CALLUS INDUCTION, PLANT REGENERATION, 
AND GROWTH ON BARLEY (Hordeum vulgare L.)

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Abstract. Six barley (Hordeum vulgare L.) genotypes: 'Mari', 'Acsad 176', 'Acsad 1164', 'Harmal', 'Acsad 1176' and 'Rum' were used to study callus induction, plant regeneration and growth. Calli were induced on Murashige & Skoog (MS) media containing different concentration (1.0, 2.0, 3.0, 4.0 or 5.0 mg/l) of 2,4-dichlorophenoxy acetic acid, (2,4-D) under dark conditions. After 40 d culture, 'Rum' genotype produced higher callus fresh and dry weight (286.3 and 21.7 mg, respectively) than other genotypes. 'Acsad 176' genotype produced the lowest callus fresh and dry weight (112.1 and 7.8 mg, respectively). Significant variations were observed among genotypes. No callus was induced on 2,4-D-free MS medium. After 30 d of culture, the highest callus fresh weight was obtained when 'Acsad 1164' was transfereed on a solid media containing 2.0 mg/l 2,4-D. Whereas other genotypes achieved the highest fresh weight on hormone free medium. Only 'Mari' was able to produce shoots on media lacking 2,4-D. 'Harmal' produced the highest shoot number (8.2 plantlet) at 3.0 mg/L TDZ. Plant regeneration in 'Acsad 176' were highly (4.2 plantlet) promoted by the addition of 3.0 mg/L BA. 'Harmal' produced 13.4 plantlets on medium supplemented with 2.0 mg/L 2iP.

Key words: Barley, Callus induction, Genotype, Hordeum vulgare, 
Plant regeneration.

INTRODUCTION

Plant tissue culture has been applied for the production of virus free plants 
and clonal propagation, it provides a source of standardized plant material
for the analysis of plant metabolism and other cellular processes and responses (Taji et al. 1992). *In vitro* cultures offer greater control and precise measurement for growth and development of plant tissues (Biagioli et al. 2006, Shatnawi et al. 2011). The use of tissue culture can help to focus on the physiological and biochemical mechanisms. Plant production via tissue culture is advantageous over traditional propagation methods because it leads to the production of disease and virus–free plants. Also, it allows the production of a high number of plants, in a short period of time and in a very limited propagation space. In addition, rapid multiplication rate of plants that are difficult to propagate conventionally can be easily achieved via *in vitro* culture (Taji et al. 1992, Shatnawi 2006).


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1985, Ward & Jordan 2001, Chauhan & Kothari 2004). For successful application of the tissue culture techniques in crop breeding, the callus growth and plant regeneration potential of each crop must be determined (Abe & Futsuhara 1986, Jha et al. 2007). Therefore, this study was conducted to establish callus induction and growth systems in six Jordanian barley (Hordeum vulgare L.) genotypes.

MATERIALS AND METHODS

Plant material

Six barley (Hordeum vulgare L.) genotypes, 'Acsad 1176', 'Acsad 1164', 'Mari', 'Harmal', 'Acsad 176' and 'Rum' were obtained from the Center for Agricultural Research and Extension (NCARE) Baqa, Jordan and were used in this study. Mature seeds of each genotype were washed thoroughly under a running tap water for 30 minutes along with few drops of mild detergent. Seeds were then rinsed with sterile distilled water for three times (10 min. each time). Seeds were then transferred into 70 % ethanol for one min under the laminar air-flow cabinet. After that, seeds were disinfested with 5% sodium hypochlorite plus 0.1 % Tween-20 (surfactant) for 30 minutes and then rinsed with sterile distilled water three times (10 min. each time).

