Effect of Growth Hormones i.e., GA₃, IAA and Kinetin on 1. Length and Diameter of Shoot, 2. Early Initiation of Cambium and Maturation of Metaxylem Elements in *Cicer arietinum* L.

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Abstract: Effects of growth regulators i.e., GA₃, IAA and Kinetin were studied on the shoot of *Cicer arietinum* L. after 45 and 60 days of treatment and was compared with control as well as among themselves. The following concentrations of growth hormones were applied individually 100 ppm GA₃, 100 ppm IAA and 20 ppm Kinetin. In combinations the concentrations used were 100 ppm GA₃+100 ppm IAA, 100 ppm GA₃+20 ppm Kinetin, 100 ppm IAA+20 ppm Kinetin and 100 ppm GA₃+100 ppm IAA+20 ppm Kinetin. Applied GA₃ increased the length of shoot significantly but in the internal morphology GA₃ revealed no positive effect in cambium enhancement and maturation of metaxylem elements. Applied IAA and Kinetin more or less promoted expansion in diameter but decreased the extension growth. IAA and Kinetin promoted initiation of cambium and maturity of metaxylem elements. In the mixed doses of GA₃+IAA and GA₃+Kinetin, increase in length as well as in the diameter was observed. The diameter of cambial region also revealed some expansion. In GA₃+IAA+Kinetin no definite pattern was observed with regard to length, diameter and cambium.

**Key words:** Cambium, metaxylem elements, growth hormones, gibberellic acid (GA₃), indole-3-acetic acid, kinetin

**Introduction**

The prospect of employment of gibberellins as general stimulants of plant growth has been explored, mostly the increased height of shoot has been observed. However, auxins have not stimulated the increase in length. GA₃ causes remarkable elongation has been reported by a number of workers (Brian and Hemming, 1958; Pinney, 1956; Singh et al., 1979; Almeida and Pereira, 1987). Inhibition in length with IAA has been reported by Bairathi and Nathawar (1980) and Pilet and Saugy (1985). Gibberellins either promote or inhibit cambial growth in plants. Stimulation of cambial growth by gibberellins has been reported in shoots of Pinus sylvestris by Wang et al. (1997). More recently Awan et al. (1999) have reported that GA₃ promotes cambial growth. Contrarily, inhibition of cambial region by applying GA₃ has been reported by Kaufman (1985) and Morris and Arthur (1985). Similarly, Ozeki and Komamine (1986) reported no effect of GA₃ on cell division in carrot suspension cultures. Acceleration of cell division in cambial region with auxins (IAA) has been reported by a number of workers. Tuominen et al. (1997) reported that a radial concentration gradient of IAA is related to secondary xylem development in Hybrid Aspen. IAA also promotes the diameter of metaxylem elements (Wareing and Roberts, 1956). The increase in metaxylem elements by applying IAA can be attributed to increased cell division in cambial region (Miller et al., 1955). More recently, Uggla et al. (1998) also reported that IAA controls cambial growth and acts as positive morphogen in cambial development. Cytokinins are also known to promote cell division in cambial region (Makarova et al., 1988).

**Materials and Methods**

The motif of the present work is to study the effect of growth hormones i.e., GA₃, IAA and Kinetin on the external and internal morphology of shoot of *C. arietinum*. Individually the following composition of growth hormones was used 100 ppm GA₃, 100 ppm IAA and 20 ppm Kinetin. In combination the concentrations used were 100 ppm GA₃+100 ppm IAA, 100 ppm GA₃+20 ppm Kinetin, 100 ppm IAA+20 ppm Kinetin and 100 ppm GA₃+100 ppm IAA+20 ppm Kinetin. Applied GA₃ increased the length of shoot significantly but in the internal morphology GA₃ revealed no positive effect in cambium enhancement and maturation of metaxylem elements. Applied IAA and Kinetin more or less promoted expansion in diameter but decreased the extension growth. IAA and Kinetin promoted initiation of cambium and maturity of metaxylem elements. In the mixed doses of GA₃+IAA and GA₃+Kinetin, increase in length as well as in the diameter was observed. The diameter of cambial region also revealed some expansion. In GA₃+IAA+Kinetin no definite pattern was observed with regard to length, diameter and cambium.

