An Ontogenetic Study of Canavanine Formation in the Fruit of Jack Bean, *Canavalia ensiformis* (L.) DC.¹

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ABSTRACT

An ontogenetic study of canavanine formation in the fruit of jack bean, *Canavalia ensiformis* (L.) DC. was conducted. Evidence was presented to show that the ovary wall is the reservoir for seed canavanine. The testa possesses sufficient canavanine to account for the continued elevation in seed canavanine after the pod senesces. The seed canavanine concentration is not constant inasmuch as the canavanine content per milligram dry weight or soluble protein increases abruptly with seed growth and levels off only with the onset of fruit ripening.

Canavanine, the guanidinoxy structural analogue of arginine, is the preponderant nonprotein amino acid of the jack bean seed, *Canavalia ensiformis* (L.) DC. (7). Studies of canavanine formation in developing jack bean fruit by Williams and Hunt (12) suggested that canavanine is synthesized in the pod and is transported to the maturing seed. In addition, after all detectable canavanine disappeared from the senescing pod, a significant increase in seed canavanine was observed. This led to the suggestion that the seeds themselves may also be capable of canavanine synthesis. Williams and Hunt (12) segregated their fruit samples into arbitrarily assigned groups predicated upon fruit color and seed length. They did not provide data on the relationship between fruit age and canavanine formation.

The results of Johnstone's ontogenetic study of jack bean nitrogen metabolism (3) were principally qualitative; moreover, much of the data could not be confirmed by this author (8). Naylor (6) published an accurate picture of canavanine ontogeny, but his presentation was descriptive.

A detailed ontogenetic investigation was needed to elucidate the quantitative pattern of canavanine formation in both the ovary wall and the seeds. Such an ontogenetic study might also provide information on the jack bean tissue suitable for use in studies of the metabolic pathway of canavanine production.

METHODS AND MATERIALS

Plant Material. Jack bean seeds were soaked in slowly flowing, aerated, distilled water for 20 hr at 25°C. The hydrated seeds were grown in thoroughly washed vermiculite for 10 days. Uniformly sized plants were transplanted into 31-cm diameter clay pots containing Bacclo potting soil. The jack bean plants were grown for 6 months under greenhouse conditions, except for 4 hr of supplemental fluorescent illumination during the final 10 weeks of growth. Every 2 or 3 weeks, the plants were fertilized with 0.1 N Johnson's solution (2).

Approximately 7 weeks after seed planting, the first flowers appeared. The jack bean plants were inspected daily; the newly emerged flowers were tagged and dated. The unfertilized jack bean flower is highly ephemeral; abscission occurs within 48 hr after anthesis. This limited the dating error caused by a flower being overlooked and subsequently tagged. At the indicated intervals, the pods were harvested, washed with distilled water, and stored at -25°C until assayed. Seeds from 11-week-old fruit and older were hydrated overnight prior to assay.

Preparation of the Plant Extract. The jack bean fruit was separated into seed coat, remaining seed, and ovary wall. Each sample was weighed and a portion was set aside for 7 days at 55°C for dry weight determinations. The remaining material was ground exhaustively in a Servall Omni-mixer for 3 to 7 min with 50 to 200 ml of deionized water, depending upon the sample bulk. After expressing the resulting slurry through several layers of cheesecloth, the filtrate was saved, and the cake was carefully collected and reground for 1 to 3 min with 50 ml of deionized water. The slurry from the second grinding was expressed as above; the combined filtrates were clarified by centrifugation at 23,000g for 15 min. When necessary, the supernatant solution was filtered over Whatman No. 541 filter paper and was brought to a final volume of 100 to 250 ml with deionized water.

Triplicate samples of the plant extract were assayed for protein content by the procedure of Lowry et al. (4). The plant extract was then deproteinized and assayed for canavanine as previously described (8).

RESULTS

The formation of canavanine in the developing pod and seed is given in Figure 1. Two weeks after flower fertilization, canavanine can be detected in the maturing pericarp. The canavanine level increases steadily during the first 6 weeks of growth prior to a 4-week period of marked depletion. The appearance of appreciable seed canavanine, however, is delayed. Thus, after 5 weeks of growth only 17% of the matured seed canavanine level is attained, whereas almost 85% of the eventual pod canavanine is present.

Nine weeks after fertilization, the pod begins to senesce. The senescent process is characterized by a pronounced decomposition in pericarp chlorophyll, a severe attenuation in both pod and seed water content (Fig. 2), a completion of most seed growth (Fig. 3), and movement of canavanine into the seed.
Fig. 1. Canavanine content in the pod (•) and seed (○) of maturing jack bean fruit. Sample material, harvested at the indicated time interval, was assayed for canavanine content as described in the text. Each point represents the average of at least six separate determinations with 6 to 18 pods and 39 to 74 seeds and testas. Inset: Canavanine content in the testa of developing jack bean seed.

Fig. 2. Fresh weight of the pod (■) and seed (○) of maturing jack bean fruit. (Fig. 1). Yet, the rapid attenuation in pod canavanine level occurs before pod ripening. As a result, pod canavanine mobilization occurs before the completion of canavanine translocation into the seed. Moreover, once the pod canavanine is utilized, little additional seed canavanine increment occurs (Fig. 1). These results are consistent with the probable role of the ovary wall as a reservoir for seed canavanine.