Callus induction

Seeds were placed into 15 x 150 mm test tube (one seed in each test tube) containing 10 ml of MS (Murashige & Skoog 1962), incubated in dark in a growth room at 22 ± 2 °C, which was supplemented with different concentrations of 2,4-D (2,4-dichlorophenoxy acetic acid) (0.0, 1.0, 2.0, 3.0, 4.0 or 5.0 mg/l). Seeds of each genotype were placed on the surface of the sterile solid media. After callus formation, calli were separated from roots of germinated seeds. Each treatment contained ten completely randomized replicates. Data were recorded after forty days of incubation for callus induction percentage, texture, color and germination percentage. Callus fresh weight were recorded for 3 randomly selected replicates. Callus samples were dried to a constant weight at 70 °C and dry weights were recorded.

Callus growth

Initiated calli were separated from roots and were subcultured on 20 ml solid MS containing 3.0 mg/l 2,4-D and 30 g/l sucrose. The calli (250 mg approximately) were transferred to fresh MS media supplemented with different concentrations (0.0, 1.0, 2.0, 3.0, 4.0, or 5.0 mg/l) of 2,4-D. Each treatment consisted of five completely randomized replicates and each replicate contained four samples. Data on callus fresh weight, texture and color was recorded after thirty days of incubation period. Callus dry weight was recorded for 20 randomly selected replicates and
each replicate consisted of four samples.

**Effect of time**

After selection growth regulator which gave the highest callus growth rate of each genotype, callus growth over time was studied. Calli (approx. 55 mg) were transferred to fresh media containing 2.0 mg/l 2,4-D for 'Acsad 1164' and hormone free media for the other genotypes. This is because 2,4-D at 2.0 mg/l produced the highest callus growth in 'Acsad 1164', however, calli of other genotypes grow better on 2,4-D free-media (Table 4). Callus fresh and dry weight were recorded every four day through 24 of culture. Values were the mean of twenty completely randomized replicates with four sample in each replicate.

**Shoot regeneration**

Calli (approx 250 mg) were subcultured into solid MS medium without any growth regulators (control), or to solid MS medium containing 0.1 mg/l 2,4-D in combination with different concentration of 6-Benzylaminopurine (BA), Thidiazuron (TDZ) or 6 (γ,γ-dimethylallylamino) purine (2ip) at 0.0, 1.0, 2.0 or 3.0 mg/l. The cultures were incubate in a culture room at 22 ± 2 °C with photoperiod 16 h light (photosynthetic photo flux density; PPFD= 40-45 μmol m⁻² s⁻¹). The number of regenerated roots for each treatment, which for each genotype, consisted of 5 replicates arranged randomly, which was recorded after 30 d.

**Statistical Analysis**

Each experiment was set up as a completely randomized design (CRD). Data were subjected to ANOVA. Means were compared using the least significant difference (LSD) test at 0.01 level of probability. Data were analyzed using STATISTICA (StatSoft 1995).

**RESULTS AND DISCUSSION**

**Callus induction**

Callus induction results showed that, seeds formed different percentage of calli ranged from 30 to 100 % among genotypes (Table 1), whereas no callus formation on free hormone medium. At 3.0 mg/l 2,4-D, all seeds produced calli in the different genotypes except for 'Acsad 176' (Table 1). These results similar to previous results obtained by Abe & Futsuhara (1986) on *Oryza sativa* were the frequency was almost 100 % in all tested rice varieties. Sears & Deckard (1982) reported that, wheat embryos developed calli ranged from 0 to 97 % among the differenent genotypes tested with an average of 45 %.

Embryo size (age), genotype, type of medium, conditions in which donor
Callus induction, plant regeneration, and growth on barley (Hordeum vulgare L.)

Table 1. Effect of different 2,4-D concentrations on callus induction in six barley genotypes after 40 days incubation period of mature seeds on MS solid media.

