Flowering and Growth Response of Peanut Plants (Arachis hypogaea L. var. Starr) at Two Levels of Relative Humidity

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ABSTRACT

Peanut plants (Arachis hypogaea L. var. Starr) grown under two different relative humidities were used in all experiments. All plants were germinated and grown to flowering in the greenhouse. At this time, one group was moved to a growth room under 95% relative humidity. After 50 days the humidity of the growth room was lowered to 50%. The second group was moved into a growth room at 50% relative humidity and after 50 days the humidity was raised to 95%.

Flowering rates of plants under high humidity were greater than rates of those plants under low humidity. Flowering was stimulated by transfer from low to high humidity, and these plants set the largest percentage of pegs, maintained a high rate of ethylene production by 2-centimeter peg sections, a high growth rate of intact pegs, and they had a higher mean content of gibberellins than plants transferred from a high to a low humidity. The plants in the high to low transfer had the least number of flowers, formed the lowest percentage of pegs, had about 50% less ethylene production by 2-centimeter peg sections, and the peg growth rate declined about 50%.

Maximum ethylene production occurred during initial stages of peg growth (1- to 5-millimeter sections), and gibberellin content was generally higher in these peg sections. Thus, high humidity enhanced flowering, peg formation, and peg growth rate. A causal relationship between these effects of high humidity and the growth regulator status of the developing fruit is indicated.

The peanut peg is a reproductive organ displaying positive geotropism. After fertilization it grows rapidly toward the soil. The peg includes developing embryos of the peanut plant. Once the peg has entered the soil, there are usually two seeds per pod at the tip of the peg. The peg is frequently referred to as a gynophore but this is not strictly correct. Since the peg displays some of the properties of both the root and the shoot as well as being a reproductive organ, its growth hormone status is of interest.

The literature relating humidity to the growth responses and hormone production of peanut plants, or plants in general is very limited. Preliminary studies with peanut plants indicated that humidity influenced flowering and pegging of the plants.

Although ethylene production has been reported for flowers, seeds, roots, tubers, and leaves of many plants (6, 13, 17, 18), data relating ethylene production to the humidity under which the plant tissue was grown has not to our knowledge been reported.

Jacobs (12) first isolated auxins in the distal tip of the peanut peg. He presented evidence that auxin is found in the distal centimeter of the peanut peg but presented no correlations between hormone content and environment.

The influence of environmental conditions on endogenous gibberellins in other plants have been reported by several workers (3, 5, 10). Gibberellins have been shown to be important in the reproductive development of the cucumber (1, 2). Gibberellins have also been isolated from dormant seeds (10) and sunflower roots (20).

This study was planned to characterize the effect of relative humidity on flowering, peg formation, and peg growth of peanut plants. The extent to which humidity affected some plant hormone contents of peg sections, excised at three stages of growth, was also determined.

MATERIALS AND METHODS

Plant Culture. Arachis hypogaea L. var. Starr were used in all experiments. Plants were grown in a mixture of agricultural vermiculite/vermiculite (Terra-Lite) and sand (1:1:1, v/v). Nutrients and water were supplied as a nutrient solution. The plants were grown in the greenhouse until they had begun to flower. At this time, the plants were transferred to growth rooms where day length, temperature, and relative humidity were controlled. The light source was a mixture of Power-Groove fluorescent tubes and 100-w incandescent bulbs separated from the room by a Plexiglas barrier. The light energy 457 mm from the barrier, between 400 and 700 nm, was 6 mw/cm². This was about 200 mm above the top of the plants.

One set of plants was given 14 hr of light, a constant temperature of 30 ± 2°C and a relative humidity of 97 ± 3%. Flowering, peg formation, and peg growth measurements were recorded and peg samples were taken for determination of hormone content. The relative humidity was then lowered to 50 ± 3%. All other factors were kept the same and another set of data collected. Using a second set of plants, the same data was collected; first at the lower humidity and then at the higher humidity. Duplicate sets of data were collected under each condition. The designations for plant age or treatment as defined in Table 1 will be used in all subsequent tables.
Peg Measurement and Sample Collection. Flowering rate (flowers achieving anthesis/10-day interval) on two sets of 93 plants each for an 80-day period was determined. Flowers from the third or higher nodes used to measure peg formation and growth were tagged over a 2- to 5-day period of high flowering. Use of these pegs allowed measurement of growth rates while the pegs were in an aerial position. Sixty of these pegs were randomly selected and daily growth measurements made, starting when the pegs first became visible and continuing for 8 days. All flowers not tagged were counted and removed.

The remainder of the pegs from tagged flowers not used for the growth measurements were used for the analyses described below. As soon as possible after initiation of growth (0.1–0.5 cm in length) a 2-g sample of pegs was randomly selected and harvested. A second 2-g sample (tip 2 cm) was taken 4 days after initiation of growth. The final 2-g sample (tip 2 cm) was taken 4 days later (8 days of growth). The 2-cm tip section of the peg was selected because in preliminary tests this section of the peg was found to produce the most ethylene. The portions of the samples that were not used for ethylene and CO2 measurement were placed in air tight containers, quick frozen in liquid nitrogen, and stored at −20 C.

