IN-VITRO RESPONSE OF POTATO VARIETIES TO VARIOUS AGAR CONCENTRATIONS

Shafqatullah Khan*, Raziuddin* and Hamidullah Jan**

ABSTRACT
A study was conducted to investigate the in-vitro response of two potato cultivars viz Desiree and Cardinal to water stress induced by various concentrations of agar (4 to 20 g l⁻¹ with increments of 2 g l⁻¹) supplemented to MS medium. Nine agar concentrations were tested in RCBD with three replications. The results showed that mean square values differed significantly (P≤0.05) for genotypes, agar treatments and interaction of these two for root initiation, number of days to transplantation, number of nodes plantlet⁻¹, number of leaves plantlet⁻¹ and number of roots plantlet⁻¹. Mean squares were significant only for agar treatments for shoot initiation whereas values were non significant for plantlet height on transplantation. Variety Desiree surpassed variety Cardinal for shoot initiation, number of leaves plantlet⁻¹ and number of roots plantlet⁻¹. Variety Cardinal exceeded variety Desiree for root initiation, number of days to transplantation, number of nodes plantlet⁻¹ and plantlet height on transplantation. The data indicated that most of the parameters responded favorably to the lower concentrations, however higher agar concentration had detrimental effect and the characters under study were negatively affected. The increased concentrations of agar made the media stiff and created drought like conditions, making the availability of water and nutrients difficult.

Key words: In vitro, Solanum tuberosum L., Agar, Desiree, Cardinal, MS media

INTRODUCTION
Potato (Solanum tuberosum L.) is a leading vegetable and the main cash crop of farmers, especially in hilly areas of Pakistan. Three potato crops are grown in a year; out of which, autumn crop has assumed the top most position with about 70% of the total potato area. Potato being 4th leading crop of the world is a wholesome food and a good source of carbohydrates, vitamins, minerals and proteins. Moisture accounts for about 80% of the bulk and the remaining 20% dry matter contains 17% carbohydrates and 2% proteins whereas rest is composed of fiber, minerals, vitamins and fats (Malik, 1995).

The average yield of potato has been around 9-10 t ha⁻¹ which is considerably low because of a series of biotic and abiotic stresses. Of the biotic stresses, several viral and other diseases are the main factors whereas regarding abiotic stresses, drought, salinity and heat are the main constraints of high yield (Ahmed and Rashid 1990). Drought is another major factor that causes severe yield losses in the areas where the water availability to this crop is scarce. During the year 1999 and onward in Pakistan, farmers suffered a big loss due to drought stress in the country.

Plant breeders around the world are trying to develop high yielding and stress tolerant genotypes. For these purposes both conventional and non-conventional approaches are applied. Of the non-conventional techniques, in vitro or tissue culture approach is widely applied to improve potato production by means of micro-propagation in order to produce large number of plants in a very short time, to speed up the production and supply of new varieties into the market, and to maintain disease free stock of seed potatoes for further multiplication. Keeping in view the importance of drought stress in potato development, this study was undertaken to explore the in-vitro drought tolerance potential in two varieties of potato. When we increase the quantity of agar, it also increases the bonding force between the water molecules and the agar, which ultimately makes it difficult for the plantlet to draw water from the solution and a state of drought is created for the plantlet in vitro. The greater the amount of agar in the medium the higher will be the intensity of drought. Therefore, to calculate the effects of agar concentrations on potato an experiment was conducted.

MATERIALS AND METHODS
This study was conducted in the tissue culture laboratory at Potato Research Center, Abbottabad. The MS medium as described by Murashige and Skoog (1962) was used for the purpose where all the constituents remained the same except the agar concentrations that ranged from 4g to 20g l⁻¹ with an increment of 2g l⁻¹. The lowest concentration of agar i.e. 4g l⁻¹ used was the standard (Sajid and Jan, 1998) in the experiment while others (6g to 20g l⁻¹) were the higher concentrations used for the screening of in-vitro plantlets of two potato varieties i.e. Desiree and Cardinal for drought tolerance. The experiment was conducted with a total of 9 treatments (4 g l⁻¹ to 20 g l⁻¹ of agar in MS medium) and three replications in randomized complete block design. Plantlets were grown in 20 test tubes for each treatment with a total
of 180 test tubes for one replication. The experiment was repeated thrice in the laboratory. The data were recorded on various parameters including number of days to shoot initiation, number of days to root initiation, number of days to transferable plant height, number of leaves plantlet\(^{-1}\) at transferable plant height, number of roots plantlet\(^{-1}\) at transferable plant height, number of nodes plantlet\(^{-1}\) at transferable plant height, and plantlet height at transplantable stage. The data thus recorded were analyzed according to the Fisher’s LSD test (Ott, 1988).