<table>
<thead>
<tr>
<th>2,4-D mg/l</th>
<th>Genotype</th>
<th>1.0</th>
<th>2.0</th>
<th>3.0</th>
<th>4.0</th>
<th>5.0</th>
<th>Mean</th>
</tr>
</thead>
</table>
| Callus induction %
| 'Mari'     | 80       | 100 | 100 | 100 | 80  | 92  |
| 'Acsad 176' | 60       | 70  | 70  | 90  | 80  | 74  |
| 'Acsad 1164' | 100     | 100 | 100 | 100 | 100 | 100 |
| 'Harmal'   | 30       | 50  | 100 | 100 | 60  | 68  |
| 'Acsad 1176' | 70      | 70  | 100 | 90  | 80  | 82  |
| 'Rum'      | 80       | 100 | 100 | 100 | 100 | 96  |

plants were grown, and 2,4-D concentrations in culture media are factors that affect callus initiation (Rines & McCoy 1981, Sears & Deckard 1982, Chauhan & Kothari 2004). All genotypes showed significant differences in respect to callus fresh (Table 2) and dry weight (Table 3), regardless the concentration of 2,4-D in the media. 'Rum', 'Acsad 1164' and 'Acsad 1176' produced calli of 286.3, 273.7 and 204.6 mg fresh weight per seed fresh respectively. However, fresh weight of calli which were developed from 'Harmal', 'Mari' and 'Acsad 176' were considerably lower 165.9, 135.7 and 112.1 mg fresh weight per seed, respectively (Table 2). Calli developed from Rum had the highest callus dry weight, whereas, callus from 'Acsad 176' had the lowest callus dry weight (Table 3). Similar findings were reported by Al-Karaki & Abu-Ein (1999) on wheat. They reported that, 3 seed per jar were able to produce a minimum of 1.0 g of calli in the different wheat genotypes tested after 30 to 40 days incubation when culture was supplemented with 2,4-D. Another study by Abe & Futsuhara (1986) showed that, most rice (Oryza sativa) varieties, formed a callus of more than 100 mg after 30 days after inoculation period.

Callus growth

After 30 days of subculture calli developed from 'Rum' had the higest fresh weight (355.1 mg) compared to that of all other genotypes (Table 4). Calli from 'Harmal', 'Acsad 1176' and 'Mari' had the lowest fresh weight (285.6, 288.7 and 296.8 mg fresh weight, respectively) (Table 4), while fresh weight of those from 'Acsad 176' and 'Acsad 1164' were in between (324.6
Table 2. Effect of different 2.4-D concentrations on callus fresh weight in six barley genotypes after 40 days of seed incubation period on MS solid media.

<table>
<thead>
<tr>
<th>2.4-D mg/l</th>
<th>Genotype</th>
<th>1.0</th>
<th>2.0</th>
<th>3.0</th>
<th>4.0</th>
<th>5.0</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Callus induction %</td>
<td>'Mari'</td>
<td>162.3 ab</td>
<td>159.0</td>
<td>106.0</td>
<td>107.3</td>
<td>143.7</td>
<td>135.7</td>
</tr>
<tr>
<td></td>
<td>'Acsad 176'</td>
<td>117.7</td>
<td>132.3</td>
<td>110.0</td>
<td>116.7</td>
<td>84.0</td>
<td>112.1</td>
</tr>
<tr>
<td></td>
<td>'Acsad 1164'</td>
<td>299.0</td>
<td>297.7</td>
<td>358.7</td>
<td>233.0</td>
<td>180.0</td>
<td>273.7</td>
</tr>
<tr>
<td></td>
<td>'Harmal'</td>
<td>197.0</td>
<td>137.3</td>
<td>243.3</td>
<td>132.0</td>
<td>119.7</td>
<td>165.9</td>
</tr>
<tr>
<td></td>
<td>'Acsad 1176'</td>
<td>160.3</td>
<td>298.3</td>
<td>182.3</td>
<td>219.3</td>
<td>162.7</td>
<td>204.6</td>
</tr>
<tr>
<td></td>
<td>'Rum'</td>
<td>261.3</td>
<td>214.7</td>
<td>337.0</td>
<td>332.3</td>
<td>286.0</td>
<td>286.3</td>
</tr>
</tbody>
</table>

a. Genotypes are significant at LSD = 91.3.
b. Variations among 2,4-D levels are not significant.
ab. Interaction between genotypes and 2,4-D levels are not significant.