Growth Regulator and Respiration Measurement. After the pegs were removed from the plants, three replications of six pegs each were randomly selected for ethylene and CO2 measurement. Each peg sample was weighed and placed in a 50-ml Erlenmeyer flask on moistened filter paper. Production of ethylene due to wounding was maximized at about 2.5 hr after excision, then it declined, and was stabilized after 4 hr. The samples were then aerated with air free of ethylene and CO2 and sealed for 2 hr. At the end of the 2-hr incubation period, a 1-ml gas sample was removed and its ethylene content measured according to Ketring and Morgan (15). Ethylene produced by the pegs was identified by its retention time and by its cochromatography with reagent grade ethylene. The gas from peanut pegs was also identified as ethylene by its reaction with cold mercuric perchlorate and recovery by addition of 4 N LiCl (22).

Respired CO2 was assayed by gas chromatography using a thermal conductivity detector and a 6 ft × ½ in. silica gel column. Amounts of ethylene and CO2 were determined from standard curves based on peak areas produced by known amounts of ethylene and CO2.

For IAA and GA analyses, the frozen samples were extracted by a method similar to that of Goldschmidt and Monselise (11). A wheat coleoptile growth bioassay, as described by Bentley (4), was used to determine IAA content of the pegs. A barley endosperm bioassay (9) was performed on the crude gibberellin extract. Hordeum vulgare L. "Era" seeds were used for the endosperm bioassay. The procedure was similar to that of Coombe et al. (9). After incubation with the gibberellin fraction, the induced amylase activity was measured by determining the reducing sugar content with a Nelson-Somogyi type (16) color reaction. The activity of the crude extract was compared with that of known amount of GA.

RESULTS AND DISCUSSION

Flower Production and Peg Formation. It was observed in preliminary studies that a change of environment resulted in an increase of flowering and pegging of peanut plants. Table II shows that plants transferred to the greenhouse (treatment b) had five times as many pegs formed as those remaining under controlled environment (treatment a). At harvest nearly three times as many pegs had reached the soil in treatment b. One major difference was that plants in treatment b received 90 to 95% relative humidity at night (Table II). In contrast, plants in treatment a received a constant lower humidity of 60 ± 10%. The following work was undertaken for more careful study of the effects of humidity on peanut plants.

Figure 1 shows that a peak rate of flower production (2200 flowers/10 days) occurred during the 20- to 30-day period after initiation of flowering for stage I of plants initially receiving high humidity (curve A) (see Table I for definitions of plant stages and sets). The flowering rate was perhaps unusually high, due to the daily removal of flowers early in the flowering period. The extended period of flowering, and the bushy appearance due to the formation of many more laterals as previously noted by Smith (21) and Bolhuis (8), was observed following flower removal. Following the peak in flowering a steady decline occurred (Fig. 1, curve A). Flowering continued to decline when the humidity was lowered, and flowering decreased to about 100 flowers/10 days during stage II of these plants, which were now receiving low humidity.
A decrease in flowering was accompanied by a yellowing and abscission of some leaves. Figure 1 also shows a flower peak of 1200 flowers/10 days at 30 to 40 days after flowering initiation followed by a slight decline during stage I of those plants initially receiving low humidity (curve B). Following the shift to high humidity, however, there was a sharp increase in flowering to a maximum rate of 3400 flowers/10 days at 60 to 70 days after flowering initiation (Fig. 1, curve B). After this peak there was a sharp decline in flowering.

The period of maximum flowering for stage I plants under low humidity (curve B) occurred 10 days later than the flower peak for plants started under high humidity (curve A). During stage I of plants initially under low humidity (set B) there was only about one-half of the flowering rate of plants of comparable age under high humidity (set A). The slight decrease shown in curve B after 40 days of flowering can probably be explained as the start of a decline in flowering as in curve A. However, following the increase in humidity (stage II) there was a striking stimulation of the flowering rate of plant set B. As a result, the total flower number of plant set B, for the 80-day period, exceeded the number of flowers produced by plant set A in the same period. The sharp increase in flowering was accompanied by an observed increase in vegetative growth of the plants. No signs of senescence were noted in the plants until after the final flowering peak under high humidity (stage II, plant set B).

The highest percentage of peg formation (pegs formed/flowers × 100) was 66% (Table III). This was achieved during stage II of plant set B under high humidity. During stage I, humidity did not appear to affect peg formation, which was about the same for either plant set A (56%) or plant set B (54%). However, there were less total flowers for plant set B during stage I. The lowest percentage of peg formation (22%) was for stage II of plant set A. Thus, during early flowering, high humidity enhanced the number of flowers but not pegs formed. However, after an initial peak of flowering under low humidity, a change to high humidity resulted in enhanced flowering and peg formation.