**RESULTS AND DISCUSSION**

**Number of Days to Shoot Initiation**

Mean square values presented in Table-I shows that for shoot initiation values differed significantly (P ≤ 0.05) for agar concentrations whereas for varieties and interaction, values were non significant. However, mean values (Table-II) shows that variety Cardinal took more number of days (24) to shoot initiation, as compared to variety Desiree (16.4). However, in general, the variety Desiree took maximum number of days to shoot initiation (43.0) in a medium containing 20 g l\(^{-1}\) agar. On the other hand, variety Cardinal, took 76.0 days in response to 14 g l\(^{-1}\) agar in the medium regarding number of days to shoot initiation. In contrary the least number of days to shoot initiation in variety Desiree were obtained in the medium containing 4 g l\(^{-1}\) and 6 g l\(^{-1}\) agar, which were 4.0, 4.3, respectively.

The results were highly significant for most of the parameters studied during this investigation. It was observed that variety Desiree took more time for shoot initiation. Shoot initiation in variety Cardinal was early as compared to variety Desiree because the variety Cardinal has narrow roots and it can penetrate inside the hard medium easily. Considering the various treatments, it was observed that 4 and 6 g l\(^{-1}\) of agar in the medium took fewer days to shoot initiation while maximum days were observed under 18 and 20 g l\(^{-1}\) of agar in the medium.

**Number of Days to Root Initiation**

Significant differences (P ≤ 0.05) were recorded between the two varieties as well as among agar concentration used in this study (Table-I). Moreover, the interaction between varieties and the treatments was also highly significant. Mean values given in Table-II depict that variety Desiree took more number of days for the root initiation with a value 33.3 while variety Cardinal took only 19.9 days towards root initiation. It was observed that 20 and 16 g l\(^{-1}\) of agar showed more number of days to root initiation with the value of 40.7 and 40.5, respectively, followed by agar concentration of 14 and 18 g l\(^{-1}\). Taking the interaction into consideration, it is clear from the Table-2, that variety Desiree in 20 g l\(^{-1}\) of agar took maximum number of days for root initiation (81.3) while earliest rooting (12 days) was observed in 4 g l\(^{-1}\) of agar. The most delayed rooting in variety Cardinal was observed in response to 14 g l\(^{-1}\) agar in the medium. Maximum number of days to root initiation was observed in 20 g l\(^{-1}\) of agar followed by 18 g l\(^{-1}\) of agar in Desiree. For variety Cardinal, 16 g l\(^{-1}\) and 14 g l\(^{-1}\) of agar in the medium showed maximum number of days to root initiation.

**Number of Days to Transplantable Height**

Mean squares for number of days for transplantable height (Table-I) had significant differences were between the varieties, treatments and interaction between the varieties and the various treatments at P ≤ 0.05. Mean values presented in Table-II showed that variety Desiree took more number of days to attain transplantable plantlet height with a value of 42.8 in comparison to variety Cardinal which took 28.3 days to attain transplantable plantlet height. Regarding agar concentrations, data ranged from 49.7 to 32.0 days for 14 g l\(^{-1}\) to 14 g l\(^{-1}\), respectively). Agar treatments could be ranked as 14 g l\(^{-1}\) followed by 12 g l\(^{-1}\) and 16 g l\(^{-1}\), 10 g l\(^{-1}\), 18 g l\(^{-1}\), 8 g l\(^{-1}\), 6 g l\(^{-1}\) and 4 g l\(^{-1}\).