Table 3. Effect of different concentrations of 2.4-D on induced callus dry weight in six barley genotypes after 40 days incubation periods on MS solid media.

<table>
<thead>
<tr>
<th>2.4-D mg/l</th>
<th>Genotype</th>
<th>1.0</th>
<th>2.0</th>
<th>3.0</th>
<th>4.0</th>
<th>5.0</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Callus induction %</td>
<td>'Mari'</td>
<td>17.0 ab</td>
<td>10.0</td>
<td>7.3</td>
<td>7.7</td>
<td>11.3</td>
<td>10.7</td>
</tr>
<tr>
<td></td>
<td>'Acsad 176'</td>
<td>6.7</td>
<td>9.0</td>
<td>8.3</td>
<td>8.7</td>
<td>6.3</td>
<td>7.8</td>
</tr>
<tr>
<td></td>
<td>'Acsad 1164'</td>
<td>20.0</td>
<td>18.3</td>
<td>23.3</td>
<td>13.0</td>
<td>13.0</td>
<td>17.5</td>
</tr>
<tr>
<td></td>
<td>'Harmal'</td>
<td>14.0</td>
<td>10.0</td>
<td>18.7</td>
<td>8.7</td>
<td>8.7</td>
<td>12.0</td>
</tr>
<tr>
<td></td>
<td>'Acsad 1176'</td>
<td>16.3</td>
<td>18.3</td>
<td>13.0</td>
<td>19.0</td>
<td>14.3</td>
<td>16.2</td>
</tr>
<tr>
<td></td>
<td>'Rum'</td>
<td>22.0</td>
<td>18.3</td>
<td>25.0</td>
<td>24.0</td>
<td>19.3</td>
<td>21.7</td>
</tr>
</tbody>
</table>

a. Genotypes are significant at LSD = 91.3.
b. Variations among 2,4-D levels are not significant.
ab. Interaction between genotypes and 2,4-D levels are not significant.

and 319.5 mg fresh weight, respectively) (Table 4). In contrast, calli from 'Acsad 176' and 'Rum' had the highest dry weight (22.2 and 22.8 mg, respectively) compared with those from 'Acsad 1176' which after 30 d
Table 4. Effect of different concentrations of 2,4-D on callus growth (fresh weight) in six barley genotypes after 30 days incubation on MS solid media.

<table>
<thead>
<tr>
<th>2,4-D (mg/l)</th>
<th>Genotype</th>
<th>0.0</th>
<th>1.0</th>
<th>2.0</th>
<th>3.0</th>
<th>4.0</th>
<th>5.0</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Callus fresh weight (mg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>'Mari'</td>
<td>331.7 ab</td>
<td>302.5</td>
<td>279.0</td>
<td>287.3</td>
<td>304.7</td>
<td>275.6</td>
<td>296.8</td>
<td></td>
</tr>
<tr>
<td>'Acsad 176'</td>
<td>359.4</td>
<td>317.5</td>
<td>313.9</td>
<td>321.5</td>
<td>311.2</td>
<td>324.4</td>
<td>324.6</td>
<td></td>
</tr>
<tr>
<td>'Acsad 1164'</td>
<td>310.8</td>
<td>308.6</td>
<td>354.3</td>
<td>317.0</td>
<td>334.8</td>
<td>291.6</td>
<td>319.5</td>
<td></td>
</tr>
<tr>
<td>'Harmal'</td>
<td>283.4</td>
<td>278.9</td>
<td>288.8</td>
<td>285.4</td>
<td>286.1</td>
<td>291.0</td>
<td>285.6</td>
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<tr>
<td>'Acsad 1176'</td>
<td>311.6</td>
<td>290.6</td>
<td>289.4</td>
<td>274.8</td>
<td>285.5</td>
<td>280.0</td>
<td>288.7</td>
<td></td>
</tr>
<tr>
<td>'Rum'</td>
<td>370.0</td>
<td>332.4</td>
<td>359.9</td>
<td>380.6</td>
<td>354.0</td>
<td>333.6</td>
<td>355.1</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>327.8</td>
<td>305.1</td>
<td>314.2</td>
<td>311.1</td>
<td>312.7</td>
<td>299.4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a. Genotypes are significant at LSD = 14.5.
b. Variations among 2,4-D levels are not significant.
ab. Interaction between genotypes and 2,4-D levels are significant LSD = 35.5

