Table 2. Days taken for in vitro flowering and pod formation in cauliflower

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Initiation of flowering</th>
<th>Opening of flowers</th>
<th>Complete pod formation</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>80 ± 7.5</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>B</td>
<td>45 ± 4.9</td>
<td>56 ± 5.8</td>
<td>95 ± 8.2</td>
</tr>
<tr>
<td>C</td>
<td>32 ± 4.3</td>
<td>40 ± 4.8</td>
<td>65 ± 6.2</td>
</tr>
</tbody>
</table>

Table 3. Details of in vitro flowering and pod formation in cauliflower

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No of flowers/plantlets</th>
<th>Length of flower stalk (cm)</th>
<th>No of pods/plantlets</th>
<th>Pod length (cm)</th>
<th>No of seeds/pod</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(after 60 days)</td>
<td></td>
<td>(after 90 days)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>3 ± 0.4</td>
<td>4 ± 0.4</td>
<td>1 ± 0.3</td>
<td>21 ± 0.4</td>
<td>3 ± 0.3</td>
</tr>
<tr>
<td>C</td>
<td>7 ± 0.6</td>
<td>8 ± 0.9</td>
<td>5 ± 0.6</td>
<td>4 ± 0.4</td>
<td>4 ± 0.5</td>
</tr>
</tbody>
</table>

Culture initiation. Similarly, the number of pods/plantlets (5.2 ± 0.6) and the pod length (4.8 ± 0.4) were also more in treatment C than in treatment B (1.8 ± 0.3 and 2.1 ± 0.4, respectively) after 90 days of culture initiation.

After 90 days, shedding of leaves and other floral parts was observed in treatment C and pods were ready to harvest. After harvesting, on an average 4.6 ± 0.5 seeds were counted in treatment C only (Table 3). By this time, pods in treatment B were green and immature.

The in vitro developed flowers were typical of cruciferous family, having 4 sepals, 4 petals, 6 stamens, of which 2 were short, and 2 carpels with superior ovary. The seeds in the fruit (silica) were small, globular, smooth and dark brown in colour.

Flowering has also been induced in vitro under appropriate conditions through culture of thin cell layers of epidermal tissues excised from flowering stems of tobacco and potato. It is hypothesized that the amount of native gibberellins present in the plant appears to be a deciding factor in whether a plant would remain vegetative or bolt and flower.

The effect of gibberellins and other growth retardants on in vitro flowering of vegetative apex of Japanese pear has also been reported. In our study, in vitro flowering in cauliflower through culture of curd explants appears to be under the control of different hormonal regimes, i.e. 3 mg l⁻¹ IAA and 0.5 mg l⁻¹ kinetin in MS medium.

Though in vitro pollination and fertilization gives a small amount of seed, plant breeders can exploit this phenomenon when this is difficult in vivo. Further, the problem of isolation distance is also overcome and the long period for flowering and pod formation under field conditions is considerably cut short this way.


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Testosterone biosynthesis in triploid sterile male tulip

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Histochemical localization of strategically important steroid dehydrogenase (3β-HSD) and immunohistochemical studies using testosterone antisera confirmed the active nature of triploid interstitial cells and the presence of 5-ene and 4-ene pathways leading to the biosynthesis of testosterone in the testis of triploid sterile tulip. Hence, the partial sterility suffered by triploid male tulip is not due to the lack of steroid hormone involved in spermatogenesis.

Triploidy has been induced by subjecting freshly fertilized eggs to thermal (heat or cold), pressure, electrical or chemical shock; in these shocked eggs the extrusion of second polar body has been suppressed as evidenced by the metaphase-spread preparations. Triploidy is also known to result in complete sterility in females (e.g. rainbow trout, tilapia), and partial sterility in males (e.g. rainbow trout, tilapia). The partial sterility suffered by males has generally been attributed to endocrine and cytological incompatibilities, although no
Figure 1 a–i. Selected sections of diploid (2n) and triploid (3n) tilapia. Bar = 5 μm.
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direct evidence has so far been reported. Lincoln and Scott were perhaps the first to show the occurrence of testosterone in the partially sterile male triploid Onchorynchus mykiss using radioimmunoassay. This observation has been subsequently confirmed in the same and Perca fluviatilis. This communication reports the occurrence of strategically important hydroxy steroid dehydrogenase, which indicates that the partial sterility suffered by triploid male tilapia is not due to the lack of steroid hormone involved in spermatogenesis.