For both varieties, Desiree and Cardinal, earliest desirable transplantable height was seen in least amount (4 g l\(^{-1}\)) of agar. As the concentration of the agar in the medium increased, the getting of transplantable height delayed and hence the most delayed height was seen in response to 18 g l\(^{-1}\) of agar for desire whereas in case of Cardinal agar concentration of 4 g l\(^{-1}\) had the most delayed effects. Agar concentration, 20 g l\(^{-1}\) in case of Desiree and 18 g l\(^{-1}\) and 20 g l\(^{-1}\) for Cardinal were found most destructive since in the presence of these agar concentrations plantlets could not attained the desirable plantlet height.

In the present experiment, earliest shoot and root initiation was observed in MS medium containing 4-6 g l\(^{-1}\) agar. Similarly, 4-6 g l\(^{-1}\) agar in the medium was found suitable for days to get transplantable heights of plantlets. As the agar concentration increased, it not only hardened the surface of the medium but also restricted supply of nutrients to the calli and hence the shoot/roots initiation delayed. Shoot initiation was earlier in variety Cardinal than variety Desiree which could be due to longer and narrow roots of Cardinal which penetrated deeper in the medium to draw water and nutrients. Similarly, Jain, et al. (1996) found lower concentrations of agar in the medium simulative for shoot/root initiative. According to Grillo, et al. (1994) and Banasal, et al.
Significant differences were found for varieties, various treatments and the interaction between the varieties, various treatments as well as among the interaction between the varieties and the various treatments were found (Table-I). From the Table-II, it was observed that variety Desiree was having comparatively large number of leaves with a value of 6.6. On the other hand variety Cardinal produced only 4.6 leaves. Taking the various treatments into consideration, it was observed that 4 g l⁻¹ of agar produced maximum number of roots (7.2), followed by 8 g l⁻¹, 10 g l⁻¹ and 12 g l⁻¹ of agar. In contrary, 20 g l⁻¹ of agar was found the most destructive since in this treatment leaves could not produced.

Taking the interaction into consideration it can be observed from Table-II that variety Desiree under 4 g l⁻¹ of agar produced the maximum leaves (8.0) while the same variety did not produce any leaf in response to 20 g l⁻¹ of agar. Similarly variety Cardinal produced maximum number of leaves (6.7) in the presence of 6 g l⁻¹ of agar while least number of leaves (0.7) was produced in response to 20 g l⁻¹ of agar.

**Number of Leaves Plantlet**
The data given in the Table-I revealed that there were significant differences between the two varieties, agar treatments and the interaction between the varieties and the various treatments (P<0.05). It was observed that variety Desiree was having comparatively large number of roots (5.6 nodes) in comparison to nodes produced by the variety Desiree (5.1 nodes) (Table-II). Taking the various agar treatments into consideration, it was observed that 6 g l⁻¹ and @ 8 g l⁻¹ of agar in the medium produced maximum number of nodes (6.8 each). In contrary the least number of nodes were obtained in the presence 20 g l⁻¹ and 18 g l⁻¹ of agar (0.3 and 3.7, respectively).

Taking the interaction into consideration, from Table-II it can be observed that the maximum number of nodes (6.7) produced by variety Desiree were in the presence of 16 g l⁻¹ of agar whereas variety Cardinal produced maximum number of nodes in the presence of 6 g l⁻¹ of agar in the medium. 20 g l⁻¹ of agar had the most negative effects on nodal formation in both varieties.

In the present experiment maximum number of nodes was observed in 6 g l⁻¹ and 8 g l⁻¹ of agar in the medium whereas higher concentrations of agar in the medium were found harmful for nodal production. Variety Cardinal produced maximum nodes in the presence of 6 g l⁻¹ agar while variety Desiree produced maximum nodes in the medium that contained 8 g l⁻¹ agar. Since higher concentrations of agar impose stressful conditions in the medium therefore its negative effects of plant growth was expected. Similar results were reported earlier by Grospietsch, et al. (1999) and Jain, et al. (1996).

**Number of Roots Plantlet**
The data given in the Table-I revealed that there were significant differences between the two varieties, agar treatments and the interaction between the varieties and the various treatments (P<0.05). It was observed that variety Desiree was having comparatively large number of roots (5.4) as compared to the number of roots produced by the variety Cardinal (3.6) roots. Taking the various treatments into consideration, it was observed that maximum roots were produced in response to 4 g l⁻¹ of agar whereas no roots were developed in the presence of the maximum dose of agar (20 g l⁻¹). Root formation was showed negatively relationship with the agar in the medium because number of root formation decreased as the agar concentration increased in the medium.