culture attained the lowest dry weight (15.7 mg) (Table 5). Our results indicated that there were significant differences among genotypes in regard to callus growth when the medium was supplemented with different concentration of 2,4-D (Tables 4 and 5). Our results agreed with Nasircilara et al. (2006), and Sharma et al. (2005), reported a significant difference among genotypes in percentage of callus induction and regeneration capacity. In addition, similar results were reported by Sears & Deckard (1982) where selected wheat genotype (ND7532) demonstrated higher percentage of callus induction and faster in vitro growth rates and higher frequency of totipotent calli than other wheat genotypes tested in their study.

Result show differences among genotypes and genotypes X 2,4-D interactions. No significant differences were detected between 2,4-D levels in regard to callus fresh weight (Table 4). Similar results were obtained for callus dry weight, however, there were no variation between genotypes and 2,4-D levels interaction (Table 5). For 'Acsad 1164' with the using of 2.0 mg/l 2,4-D reported the highest callus growth (fresh weight basis) compared with other concentrations (Table 4). However transferring calli of other genotypes to 2,4-D free-media were the best for calli growth (Table 4). This could be attributed to the carry over effect of 2,4-D (Shibli & Ajlouni 2000). Sears & Deckard (1982) reported that the ND7532 wheat genotype seems to grow satisfactory at 0.5 mg/l 2,4-D, whereas Alabasskaja and PI 86207 genotypes required higher levels for suitable cell growth. Hanzel et
al. (1985) indicted that the type (quality) of barley callus grown on MS media was not affected by 2,4-D concentration. Bregitzer (1992) and Nasircilara et al. (2006) reported that genotype was the most important factor determining the friability and morphology of barley callus cultures.

Table 5. Effect of different 2.4-D concentrations on callus growth (dry weight) in six barley genotypes after 30 days incubation on MS solid media.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>0.0</th>
<th>1.0</th>
<th>2.0</th>
<th>3.0</th>
<th>4.0</th>
<th>5.0</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
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<td>20.4</td>
<td>22.5</td>
<td>17.3</td>
<td>18.5</td>
<td>19.5</td>
<td></td>
</tr>
<tr>
<td>'Acsad 176*'</td>
<td>22.5</td>
<td>22.3</td>
<td>21.8</td>
<td>23.0</td>
<td>23.3</td>
<td>22.2</td>
<td></td>
</tr>
<tr>
<td>'Acsad 1164*'</td>
<td>19.3</td>
<td>18.1</td>
<td>17.5</td>
<td>15.0</td>
<td>13.8</td>
<td>17.1</td>
<td></td>
</tr>
<tr>
<td>'Harmal'</td>
<td>17.4</td>
<td>19.6</td>
<td>17.5</td>
<td>18.3</td>
<td>18.9</td>
<td>18.0</td>
<td></td>
</tr>
<tr>
<td>'Acsad 1176*'</td>
<td>18.0</td>
<td>14.5</td>
<td>14.9</td>
<td>16.0</td>
<td>14.9</td>
<td>15.7</td>
<td></td>
</tr>
<tr>
<td>'Rum'</td>
<td>22.6</td>
<td>21.0</td>
<td>22.9</td>
<td>26.5</td>
<td>22.3</td>
<td>21.4</td>
<td>22.8</td>
</tr>
</tbody>
</table>

a. Genotypes are significant at LSD = 2.3.
b. Variations among 2,4-D levels are not significant.
ab. Interaction between genotypes and 2,4-D levels are not significant.