Triploidy was induced in Orechromis mossambicus following the procedure described by Varadaraj and Pandian. These triploids were reared until they attained sexual maturity. Testes from confirmed triploids were sectioned in a cryotome (International Equipment, USA). H-E staining was done to obtain the structural details. Fresh frozen sections (16 μm) were incubated in an appropriate incubation medium for 2–3 h according to the method of Baillie et al. Pregnenolone was selected as a substrate for histochemical localization of 3β-HSD. The incubation medium contained 0.1 M sodium phosphate buffer (pH 7.2), 0.14 mM pregnenolone, 0.6 mM nitro blue tetrazolium and 7 mM NAD. The sections were fixed in 10% formalin after the completion of reaction. Immunohistochemical staining was done according to the method of Kuhlmann. Sections (16 μm thick) were first neutralized of their endogenous peroxidase activity by treating with 1% hydrogen peroxide in PBS. To reduce nonspecific staining, sections were blocked using normal goat serum and 1% BSA. This was followed by incubation with testosterone antisera (Sigma, USA, in 1:10 dilution of the reconstituted lyophilized stock) for 2 h at room temperature. Finally, sections were treated with 1:100 dilution of HRP-coupled goat antirabbit IgG (Genei, Bangalore) for 1 h and developed in a solution containing 30 mg 4-chloro-1-naphthol, 10 μl methanol, 30 μl of 30% hydrogen peroxide and 40 μl of 1× PBS for 15 min in dark. After each treatment the sections were washed thoroughly with PBS (pH 7.2) to remove the unbound protein molecules from the sections.

H-E staining of the testis of 5-month-old triploids revealed the presence of numerous interstitial cells as in diploid controls (Figure 1a, b). Their active nature was confirmed by lipid and histochemical staining. There were a few irregular-sized spermatozoa in triploid testis, indicating their aneuploid nature arising due to abnormal meiosis (Figure 1a, d). In contrast, the diploid sections showed the presence of abundant, regular-sized spermatozoa (Figure 1a, c). The 3β-HSD activity was slightly stronger in the triploid interstitial cells (Figure 1f) than that of the diploids (Figure 1e). Control sections incubated in substrate-free medium showed no reaction. Testosterone immunoactivity was slightly stronger in the triploid sections (Figure 1j) than that in the diploids (Figure 1h). Control sections incubated in substrate-free medium showed no reaction (Figure 1g).

Hence, the triploid interstitial cells are active and produce testosterone through the 5-ene and 4-ene pathways.

Hyder et al. observed an increase in the plasma testosterone titre in correlation with an enlargement of interstitial cells when human chorionic gonadotropin was administered. Hence, the presence of larger interstitial cells in triploid tilapia might be due to their active involvement in testosterone biosynthesis.

Conversion of pregnenolone to progesterone is a key step in the formation of all biologically active steroids. As 3β-HSD brings about this conversion, its activity has been used to confirm the interstitial cells and is also considered as a measure of steroid biosynthesis by many workers. The stronger activity of this enzyme in triploid interstitial cells indicates the presence of highly active 5-ene and 4-ene pathways in triploids. Stronger immunohistochemical staining for testosterone confirms the conclusion.

In conclusion, the triploid interstitial cells and the 5-ene and 4-ene pathways leading to the biosynthesis of testosterone are active. The partial sterility suffered by triploid male tilapia may be attributed to the problems in meiosis, and not due to the lack of steroid hormone involved in spermatogenesis.


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