ADVENTITIOUS SHOOT REGENERATION FROM DIFFERENT EXPLANTS OF WILD LENTIL

*(LENS CULINARIS SUBSP. ORIENTALIS)*

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**ABSTRACT**

This study reports an efficient adventif shoot regeneration system from cotyledonary node and stem node explants of Wild lentil using 0.05 mg/l TDZ concentration in MS medium. It is expected that the reported procedure would be effectively used for conservation of Wild lentil germplasm, which is under threat of extinction primarily through habitat destruction.

**Introduction**

Lentil is grown these days mainly in the region extending from the Atlantic coast of Spain and Morocco in the west through to India in the east. *Lens culinaris* is indigenous to the Near East and central Asia. The wild subspecies *Lens culinaris orientalis* is found in Turkey, Syria, Lebanon, Israel, Jordan, Iraq, Iran, Afghanistan and central Asia (16). They are thought to have been brought into cultivation somewhere in southeast Turkey or northern Syria near the Tigris and Euphrates rivers. The crop was domesticated from a wild lentil species which grows naturally in the area (*L. culinaris* subsp. *orientalis*). This wild progenitor still exists in the region and further afield (23). It contains a huge reservoir of genetic variability which can be used in lentil-improvement programs. Narrow genetic base of cultivated lentil calls for the transfer of useful genes from other sources (20) especially wild relatives of lentil for improving resistance against certain type of stresses. Wild lentils are continuously under threat of erosion and are needed to be conserved before they are completely lost, primarily through habitat destruction.

In vitro culture of lentils has proved difficult. In the last 25 years techniques have been progressively improved starting with Bajaj and Dhanju (2) various workers like Williams et al. (19), Saxena and King (17), Polanco et al. (14), Malik and Rashid (11), Malik and Saxena (9, 10), Ahmad et al. (1), Polanco and Ruiz (15), Halbach et al. (5), Polanco (13), Ye et al. (20, 21), Khawar and Özcan (7), Khawar et al. (8), Fratini and Ruiz (4) have reported multiple shoot formation from various explants including shoot meristem, hypocotyl, epicotyls, embryonic axes, first node, first pair of leaves, seed culture and cotyledonary node etc. None of these report any procedure for *in vitro* shoot regeneration from wild lentils.

The study was designed for the development of efficient in vitro regeneration procedure for the wild lentil.

**Materials and Methods**

**Plant regeneration**

Wild lentil Accession No.P1 572366 95i Hoffman No. 10 of ICARDA obtained
Shoot regeneration from different explants of wild lentil *Lens culinaris* subsp *orientalis* (Accession No.P1 572366 951 Hoffman No. 10 of ICARDA) after 8 weeks in culture on MS medium supplemented with various concentrations of TDZ

<table>
<thead>
<tr>
<th>Explants</th>
<th>Frequency of shoot regeneration</th>
<th>Mean number of shoots per explant</th>
<th>Mean shoot length</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TDZ concentration (mg/l)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.00</td>
<td>0.05</td>
<td>0.10</td>
</tr>
<tr>
<td>Stem</td>
<td>0.00</td>
<td>0.00c</td>
<td>0.00c</td>
</tr>
<tr>
<td>Stem node</td>
<td>0.00</td>
<td>83.33b</td>
<td>75.00b</td>
</tr>
<tr>
<td>Cotyledon node</td>
<td>0.00</td>
<td>100a</td>
<td>91.66a</td>
</tr>
</tbody>
</table>

Each value is the mean of 3 replications of 3 Petri dishes with 5 explants each.

1 From explants which regenerated shoots.
2 Values within a column followed by different letters are significantly different at the 0.01 probability level using Least significant Difference (LSD) test ($p < 0.01$).

Results and Discussion

Multiple shoot induction

The frequency of shoot regeneration, number of shoots per explant and mean shoot length were visibly affected by the type of explant and concentration of TDZ in the regeneration medium. Multiple shoots were induced on each of the two TDZ concen-
trations from cotyledonary node and stem node with no regeneration from stem explants (Table). The effects of TDZ concentrations were significant on frequency of shoot regeneration, the number of shoots per explant and the mean shoot length (p<0.01). The regeneration from cotyledonary nodes was preceded by 7 to 10 days callusing followed by green shoot primordia on callus within two weeks, which later developed into normal multiple adventitious shoots after 8 weeks of culture (Figure). Higher TDZ concentrations reduced shoot regeneration and resulted in stunted shoots in both genotypes, as has been reported for pea, chick pea and lentils (9). Growth regulator free media failed to induce shoot regeneration on any explant (Table).

Mallick and Rashid (11) also obtained multiple shoot regeneration from cotyledonary nodes of lentil on a medium containing BAP (data is not given). Polanco (13) achieved 5-20 shoots per immature seeds of four lentil genotypes on media supplemented with BAP. We have obtained higher frequency of shoot regeneration from cotyledonary node of wild lentil explants using TDZ, which has not been reported previously.

Comparing the number of shoots induced using 0.05 mg/l concentration of TDZ (Table) with 0.1 mg/litre TDZ, the frequency of shoot regeneration, mean number of shoots per explant and mean shoot length decreased with increased TDZ concentration. The highest number of 55.34 shoots per cotyledon node explant at 0.05 mg/litre (Figure) reduced to 19.31 at 0.10 mg/litre TDZ.

TDZ induced multiple shoot formation from cotyledon node explant and stem node with no shoots from stems. The effectiveness of TDZ on shoot induction in lentil tissue culture has been well documented (10, 7, 8). Our results underline the importance of TDZ and suggest that a lower dose of TDZ induces high frequency of shoot regeneration from cotyledonary nodes. Malik and Saxena (10) obtained the highest shoot regeneration from nodal and basal regions of primary shoots developed from seed cultures of *Lens culinaris*, *Pisum sativum*, *Cicer arietinum*, and *Phaseolus vulgaris* on media supplemented with relatively low concentrations of TDZ. Similarly Khawar and Özcan (7) and Khawar et al. (8) obtained high frequency of shoot regeneration from cotyledon nodes using TDZ. These results implied that a universal protocol for wild lentil in vitro culture could be developed based on cotyledon node using a TDZ containing medium, and that lines/accessions screening for good multiplication performance will greatly improve the success rate of in vitro culture. This observation can be exploited to increase the efficiency of multiplication in a future study.

Excising regenerating adventitious shoots and the subculture of the explant onto a suitable medium gave negative results. Adventitious shoot regeneration was either completely lost or reduced and stunted with browning of explants. One reason may be the external application of cytokinins was hard for the explant to survive resulting in the browning of callus tissue leading to the gradual death of cells or the calli were exhausted after one regeneration once the shoots were excised.
Conclusions
In conclusion, the present study underlines the importance of lower concentrations of TDZ for high shoot regeneration preferably from cotyledonary nodes of wild lentil. TDZ induced multiple shoot formation in wild lentil from cotyledon node culture. This suggests that this protocol can be usefully applied to conserve the narrow genetic base of wild lentils.

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REFERENCES