In the present experiment maximum number of nodes was observed in 6 g l⁻¹ and 8 g l⁻¹ of agar in the medium whereas higher concentrations of agar in the medium were found harmful for nodal production. Variety Cardinal produced maximum nodes in the presence of 6 g l⁻¹ agar while variety Desiree produced maximum nodes in the medium that contained 8 g l⁻¹ agar. Since higher concentrations of agar impose stressful conditions in the medium therefore its negative effects of plant growth was expected. Similar results were reported earlier by Grospietsch, et al. (1999) and Jain, et al. (1996).
Plantlet Height at Transplantable Stage

As given in Table-I, there were no significant differences ($P \leq 0.05$) between the two varieties, among agar treatments and the interaction between the varieties and the treatments. Mean values given in Table-II showed that variety Cardinal attained more plantlet height (8.5 cm) in comparison to the plantlet height attained by variety Cardinal (8.3). Taking the various treatments into consideration, it was observed that the maximum plantlet height (12.3 cm) was seen in the presence of 6 g l$^{-1}$ of agar followed by (11.7 cm) in response to 4 g l$^{-1}$ of agar in the medium. After 6 g l$^{-1}$ of agar in the medium, plantlet height was linearly but negatively declined with each increment of agar in the medium and hence the minimum height was seen in response to 20 g l$^{-1}$ of agar. Taking the interaction into consideration it can be observed that in the presence of 6 g l$^{-1}$ of agar, variety Desiree produced maximum plantlet height (12.7 cm) while in case of Cardinal maximum plantlet height (12.7 cm) was attained in response to 6 g l$^{-1}$ of agar. The minimum plantlet height was recorded in the presence of the maximum dosage of agar i.e. 20 g l$^{-1}$, in both varieties.

The present study showed that 4 and 6 g l$^{-1}$ agar in the medium were found best for leaf proliferation by producing maximum number of leaves whereas higher doses had negative impact on leaf production. Similarly, 4 and 6 g l$^{-1}$ agar in the medium was found best for producing maximum number of roots. The negative response of the agar in the medium is obvious. Our results further showed that both the varieties responded differently to agar induced stress however higher concentrations imposed negative effects on leaf and root formation. The difference among the various treatments could be attributed to the differential response of the two genotypes used in this study. The number of leaves in Desiree was less because the leaves of this variety are broad as compared to Cardinal. By this way they need more nutrients from the roots. The drought created by increasing agar concentrations inhibited the supply of water as well as nutrients to the plantlet. Similarly, higher concentrations of agar such as 14 g l$^{-1}$ and above had negative effects for attaining plantlet height whereas in response to lower doses of agar plantlets get reasonable heights in given time. The negative impact of stresses such as drought imposed by different chemical compounds in the medium is reported earlier by Scaramigli, et al. (2000) and Grospietsch, et al. (1999).

From the present experiment it could be concluded that lower concentrations of agar such as 4-6 g l$^{-1}$ in the medium had positive effects for majority of the traits studied whereas higher dosage of agar imposed negative effects. By comparing two varieties, variety Cardinal surpassed variety Desiree for majority of the traits and was found more responsive/tolerant to the agar induced drought stress in the medium.
Table-I  Mean square values of different traits for in-vitro response of potato varieties Desiree and Cardinal to various agar concentrations