Effect of time

Different genotypes showed significant differences in callus growth with different culture periods (Table 6). Cultivar 'Rum' produced significantly higher callus growth (63.0 mg) compared to the other genotype regardless of the duration of culture (Table 6). 'Harmal' had the lowest callus growth (59.6 mg) (Table 6). However this study showed the need for subculturing of callus to new fresh media after 16 days of inoculation period for 'Acsad 1176', 20 days for 'Harmal', and 24 days for other cultivars ('Mari', 'Acsad 176', 'Acsad 1164', and 'Rum') (Table 6). Hanzel et al. (1985) reported that barley callus cultures were maintained by subculturing on fresh media at monthly intervals. A study by, Sears & Deckard (1982) indicated that many of the wheat varieties examined exhibited a declining growth rate after 90 days growth period in culture and necrotic spots on calli were observed. This study show that the formation of callus in H. vulgare plants depend greatly on the genotype, and hormonal requirements.
Table 6. Increase in growth (fresh weight) of six barley (*Hordeum vulgare* L.) genotypes during 24 days incubation on MS solid media containing 2.0 mg/l 2.4-D for ACSAD 1164 and hormone free media for other cultivars.

<table>
<thead>
<tr>
<th>Time (days)</th>
<th>Genotype</th>
<th>0.0</th>
<th>4</th>
<th>8</th>
<th>12</th>
<th>16</th>
<th>20</th>
<th>24</th>
<th>Mean</th>
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<tr>
<td></td>
<td></td>
<td>Callus fresh weight (mg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>'Mari'</td>
<td>55.0</td>
<td>58.1</td>
<td>59.5</td>
<td>60.1</td>
<td>60.5</td>
<td>62.0</td>
<td>63.4</td>
<td>59.8</td>
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<td>'Acsad 176'</td>
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<td>57.3</td>
<td>60.3</td>
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<td>61.1</td>
<td>62.0</td>
<td>59.7</td>
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<td>57.8</td>
<td>59.3</td>
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<td>64.1</td>
<td>66.8</td>
<td>60.7</td>
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<tr>
<td></td>
<td>'Harmal'</td>
<td>55.0</td>
<td>56.5</td>
<td>59.0</td>
<td>59.6</td>
<td>60.6</td>
<td>63.1</td>
<td>63.3</td>
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<td>58.4</td>
<td>62.3</td>
<td>63.6</td>
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<td>64.1</td>
<td>64.9</td>
<td>65.8</td>
<td>69.5</td>
<td>63.0</td>
</tr>
</tbody>
</table>

a. Genotypes are significant at LSD = 1.4.
b. Variations among time durations are significant at LSD = 1.4.
ab. Interaction between genotypes and time durations are not significant.

Shoot regeneration

Calli from six barley (*H. vulgare*) genotype were subculture twice on fresh MS medium prior transfere to the regeneration medium that contained the required hormone, in order to increase the regeneration capacity (Goldstein & Kronstad 1986). 'Mari' was the only which regenerate shoots on a hormone free medium (Table 7). 2,4-D reported to stimulate callus induction in Gramineae species (Ahn et al. 1987). The effect of TDZ on plant regeneraton differed significantly espicially among 'Harmal' and 'Mari' from the one hand and the other genotypes from the other. 'Harmal' produce an average of 2.75 plantlets, and 'Mari' produce 2.7, while 'Acsad 1176', 'Acsad 176' and 'Rum' produce 0.2, 0.35 and 0.85 planlets, respectively. No plant regeneration was observed in 'Acsad 1164'. These results are simislar to previouse finding by Ma et al. (1987) where the ability of regeneration was shown to be affected by herittability. Maximumm regeneation capicity was observed when media were supplemented with 3.0 mg/l TDZ (Table 7). The interaction between TDZ and genotype was highly significant. Morover, 'Harmal' and 'Mari' attained the highest frequency of shoot formation, while there were no significant diffrences among 'Acsad 176', 'Acsad 1164', 'Acsad 1176' and 'Rum' (Table 7). This is similar to the result observed by Shan et al. (2000). Fahmy & El-Shihy (2006), reported that the cultivar Sids1 produced highest number of shoots.
per callus (4.5) in TDZ containing medium (0.2 mg/l). Also they reported important differences in regeneration characteristics observed between the two genotypes.

