The Auxin Induced Curvature of Citrus Petals

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Abstract. The curvature of citrus petals was studied, using petals implanted in an agar-sucrose medium. Applications of indoleacetic acid and p-chlorophenoxyisobutyric acid and determinations of endogenous auxins indicate that the bending of citrus petals is an auxin-mediated process.

The opening of the flower is caused usually by the curvature of the petals. This curvature is preceded or accompanied by elongation of the petals. The curvature of the petal results from a faster growth in its inner (=ventral) side (4,8,17). Differences in temperature sensitivity between the inner and outer sides of the petal cause a daily cycle of opening and closing in certain flowers (17).

As already pointed out by Fogg (5) it is rather surprising that auxin and other growth hormones have not yet been implicated in the process of flower opening. In a preliminary communication we reported that citrus petals implanted in agar respond to applied auxin by an enhanced curvature (6). We now present data which show that the curvature of citrus petals is controlled by auxin.

Citrus petals have several properties which make them suitable for experimental studies. A) Petals can curve even when detached. Therefore we were able to use petals implanted in an agar-sucrose medium for our experiments (see fig 1A). B) Citrus petals are rather thick and elastic. The

Fig. 1. A) (above) The experimental system used. The 3 test tubes contain petals implanted in the agar-sucrose medium, curved to a different extent. On the left is an opened citrus flower and on the right a closed one; near opening. B) (below) The measurement of the angle of curvature by the protractor designed for this purpose.
thickness of the petal (about 0.5 mm for Shamouti orange petals) makes it possible to separate layers from the inner and outer sides and to study physiological differences between them. The elasticity and rigidity of the petal allow an accurate measurement of the degree of curvature. C) The elongation of citrus petals is almost finished before curvature begins, making it easy to distinguish between usual elongation and curved growth.

**Materials and Methods**

Petals of Shamouti orange and Eureka lemon have been used in the present study but other species of citrus may be used as well. Flowers were collected from the tree when they had attained maximum length, just before opening. The first stage of opening consists of an unfolding of a single petal and this is followed by the opening, within 1 day, of the others. A slight pull will separate the petal from the rest of the flower. The collection of flowers and the separation of the petals should be made just before use. However, it is possible to keep the petals for several hr, if necessary, in a humid chamber at low temperature.

The experimental system used in these studies is somewhat different from that described before (6,12). Into test tubes 14 mm in diameter and 13 mm high we pour 0.5 ml of IAA solution, any other desired solution, or water. Then, while stirring vigorously, 0.5 ml of liquid 3% agar solution containing 6% sucrose are added. The resulting 1 ml of diluted agar-sucrose, well mixed by stirring, immediately gels in the tube. Three petals are implanted in each tube and oriented so that they may freely bend outwards. The tubes are placed in a wooden stand and the whole system is placed in a humid chamber at 18 to 20° for about 24 hr, which is the usual duration of an experiment.

The degree to which the petals are curved can be measured in a way similar to that used for split pea stems (16) by measuring the angle between the tangents to the tip and to the base of the petal (17).

In order to improve the procedure of measurement we devised the protractor seen in figure 1B. In this sort of protractor the sides of the angles do not intersect at one point as usual; instead each side of an angle intersects with the base line at a different point. The procedure of measurement consists of pulling the petal along the base line from left to right until the tangent to the tip of the petal coincides with one of the lines which indicate the angle to be measured. Angles exceeding 180° and even curvatures beyond 360° can be measured easily with this protractor. After the measurement the average degree of curvature is calculated for each group of 3 petals implanted together in the same test tube.

The wheat coleoptile elongation method (13,14) has been used for the bioassay of native auxins and their inhibitors. Lanolin pastes containing Tween 20 were prepared according to Mitchell and Livingston (12).

**Results**

The combined effects of IAA concentrations and different times of exposure on the curvature of citrus petals is shown in figure 2. Citrus petals prove to be sensitive to IAA-concentrations as low as 10^-9 m. Such concentrations of IAA are amongst the lowest effective on plant material (11). The degree of curvature increases proportionally with the logarithm of IAA concentration and no sign of saturation appears even at 10^-5 m IAA.

