtuber.

<table>
<thead>
<tr>
<th>Treatment combination Protocol × Variety</th>
<th>Days to tuber initiation</th>
<th>No. of microtubers/ flask</th>
<th>Average wt of microtubers (mg)</th>
<th>Grade of microtubers by number (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt;150 mg</td>
</tr>
<tr>
<td>P₁ X Cardinal</td>
<td>12.7de</td>
<td>13.0ab</td>
<td>145.1j</td>
<td>45.7</td>
</tr>
<tr>
<td>Diamant</td>
<td>13.3d</td>
<td>13.7a</td>
<td>162.1i</td>
<td>46.7</td>
</tr>
<tr>
<td>Heera</td>
<td>12.0ef</td>
<td>11.7cde</td>
<td>165.0i</td>
<td>43.8</td>
</tr>
<tr>
<td>P₂</td>
<td>11.3f</td>
<td>12.0cd</td>
<td>160.3i</td>
<td>51.6</td>
</tr>
<tr>
<td>Cardinal</td>
<td>12.7de</td>
<td>12.3bc</td>
<td>171.3i</td>
<td>42.1</td>
</tr>
<tr>
<td>Diamant</td>
<td>11.3f</td>
<td>10.3gh</td>
<td>201.4h</td>
<td>39.2</td>
</tr>
<tr>
<td>Heera</td>
<td>16.7ab</td>
<td>10.3gh</td>
<td>216.2g</td>
<td>32.3</td>
</tr>
<tr>
<td>P₃</td>
<td>17.3a</td>
<td>10.7fg</td>
<td>235.3f</td>
<td>29.4</td>
</tr>
<tr>
<td>Cardinal</td>
<td>13.3d</td>
<td>8.7j</td>
<td>220.2g</td>
<td>35.4</td>
</tr>
<tr>
<td>Diamant</td>
<td>15.7bc</td>
<td>11.0efg</td>
<td>285.4d</td>
<td>28.1</td>
</tr>
<tr>
<td>Heera</td>
<td>16.6ab</td>
<td>11.3def</td>
<td>297.4e</td>
<td>24.2</td>
</tr>
<tr>
<td>P₄</td>
<td>15.0c</td>
<td>11.3def</td>
<td>336.5b</td>
<td>20.6</td>
</tr>
<tr>
<td>Cardinal</td>
<td>15.7bc</td>
<td>11.7cde</td>
<td>365.5a</td>
<td>19.3</td>
</tr>
<tr>
<td>Diamant</td>
<td>15.3d</td>
<td>9.6i</td>
<td>285.1d</td>
<td>24.1</td>
</tr>
<tr>
<td>Heera</td>
<td>12.0ef</td>
<td>9.6i</td>
<td>285.1d</td>
<td>24.1</td>
</tr>
</tbody>
</table>

Level of significance ** ** ** NA NA NA
CV (%) 3.57 3.14 2.16 - - -

Means bearing same letter (s) in the same column do not differ significantly at 1% level of probability. NA- Not analyzed.

performance of P₂ protocol. Dodds et al. (1988) found more number of microtubers by using 50 mg/l coumarin (P₁) instead of 500 mg/l CCC (P₂). The protocol P₃ produced the highest (365.5 mg) average weight of microtuber in the variety Diamant (Table 5). Lower average weight of microtuber was with P₁ and P₂ protocol in all varieties. Lower microtuber weight might be due to the presence of growth retardant which reduced microtuber weight (Harvey et al., 1991; Leclerc et al., 1994 and Lian-Yong et al., 1996). Higher microtuber weight with P₃ and P₄ was obviously due to the presence of high concentration of BA and sucrose (Koda and Okazawa, 1983; Wang and Hu, 1982, and Palmar and Smith, 1969). The highest percentage (52.2) of >300 mg size and lowest percentage (19.3) of <150 mg size microtuber was produced with P₅ protocol in Diamant variety of potato (Table 5). In case of all varieties, the protocol P₁ did not produce any microtuber of size >300 mg, while P₂ produced the highest percentage (51.6) of <150 mg size microtuber in Cardinal.
References