**Peg Growth.** Peg growth (measured by peg elongation) during stage II at low humidity was less than the growth rate of pegs from all other treatments (Fig. 2). Peg growth rates during stage II slowed considerably if the humidity was lowered following high humidity. This slower peg growth corresponded with the decrease in rate of flowering and peg set that was found at stage II under low humidity. The elongation rates of pegs from stage I was not affected by the humidities used in this study.

In an attempt to determine factors that may be related to the enhanced formation of pegs and increased peg growth rate, IAA and GA contents of peg sections were assayed and ethylene and CO₂ production of peg sections were measured.

**Respiration.** CO₂ evolution was measured as an indicator of metabolism of the peg sections. The average production rate of CO₂ was 541 μl/g fresh weight·hr for all samples. There were no correlations between CO₂ production and humidity or stage of peg growth. Therefore, alterations in respiratory metabolism cannot account for the changes in peg growth we observed.

**IAA and GA Content.** The average content of IAA in all of the peg sections from all plants and treatments was 30 ± 20 ng/g fresh wt. An analysis of variance (F test) indicated that there was no effect of plant age, humidity, or stage of growth of the peg on the IAA content of the pegs (95% level of confidence). Jacobs (12) found about 0.25 ng of diffusible IAA per 1.0-cm tip sections of peanut pegs. He indicated that this may be saturating levels of auxin, and that this was not a lim-

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**Table III. Effect of Relative Humidity and Plant Stage on Percentage of Flowers Forming Pegs**

<table>
<thead>
<tr>
<th>Plant Set</th>
<th>Plant Stage</th>
<th>Relative Humidity</th>
<th>Total Flowers Tagged</th>
<th>Pegs Formed From Tagged Flowers</th>
<th>Pegging %</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>I</td>
<td>High</td>
<td>386</td>
<td>216</td>
<td>56%</td>
</tr>
<tr>
<td>B</td>
<td>I</td>
<td>Low</td>
<td>375</td>
<td>202</td>
<td>54%</td>
</tr>
<tr>
<td>A</td>
<td>II</td>
<td>Low</td>
<td>200</td>
<td>42</td>
<td>22%</td>
</tr>
<tr>
<td>B</td>
<td>II</td>
<td>High</td>
<td>488</td>
<td>295</td>
<td>66%</td>
</tr>
</tbody>
</table>

1 All values represent an average of 2 experiments.
GROWTH RESPONSE OF PEANUT PLANTS

iting factor in their growth. Our results tend to support this hypothesis, but further studies are necessary.

GA. Peg sections that were harvested from pegs after 1 day of their growth on the plant had the highest gibberellin content. An analysis of variance (F test) indicated that among the parameters studied, only peg length significantly (95% level of confidence) affected gibberellin content of the pegs.

Ethylene Production. The highest production of ethylene was by pegs harvested after 1 day of growth, and this declined after 8 days of growth during stage I under high humidity (Table IV). These production rates were the highest for their respective growth periods; in all treatments ethylene production was higher for peg sections after 1 day of growth than after 8 days of growth. Thus, ethylene production was highest during the initial stages of peg growth. This is similar to the higher ethylene production found during the early stages of peanut seed germination (i.e., when the hypocotyl-radical is first emerging) (13, 14, 19). Higher gibberellin contents accompanied the increased ethylene production, and both of these were associated with rapid peg growth (Table IV and Fig. 2). Peg formation and peanut seed germination are instances of plant organs that are actively initiating growth. Burg (7), using 14-day-old pea seedlings, found a trend toward increasing ethylene production from the oldest to the youngest tissue.

In relatively young plants (stage I), ethylene production by peg sections after 1 and 8 days of growth was significantly higher when the plants were under high humidity than when they were under low humidity (Table IV). Also, an analysis of variance (F test) of the data indicated that ethylene production by the peg sections was significantly related (95% level of confidence) to plant stage, peg length and relatively humidity.

When the mean ethylene production decreased from 12.42 to 3.49 nl/g fresh weight-hr there was no change in growth rate. However, when the mean ethylene production had declined to 1.93 nl/g fresh weight-hr for peg sections after 1 day of growth, a decrease in peg growth rate had occurred. This may indicate an activation level of ethylene production between about 2 and 3.5 nl/g fresh weight-hr. Ketting and Morgan (15) indicated an enhancement of about 2 to 3 nl of ethylene production per g fresh weight per hr was correlated with at least a 10-fold increase in dormant peanut seed germination.

Analyses for IAA, gibberellins, and ethylene production of peg sections indicated that auxins were present but apparently unaffected by the parameters studied; gibberellin content and ethylene production were highest during initial stages of peg growth and significantly related to these growth stages; ethylene production was affected by plant age, peg length, and humidity in a complex manner. Only ethylene production was significantly affected by relative humidity in this study, and it may be one of the limiting factors in peg formation when peanut plants are grown under low humidity. It is clear that relative humidity influenced flowering, peg formation, and peg growth of peanut plants.

LITERATURE CITED