Table 7. Effects of different thiadiazourn (TDZ) concentrations in medium supplimented with 0.1mg/L 2.4-D on plant regeneration of six barley genototype after 30 days growth periods under ligth (16 h ligth /8 h dark).

<table>
<thead>
<tr>
<th>Genotype</th>
<th>TDZ (mg/l)</th>
<th>0.0</th>
<th>1.0</th>
<th>2.0</th>
<th>3.0</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of regenerated plantlets / 250 mg calli</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>'Mari'</td>
<td>4.0&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.0</td>
<td>2.6</td>
<td>3.2</td>
<td>2.7</td>
<td></td>
</tr>
<tr>
<td>'Acsad 176'</td>
<td>0.0</td>
<td>1.0</td>
<td>0.4</td>
<td>0.0</td>
<td>0.35</td>
<td></td>
</tr>
<tr>
<td>'Acsad 1164'</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>'Harmal'</td>
<td>0.0</td>
<td>1.6</td>
<td>1.2</td>
<td>8.2</td>
<td>2.75</td>
<td></td>
</tr>
<tr>
<td>'Acsad 1176'</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.8</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>'Rum'</td>
<td>0.0</td>
<td>0.8</td>
<td>1.0</td>
<td>1.6</td>
<td>0.85</td>
<td></td>
</tr>
</tbody>
</table>

a. Genotypes are significant at LSD = 1.08.
b. Variations among TDZ levels are significant at LSD= 1.317
ab. Interaction between genotypes and TDZlevels are significant at LSD = 2.16.

BA had a significant interaction with genotype. Shoot regeneration by calli which were developed from 'Acsad 176' (Fig. 1). 'Acsad 176', was highly promoted by BA compared to that from 'Acsad 1164' and 'Harmal' (Table 8). However, there were no variations among 'Acsad 176', 'Mari', 'Acsad 1176' and 'Rum'. When 'Acsad 1176' were cultured on 3.0 mg/l BA L produced more shoot compared to the rest concentration used in this study. Wei et al. (1986) reported that 2.0 mg/l0 BA and 0.05 mg/l IAA were the optimal hormone concentration for shoot egeneration; while in sunflower (Helianthus annuus) the best growth regulator for shoot regeneration was on MS medium supplimented with 1.0 mg/l BAP and 0.1 mg/l NAA (Table 8). This means that regeneration depends on species and hormone concentration, a conclusion which is drawn also in the present study (Shah et al. 2003).

H. vulgare show differences in shoot formaton among the six genotype when media were supplemented with 2ip (6 γ,γ-dimethylallylamino). No shoot regeneration was observed in 'Acsad 1164' and 'Acsad 1176' when culture media were with 1.0, 2.0 or 3.0 mg/l 2ip. However, 'Harmal' and
Callus induction, plant regeneration, and growth on barley (Hordeum vulgare L.)

‘Acsad 176’ produced the highest number of shoots followed by ‘Acsad 176’. Significant differences have been observed among 2ip levels and H. vulgare genotype (Table 9). ‘Rum’ had the lowest number of shoots formed compared to other genotype; no variation were observed at different concentration of 2ip (Table 9).

Table 8. Effects of different 6-benzylaminopurine (BA) concentrations in medium suplimented with 0.1 mg/l 2.4-D on plant regeneration of six barley genotype after 30 days growth periods under ligth (16 h ligth /8 h dark).