![Figure 2](image)

**Fig. 2.** Effects of different times of exposure (17.5, 26.0, and 39.0 hr) and IAA molar concentration on curvature of citrus petals. Each point represents the average value obtained from 8 replicates.

The promotive effect is evident for all times of exposure; however, the clearest response under the present conditions is evinced after 26 hr. A shorter exposure is not sufficient in order to develop a full differentiation between treatments, while a longer exposure allows excessive rolling of the controls.

The remarkable response of citrus petals to exogenous IAA raises the question whether the naturally occurring curvature is caused by endogenous auxin. Trying to get some answer to this problem we chose the approach used by Cleland (2), using auxin antagonists as p-chlorophenoxyisobutyric acid (PCIB) to block auxin mediated processes. Increasing amounts of PCIB gradually block the curvature and addition of external IAA partly counteracts the effect of PCIB (fig 3). Table I summarizes 2 additional experiments in which increasing concentrations of IAA gradually overcame the PCIB-induced inhibition and attained curvatures similar to those of the non treated controls. These
Table I. Effects of Increasing Concentrations of IAA on Curvature of Non-treated (Controls) and PCIB-treated Petals

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Control</th>
<th>PCIB 100 μg/l</th>
<th>PCIB 200 μg/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (control)</td>
<td>86 ± 7</td>
<td>46 ± 7</td>
<td>58 ± 11</td>
</tr>
<tr>
<td>IAAs conc (μM)</td>
<td>10^-6</td>
<td>10^-5</td>
<td>10^-4</td>
</tr>
<tr>
<td>0</td>
<td>137 ± 15</td>
<td>134 ± 13</td>
<td>164 ± 5</td>
</tr>
<tr>
<td>10^-6</td>
<td>70 ± 9</td>
<td>103 ± 15</td>
<td>88 ± 10</td>
</tr>
<tr>
<td>10^-5</td>
<td>112 ± 19</td>
<td>114 ± 18</td>
<td>204 ± 18</td>
</tr>
<tr>
<td>10^-4</td>
<td>97 ± 19</td>
<td>112 ± 15</td>
<td>164 ± 16</td>
</tr>
</tbody>
</table>

Fig. 3. Effects of PCIB concentrations, with or without 10^-6 M IAA, on the curvature of citrus petals. Each point represents the average value obtained from 4 replicates. Time of exposure was 24 hr.

Results confirm our expectations that endogenous auxin might be of some importance for the process of curvature.

Estimation of the hormonal balance in petal tissue should also be of interest in this connection. Bioassays of 80% methanol extracts and diffusates into agar (fig 4) show at least 2 zones of auxin activity and only little inhibition. Similar bioassay histograms have already been described for citrus petals by Monselise et al. (13). The activity zone at Rf 0.3 to 0.6 appears in other citrus tissues as well (13) and corresponds roughly to the zone of IAA (7,13), but the second activity zone (Rf 0.75-0.90) appears only in petals and is therefore of special interest. Fractionation of extracts was carried out by various techniques (1,7) but the frontal activity zone could not be detected after fractionation. A considerable amount of the auxin activity remained after solvent partition in the water soluble fraction but all the other fractions also contained promotive zones, usually at Rf 0.4 to 0.6. The mere presence of several auxins in petal extracts does not prove that these compounds play a role in the regulation

Fig. 4. Bioassay histograms of auxins and their inhibitors from petals. A) (Above) An 80% methanol extract. B) (Below) A 24 hr diffusion into agar and re-extraction with 80% methanol. Ascending paper chromatography (Whatman 3MM) was carried out with isopropanol:ammonia:water (80.0:0.1:19.9) as the developing solvent. Each strip contained extract from 1 g fresh material.
of curvature. Nevertheless, the high content of promoters and the small amount of inhibitors might form ideal conditions for the enhanced growth performed by the petals.

There is, however, some difficulty to understand why the petals, so rich in endogenous auxin, respond to additional auxin supplied from outside. The answer might perhaps be that under certain circumstances the endogenous auxin is not available and exogenous auxin replaces it efficiently.

The behavior of implanted petals at different temperatures may have some bearing to this problem. The influence of the temperature on the opening of flowers is known to be very strong (4, 17) and this is true also with citrus petals (table II), but the effect of IAA seems to have an optimal range between 18 to 20°C when the conditions for the curvature of controls are still suboptimal.