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Shoot initiation</th>
<th>Root initiation</th>
<th>Days to transplantation</th>
<th>Nodes plant(^{-1})</th>
<th>Leaves plant(^{-1})</th>
<th>Roots plant(^{-1})</th>
<th>Plantlet height</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rep.</td>
<td>2</td>
<td>599.8NS</td>
<td>0.7NS</td>
<td>82.2NS</td>
<td>1.1NS</td>
<td>1.1NS</td>
<td>4.5(^*)</td>
<td>151.4NS</td>
</tr>
<tr>
<td>Varieties (A)</td>
<td>1</td>
<td>778.2NS</td>
<td>2773.5(^*)</td>
<td>2816.7(^*)</td>
<td>2.7(^*)</td>
<td>56.0(^*)</td>
<td>46.3(^*)</td>
<td>188.9NS</td>
</tr>
<tr>
<td>Agar Con. (B)</td>
<td>8</td>
<td>1637.8(^*)</td>
<td>661.9(^*)</td>
<td>1249.6(^*)</td>
<td>27.4(^*)</td>
<td>27.4(^*)</td>
<td>36.9(^*)</td>
<td>219.6NS</td>
</tr>
<tr>
<td>AB</td>
<td>8</td>
<td>691.5NS</td>
<td>1595.8(^*)</td>
<td>1281.9(^*)</td>
<td>3.8(^*)</td>
<td>5.1(^*)</td>
<td>3.1(^*)</td>
<td>145.1NS</td>
</tr>
<tr>
<td>Error</td>
<td>34</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>149.8</td>
</tr>
<tr>
<td>Total</td>
<td>53</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*NS= Non-significant    *= Significant at P\(\leq\)0.05 probability levels

Table-II  Mean values for different traits for in-vitro response of potato varieties Desiree and Cardinal to various agar concentrations

<table>
<thead>
<tr>
<th>S.No</th>
<th>Agar</th>
<th>Days to Shoot initiation</th>
<th>Days to Root initiation</th>
<th>Days to transplantation</th>
<th>Number of Nodes plant(^{-1})</th>
<th>Number of Leaves plant(^{-1})</th>
<th>Number of Roots plant(^{-1})</th>
<th>Plantlet height (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4 g l(^{-1})</td>
<td>4.0</td>
<td>5.3</td>
<td>12.0 h</td>
<td>15.3 kl</td>
<td>32.3 e</td>
<td>31.7 el</td>
<td>5.7 cdef</td>
</tr>
<tr>
<td>2</td>
<td>6 g l(^{-1})</td>
<td>4.3</td>
<td>5.7</td>
<td>14.0 l</td>
<td>16.0 jk</td>
<td>35.3 de</td>
<td>32.3 e</td>
<td>5.7 cdef</td>
</tr>
<tr>
<td>3</td>
<td>8 g l(^{-1})</td>
<td>5.0</td>
<td>7.7</td>
<td>15.0 ki</td>
<td>19.0 I</td>
<td>37.3 cde</td>
<td>37.7 cdei</td>
<td>6.3 abcd</td>
</tr>
<tr>
<td>4</td>
<td>10 g l(^{-1})</td>
<td>6.3</td>
<td>10.0</td>
<td>17.3 j</td>
<td>22.0 h</td>
<td>38.0 cde</td>
<td>42.0 bcdce</td>
<td>6.0 bcdce</td>
</tr>
<tr>
<td>5</td>
<td>12 g l(^{-1})</td>
<td>9.7</td>
<td>9.0</td>
<td>25.0 g</td>
<td>27.0 f</td>
<td>45.3 bcd</td>
<td>45.7 bcd</td>
<td>6.0 bcdce</td>
</tr>
<tr>
<td>6</td>
<td>14 g l(^{-1})</td>
<td>12.0</td>
<td>76.0</td>
<td>34.0 d</td>
<td>31.3 e</td>
<td>50.7 b</td>
<td>48.7 bc</td>
<td>5.3 def</td>
</tr>
<tr>
<td>7</td>
<td>16 g l(^{-1})</td>
<td>26.0</td>
<td>23.0</td>
<td>40.7 e</td>
<td>40.3 c</td>
<td>68.3 a</td>
<td>47.3 bc</td>
<td>6.7 abcd</td>
</tr>
<tr>
<td>8</td>
<td>18 g l(^{-1})</td>
<td>37.0</td>
<td>30.0</td>
<td>60.7 b</td>
<td>0.0 n</td>
<td>78.0 a</td>
<td>0.0 g</td>
<td>4.3 efg</td>
</tr>
<tr>
<td>9</td>
<td>20 g l(^{-1})</td>
<td>43.0</td>
<td>24.7</td>
<td>81.3 a</td>
<td>0.0 n</td>
<td>0.0 g</td>
<td>0.0 g</td>
<td>0.0 g</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Means</td>
<td>16.4</td>
<td>24.0</td>
<td>33.3</td>
</tr>
</tbody>
</table>

*Means followed by the same letter are not significantly different from one another according to the Fisher’s LSD test at P=0.05
REFERENCES