Results

In the present work, the shoot of *C. arietinum* showed a significant increase in length with applied GA₃ i.e., the 100 ppm dose showed 66.11% increase after 45 days and 88.94% after 60 days. On the other hand, it registered inhibitory effect on length e.g. the dose of 100 ppm showed 16.66% decrease after 45 days.
Guttridge, 1959; Basford, 1961; Adams and Ross, 1983; increase in the number of internodes (Thompson and Almeida and Pereira, 1997). The obvious effect being reported by a number of workers (Brian and Hemming, 1958; Phinney, 1956; Lang, 1970; Singh, 1959). The mixed doses of GA3+IAA and GA3+Kinetin showed significant increase in length after 45 and 60 days (Table 1). Thus revealing the dominant effect of GA3 where length is concerned (Frankland and Wareing, 1960; Brian et al., 1964). The diameter of shoot also increased significantly with these mixed doses (Table 1), which may be attributed to the activity of IAA and Kinetin (Eisinger, 1983). Applied IAA caused inhibition in length after 45 and 60 days (Table 1, Fig. 2). Similar results have been reported by Bairathi and Nathawat (1980) and Pilet and Saugy (1985). However, the diameter of shoot increased significantly with IAA (Table 1). The mixed dose of IAA+Kinetin registered inhibition in length accompanied by a well-marked increase in diameter. Applied kinetin showed significant increase in length and diameter after 45 and 60 days (Table 1, Fig. 3, 4). Similar results have been reported by Makarova et al. (1988).

In the internal morphology, applied GA3 registered inhibition in cambial region after 45 as well as 60 days when compared with control. The number of metaxylem elements also decreased with applied GA3 i.e., 78 after 45 days and 98 after 60 days in comparison to control. Similarly single cambial strand also showed inhibition with applied GA3. Contrary to GA3 applied IAA showed 7.1% increase in cambial region after 45 days and 14.28% after 60 days. The number of metaxylem elements also increased i.e., 95 after 45 days and 120 after 60 days. The 20 ppm kinetin also registered increase when compared with control. The number of metaxylem elements were 98 after 45 days and 124 after 60 days (Table 1, Fig. 1).

The equally mixed dose of GA3+IAA showed 4.83% increase in cambial region after 45 days and 11.42% after 60 days, thus showing the dominant effect of IAA. The number of metaxylem elements increased upto 95 after 45 days and 108 after 60 days. The mixed dose of 100 ppm GA3+20 ppm Kinetin showed 9.87% increase in cambium after 45 days and 12.5% after 60 days. The number of metaxylem elements observed after 45 days were 88 and after 60 days they were 119 in comparison to control. The mixed dose of 100 ppm IAA+20 ppm kinetin showed a significant increase in cambium i.e., 13.54% after 45 days and 18.57% after 60 days when compared with control. The metaxylem elements showed maximum response i.e., 105 after 45 days and 125 after 60 days when compared with control. The combined dose of all three hormones showed no significant effect after 45 days, however, after 60 days it increased to 12.85%. The metaxylem elements also showed increase but this increase was not well marked when compared with control, this may be attributed to mixed GA3 dose (Table 1, Fig. 1).

Discussion
In the external morphology, application of GA3 showed significant increase in the length of shoot after 45 and 60 days. GA3 causes remarkable elongation has been reported by a number of workers (Brian and Hemming, 1958; Phinney, 1956; Lang, 1970; Singh et al., 1979; Almeida and Pereira, 1997). The obvious effect being increase in the number of internodes (Thompson and Guttridge, 1959; Basford, 1961; Adams and Ross, 1983; Hernandez, 1997). This may be attributed to the intact cell elongation (Awan et al., 1999). The marvellous increase in length on one hand was accompanied by inhibition in the diameter of shoot on the other hand. This may be attributed to the extra amount of sugars consumed during the rapid elongation of internodes, thus affecting the girth (Alisopp, 1959). The mixed doses of GA3+IAA and GA3+Kinetin showed significant increase in length after 45 and 60 days (Table 1). Thus revealing the dominant effect of GA3, where length is concerned (Frankland and Wareing, 1960; Brian et al., 1964). The diameter of shoot also increased significantly with these mixed doses (Table 1), which may be attributed to the activity of IAA and Kinetin (Eisinger, 1983). Applied IAA caused inhibition in length after 45 and 60 days (Table 1, Fig. 2). Similar results have been reported by Bairathi and Nathawat (1980) and Pilet and Saugy (1985). However, the diameter of shoot increased significantly with IAA (Table 1). The mixed dose of IAA+Kinetin registered inhibition in length accompanied by a well-marked increase in diameter. Applied kinetin showed significant increase in length and diameter after 45 and 60 days (Table 1, Fig. 3, 4). Similar results have been reported by Makarova et al. (1988).