Unavailability of the endogenous auxin at the relatively low temperatures of 18 to 20°C might be the reason for the vigorous response to IAA at higher temperatures the petals use their own auxins and exogenous treatments of IAA are far less effective. Additional work is needed, of course, to check this hypothesis.

Table II. The Influence of Different Temperatures on Curvature of $5 \times 10^{-4}$ M IAA Treated Petals and Control Petals

Each number is the average of 4 replicates. Time of exposure was 24 hr.

<table>
<thead>
<tr>
<th>Temp</th>
<th>Degrees of curvature</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-IAA</td>
<td>+IAA</td>
<td>Difference</td>
</tr>
<tr>
<td>8°C</td>
<td>50</td>
<td>70</td>
<td>20</td>
</tr>
<tr>
<td>18°C</td>
<td>210</td>
<td>275</td>
<td>65</td>
</tr>
<tr>
<td>20°C</td>
<td>210</td>
<td>315</td>
<td>105</td>
</tr>
<tr>
<td>25°C</td>
<td>445</td>
<td>475</td>
<td>30</td>
</tr>
</tbody>
</table>

The best studied example of curved growth is the *Avena* coleoptile (16): we tried to study the curvature of citrus petals in the light of the knowledge on the *Avena* coleoptile curvature. The curvature of petals also seems to stem from faster growth of 1 side (4, 8) and differences between the 2 sides might perhaps explain the differential growth performed by the curving petal.

In order to compare physiological properties of the 2 sides of the petal we punched discs with a cork borer (7 mm in diameter) and split the discs by a tangential cut (parallel to the epidermis) with a razor blade. Weight determinations showed that the discs had been satisfactorily halved.

Rather disappointingly, we could not detect major differences between the 2 sides. Peroxidase, catalase, and ascorbic acid, which have often been shown to be connected with growth processes, seem to be more or less equally distributed between the 2 sides. Bioassays showed that both sides of the petal contain considerable amounts of auxin but we have hints that the outer side contains a higher amount of inhibitors. Perhaps the only significant difference is that of dry matter percentage, 14.4% in the outer side against 12.0% in the inner side of the petal, indicating that differential water uptake might play some role in the bending of the petals (8, 15).

Yet, the existence of some difference between the 2 sides of the petal could easily be shown by another type of experiments. Lanolin paste containing either 0.1% PCIB or 0.1% IAA was applied to either the outer or the inner side of petals which were then implanted in agar-sucrose medium as usual. The results (table III) show that application of PCIB was almost equally inhibitive on both sides of the petal, but IAA had a much greater effect when applied to the inner side and this seems to prove that it is the inner side which mainly responds to IAA and causes the curvature. It could be expected that application of auxin to the outer side would inhibit the curvature or even induce a negative curvature. This did not happen, and may be the petals are not capable of performing curvature to the opposite direction. It might also be that certain amounts of IAA diffused laterally to the tissues of the inner side where they caused some curvature. It should also be kept in mind that curvature does not necessarily mean growth of only 1 side of the petal. It only means a faster growth of 1 side and thus the physiological differences between both sides of the petal might be rather small and yet produce curvature.

**Discussion**

The system of citrus petals seems to be the first known example of an auxin controlled petal curvature. It should be noted, however, that auxins have been shown to induce wilting of the perianth in orchids (10, 15). Horie (8) who studied flowers of *Tradescantia reflexa* found that the auxin α-naphthalene acetic acid enhanced fading and senescence of the petals but not the earlier stages of flower opening.

Gibberellin application does not induce curvature of citrus petals (6) though it has some indirect effect, producing longer petals which might contain higher levels of auxin and hence show excess of curvature (3; see their fig 1). Light and darkness
were also without influence on our system (6). It seems therefore that the regulation of the curvature of citrus petals is rather simple, as compared with other curvature responses like those of the *Avena* coleoptile which responds also to light (16) and that of pea tendrils which respond to both light and gibberellin, in addition to auxin (9). Further studies on flower opening will probably show that the regulation of petal curvature differs greatly in different plant families and is not always so simple.

**Acknowledgments**

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**Literature Cited**