<table>
<thead>
<tr>
<th>Genotype</th>
<th>BA (mg/L)</th>
<th>Means</th>
<th>0.0</th>
<th>1.0</th>
<th>2.0</th>
<th>3.0</th>
<th>250 mg calli</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Number of regenerated plantlets /</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>‘Mari’</td>
<td>4.0</td>
<td>0.4</td>
<td>0.6</td>
<td>0.4</td>
<td>1.55</td>
<td></td>
<td></td>
</tr>
<tr>
<td>‘Acsad 176’</td>
<td>0.0</td>
<td>0.6</td>
<td>1.8</td>
<td>4.2</td>
<td>1.65</td>
<td></td>
<td></td>
</tr>
<tr>
<td>‘Acsad 1164’</td>
<td>0.0</td>
<td>0.0</td>
<td>0.2</td>
<td>0.2</td>
<td>0.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>‘Harmal’</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>‘Acsad 1176’</td>
<td>0.0</td>
<td>0.0</td>
<td>2.6</td>
<td>2.0</td>
<td>1.15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>‘Rum’</td>
<td>0.0</td>
<td>0.4</td>
<td>1.2</td>
<td>1.0</td>
<td>0.65</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a. Genotypes are significant at LSD = 1.67.
b. Variations among BA levels are not significant.
ab. Interaction between genotypes and TDZ levels are significant at LSD = 2.114

Figure 1. Plantlet regeneration from ‘Acsad 176’ in solid MS medium supplemented with 0.2 mg/l BA.

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Table 9. Effects of different 6′γ,γ-dimethylallylamino (2iP) concentrations in medium supplemented with 0.1 mg/l 2,4-D on plant regeneration of six barley genotypes after 30 days growth periods under light (16 h light / 8 h dark).

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>2iP (mg/l)</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.0</td>
<td>1.0</td>
<td>2.0</td>
<td>3.0</td>
<td>Means</td>
</tr>
<tr>
<td></td>
<td>Number of regenerated plantlets / 250 mg calli</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>'Mari'</td>
<td>4.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>1.0</td>
</tr>
<tr>
<td>'Acsad 176'</td>
<td>0.0</td>
<td>0.0</td>
<td>4.4</td>
<td>4.8</td>
<td>2.3</td>
</tr>
<tr>
<td>'Acsad 1164'</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>'Harmal'</td>
<td>0.0</td>
<td>2.8</td>
<td>13.4</td>
<td>2.4</td>
<td>4.65</td>
</tr>
<tr>
<td>'Acsad 1176'</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>'Rum'</td>
<td>0.0</td>
<td>1.0</td>
<td>0.6</td>
<td>0.4</td>
<td>0.5</td>
</tr>
</tbody>
</table>

a. Genotypes are significant at LSD = 1.125.
b. Variations among 2iP levels are significant at LSD = 1.225.
ab. Interaction between genotypes and 2iP levels are significant at LSD = 2.25.

CONCLUSIONS

In summary no callus induced in hormone free media in all genotypes evaluated. ’Acsad 176’ and ’Rum’ have the highest callus production on dry weight basis compared to ’Harmal’ which produced lowest dry weight. However, calli started to produce plantlets after two weeks incubation period on solid MS hormone free, or on media containing different concentration of TDZ, BA and 2iP at 1.0, 2.0 or 3.0 mg/l combination with 0.1 mg/l 2, 4-D. In this study we have successfully developed a protocol for adventitious plant regeneration which differs from earlier organogenesis reports in which the explants as well as the cultivars were different. Additionally, in this investigation the frequency of callus induction and percentage of shoot regeneration in explant were more than the last reports. In our study callus induction and regeneration occur in one medium so it makes present protocol more economic by shortening regeneration duration, labor and material saving. This protocol seems to be promising and may help in V. vulgare improvement program through genetic transformation because high frequency of plantlet regeneration in this method increases the achievement possibility of transformed plantlets. The probability of undesirable somatic variation is decreased because subsequent subcultures were unnecessary, besides it provides high responsive explants with thin cell wall which are appropriate for
introgression of useful genes mediated by *agrobacterium* or other vectors. Therefore evaluation of those plants in the fields will be promising for future studies of regenerated plantlets.

**REFERENCES**


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