The maturing seed continues to increase in soluble protein, dry weight, and canavanine content, even after pod senescence is completed (Figs. 1 and 3). This type of observation formed the basis for the Williams and Hunt assertion (12) that the seeds themselves are capable of canavanine production. However, inspection of the data illustrated in the Figure 1 inset reveals that the testa possesses appreciable canavanine content. More importantly, sufficient canavanine exists in the testa to account for the continued elevation in seed canavanine after the pod yellows. Even in the fully matured seed, canavanine can be detected in the testa. Thus, the mere elevation of seed canavanine content subsequent to pod senescence does not establish a canavanine-producing capacity for the maturing seed.

Williams and Hunt (12) also reported that jack bean seeds did not show a significant change in dry weight canavanine concentration in any of the sample classes evaluated. This result, consistent with the findings of Tschiersch (9) with Colutea arborescens, led to the intriguing suggestion of a regulatory mechanism for the maintenance of constant canavanine concentration in the seed. The data of Table I reveal that on either a dry weight or soluble protein basis, the seed canavanine concentration is not constant. Rather, the canavanine content per milligram dry weight or soluble protein increases abruptly with seed growth and levels off only with the onset of fruit ripening. The pod canavanine content per milligram dry weight or soluble protein also varies appreciably (Table I).

Several additional points of interest were noted in Table I. Canavanine Concentration in the Maturing Seed and Pod

<table>
<thead>
<tr>
<th>Time (weeks)</th>
<th>Protein basis (μmoles/mg × 10^4)</th>
<th>Dry wt. basis (μmoles/mg × 10^4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>340 ± 45</td>
<td>34 ± 3</td>
</tr>
<tr>
<td>4</td>
<td>760 ± 30</td>
<td>100 ± 13</td>
</tr>
<tr>
<td>5</td>
<td>870 ± 40</td>
<td>131 ± 16</td>
</tr>
<tr>
<td>6</td>
<td>1120 ± 120</td>
<td>142 ± 21</td>
</tr>
<tr>
<td>7</td>
<td>960 ± 20</td>
<td>160 ± 17</td>
</tr>
<tr>
<td>8</td>
<td>810 ± 60</td>
<td>141 ± 13</td>
</tr>
<tr>
<td>9</td>
<td>650 ± 50</td>
<td>128 ± 11</td>
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<tr>
<td>10</td>
<td>520 ± 30</td>
<td>122 ± 14</td>
</tr>
<tr>
<td>11</td>
<td>510 ± 40</td>
<td>125 ± 16</td>
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<tr>
<td>12</td>
<td>460 ± 55</td>
<td>124 ± 12</td>
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<tr>
<td>13</td>
<td>480 ± 50</td>
<td>133 ± 18</td>
</tr>
<tr>
<td>18</td>
<td>470 ± 60</td>
<td>134 ± 15</td>
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</tbody>
</table>
Fig. 4. Dry weight (△) and protein content (●) of the maturing jack bean pod.

study. As shown in Figure 3, similar growth curves were obtained by dry weight and protein measurement of the seed. Significantly, the soluble protein assay suggested continued seed growth after the 10th week, while the dry weight procedure did not. The pod growth pattern, as assessed by dry weight determination, showed little quantitative change during fructification (Fig. 4). The protein content evaluation, however, reflected much greater pod alteration in the course of growth.

Throughout the fructescent period, the pod water content remained remarkably constant at 80 ± 3% on a weight basis. On the other hand, the water content of the seed varied continually from a peak of 86% at 3 weeks of age to approximately 50% at seed maturity. Finally, the data of Table I showed the canavanine concentration of the 6-week-old pod to be 1.91 μmoles per mg of soluble protein. This tissue contains the greatest canavanine concentration observed in any jack bean material tested, including floral parts.

DISCUSSION

Analyses of maturing pericarps confirm the assertion of several investigators that canavanine is transported from the pod to the developing seed (6, 9, 12). The role of the ovary wall in the actual synthesis of canavanine, however, has not been elucidated. Warren and Hunt (11) demonstrated the fixation of $^{14}$CO$_2$ into canavanine by cultured pericarp discs of Canavalia ensiformis. Feeding studies with $^4$C-glycine resulted in the production of labeled canavanine in excised Colutea arborescens pods (10). On the other hand, Nakatsu et al. (5) failed to find any canavanine in the pods of Canavalia gladiata, although the seeds contain canavanine (1).

Nakatsu et al. (5) proposed that the leaves are the possible site of canavanine synthesis. The newly emerged foliage leaves of Canavalia ensiformis plants contain extremely large amounts of canavanine, but the canavanine may be derived from the aging cotyledons (8). Preliminary findings from canavanine assay of older leaves indicate that the trilolate leaves directly opposite the growing pod contain only one-third of the canavanine occurring in other trilolate leaves. When the trilolate leaves in the proximity of pods older than 6 weeks were assayed, canavanine was no longer detectable in the leaf extract.

Thus, the trilolate leaves and the actively growing pericarp represent two potential sources for enzymes catalyzing canavanine synthesis. Studies are in progress, utilizing potential precursor compounds, to elucidate the site and metabolic reactions of canavanine synthesis.

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LITERATURE CITED