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Chaudhry and Khan: Cambium, metaxylem elements, growth hormones, gibberellic acid (GA$_3$)

Fig. 1: Effect of 100 ppm GA$_3$ on the shoot of *Cicer arietinum*
Fig. 2: Effect of 100 ppm IAA on the shoot
Fig. 3: Effect of 20 ppm Kinetin on the shoot
Fig. 4: Shoot of *C. arietinum* in transection (X10) showing cambial region
Fig. 5: Effect of 100 ppm GA$_3$ in transection (X10) showing cambial region
Fig. 6: Effect of 100 ppm IAA in transection (X10) showing cambial region
Fig. 7: Effect of 20 ppm Kinetin in transection (X10) showing cambial region
Fig. 8: Effect of 100 ppm IAA + 20 ppm Kinetin in transection (X10) showing cambial region
Table 1: Effect of growth hormones on the external and internal morphology of stem after 45 and 60 days

<table>
<thead>
<tr>
<th>Treatment (ppm)</th>
<th>Length of shoot (cm)</th>
<th>Diameter of first internode (µm)</th>
<th>Diameter of xylem strand (µm)</th>
<th>Numver of metaxylem elements</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>36.0 ± 0.57</td>
<td>0.20 ± 0.013</td>
<td>310.0 ± 0.35</td>
<td>105.0 ± 0.28</td>
<td>39.8 ± 0.32</td>
<td>0.22 ± 0.008</td>
<td>350.0 ± 0.12</td>
<td>105.0 ± 0.28</td>
</tr>
<tr>
<td>100 IAA</td>
<td>59.8 ± 0.79</td>
<td>0.19 ± 0.00</td>
<td>300.0 ± 0.03</td>
<td>98.0 ± 0.31</td>
<td>75.2 ± 0.88</td>
<td>0.21 ± 0.05</td>
<td>335.0 ± 0.18</td>
<td>98.0 ± 0.31</td>
</tr>
<tr>
<td>100 GA3</td>
<td>30.0 ± 0.88</td>
<td>0.26 ± 0.005</td>
<td>332.0 ± 0.75</td>
<td>120.0 ± 0.61</td>
<td>36.0 ± 0.57</td>
<td>0.27 ± 0.05</td>
<td>400.0 ± 0.23</td>
<td>120.0 ± 0.61</td>
</tr>
<tr>
<td>20 Kinetin</td>
<td>43.0 ± 0.57</td>
<td>0.24 ± 0.00</td>
<td>350.0 ± 0.28</td>
<td>124.0 ± 0.73</td>
<td>47.0 ± 0.52</td>
<td>0.25 ± 0.03</td>
<td>410.0 ± 0.74</td>
<td>124.0 ± 0.73</td>
</tr>
<tr>
<td>100 GA3 + 100 IAA</td>
<td>51.4 ± 0.32</td>
<td>0.22 ± 0.006</td>
<td>325.0 ± 0.24</td>
<td>108.0 ± 0.43</td>
<td>63.0 ± 0.57</td>
<td>0.23 ± 0.138</td>
<td>390.0 ± 0.75</td>
<td>108.0 ± 0.43</td>
</tr>
<tr>
<td>100 IAA + 20 Kinetin</td>
<td>65.0 ± 0.85</td>
<td>0.21 ± 0.003</td>
<td>340.0 ± 0.30</td>
<td>119.0 ± 0.22</td>
<td>85.5 ± 0.61</td>
<td>0.23 ± 0.012</td>
<td>395.0 ± 0.39</td>
<td>119.0 ± 0.22</td>
</tr>
<tr>
<td>100 GA3 + 20 Kinetin</td>
<td>25.5 ± 0.66</td>
<td>0.27 ± 0.02</td>
<td>352.0 ± 0.28</td>
<td>125.0 ± 0.28</td>
<td>30.5 ± 0.529</td>
<td>0.29 ± 0.011</td>
<td>415.0 ± 0.39</td>
<td>125.0 ± 0.28</td>
</tr>
<tr>
<td>100 IAA + 100 IAA + 20 Kinetin</td>
<td>45.5 ± 0.08</td>
<td>0.24 ± 0.003</td>
<td>315.0 ± 0.19</td>
<td>112.0 ± 0.21</td>
<td>49.3 ± 0.502</td>
<td>0.25 ± 0.015</td>
<td>395.0 ± 0.97</td>
<td>112.0 ± 0.21</td>
</tr>
</tbody>
</table>

L.S.D. 13.122 0.024 17.59 7.809 18.22 0.024 26.509 9.022

increase (Table 1). From all the above observations, it can be concluded that IAA, Kinetin and their combinations have a positive effect on cell division when compared with control as well as GA3.

References