INHERITANCE OF ANTI-A_1 HEMAGGLUTINATING ACTIVITY IN LIMA BEANS, PHASEOLUS LUNATUS*

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Blood group specific hemagglutinins are essential reagents in certain medical and medico-legal procedures and have found application also in anthropology. Until a few years ago hemagglutinins were obtained solely from animals treated and maintained for that purpose or from human donors.

The discovery that certain plants possess naturally-occurring blood-antigen-specific proteins¹ –⁴ revealed additional potential sources of specific blood grouping reagents. These substances can be extracted from the plant parts which contain them, usually seeds, and used in the same manner as agglutinins from animals; they have the advantage of being much more economical to produce and would be quickly available in case of a large-scale emergency. An extract of Dolichos bifloris L is in commercial production as an anti-A_1 agglutinin, and other specific plant agglutinins are known. A brief review of pertinent work on screening plants for agglutinins and a summary of the results of recent studies are presented by Schertz et al.⁷

Although inter- and intra-species differences have been observed in agglutinating activity of plants, there is no published information on the genetics of plant hemagglutinins. The purpose of this study was to investigate the inheritance of anti-A_1 agglutinating activity of lima beans, Phaseolus lunatus. In 1945 Boyd¹ observed that some lima bean seeds contained an agglutinin specific for the A antigen of human blood. The large variation in agglutinating activity among varieties of lima beans suggested the possibility that the formation of these hemagglutinins might be gene-controlled. Some lines apparently contained virtually no agglutinin and other lines possessed a high level of agglutinating activity. Lima beans are well suited for inheritance studies because pure lines with genetic markers exist, and an efficient crossing technique is available.⁹

Materials and Methods.—Two lima bean varieties, Fordhook 242 and Cowey, and one genetic stock, L-121, were used as parents in crosses. They were chosen from among 10 lines‡ on the basis of agglutinating activity and genetic markers. Fordhook 242 was selected as the “low” parent because of its low agglutinating activity and a dominant mottling of the primary leaves. Cowey and L-121 were used as parents with high agglutinating activity, the latter having a dominant purple hypocotyl characteristic.

The crossing technique used was that described by Wester and Jorgensen⁹ in which stigmas are forced out of large unemasculated flower buds and pollen is deposited on the exposed stigmas. To insure that seed produced by accidental selfing was not mistaken for hybrids, only those seeds which gave rise to plants with the specified markers were used to establish F₂ progenies.

Seeds produced by selfing were harvested from each of the three lines and assayed for agglutinating activity. Parents used in crosses between lines were selected from among those whose selfed progenies showed no segregation. Reciprocal
crosses were made between Fordhook 242 and Cowey and between Fordhook 242 and L-121.

Each seed was cut in half and the portion containing the embryo was planted. The remainder of the seed was divided into two parts and each part was assayed separately for agglutinating activity. The samples were numbered in such a way that the identity of duplicate samples (halves of the same cotyledon) was not known to any personnel of the testing laboratory until all results had been reported. The tests of agglutinating activity were performed in the laboratory of W. C. Boyd in the following manner: The samples were ground and extracted overnight with physiological saline solution in the proportion of 1 gm. of seed to 10 ml of solution. One drop of each extract was tested against one drop of a one per cent suspension of normal red blood cells in physiological saline solution. The extract was tested against A₁, A₂, and B red blood cells. The solution was diluted in steps of 10¹ (1:10) and the highest active (agglutinating) dilution was determined for each sample. The final assay value for each seed was based on the activity of the highest member of the pair of samples for a given seed; when the values did not closely correspond, the data for that seed were discarded (37 cases out of a total of 642). The exponent of the highest active dilution plus 1.5 (no reaction with undiluted equals 0.5) was used in the determination of population means. In view of the method of titers used in determining activity, averages and standard deviations were determined on exponents of 10 rather than absolute dilutions.

Only the results obtained with A₁ erythrocytes are reported here; seeds most active against A₁ were generally active against A₂ also, and sometimes even weakly active with B. It is probable that all these reactions are due to the same agglutinin.

Results.—None of the three lima bean lines used, Fordhook 242, Cowey, and L-121 showed segregation for agglutinating activity and thus were judged to be true breeding for this characteristic. Fordhook 242 had a low activity and differed significantly (P<0.01)² from the other two lines. Cowey and L-121 had high agglutinating activity and were not found to differ significantly from each other in this respect.

<table>
<thead>
<tr>
<th>Parents and F₁</th>
<th>Number of Seeds Assayed</th>
<th>Mean Highest Active Dilution As Ratio As Exponent*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parent</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fordhook 242</td>
<td>33</td>
<td>1:0.13</td>
</tr>
<tr>
<td>Cowey</td>
<td>29</td>
<td>1:190</td>
</tr>
<tr>
<td>L-121</td>
<td>27</td>
<td>1:324</td>
</tr>
<tr>
<td>F₁ Cross†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fordhook 242 × Cowey</td>
<td>15</td>
<td>1:158</td>
</tr>
<tr>
<td>Cowey × Fordhook 242</td>
<td>7</td>
<td>1:195</td>
</tr>
<tr>
<td>Fordhook 242 × L-121</td>
<td>31</td>
<td>1:182</td>
</tr>
<tr>
<td>L-121 × Fordhook 242</td>
<td>20</td>
<td>1:316</td>
</tr>
</tbody>
</table>

* ± Standard error of the mean exponent.
† Female parent listed first.

In the cross of Fordhook 242 × Cowey and its reciprocal, 21 F₁s were produced, all of which had a high A₁ agglutinating activity (Table 1). High anti-A₁ activity
was also obtained for each of the 51 seeds resulting from the cross of Fordhook 242 × L-121 and its reciprocal. By mean separation, using standard errors, no statistically significant differences were found between the various F₁ progenies nor between the F₁ progenies and the selfed progenies of the "high" parents.

The F₂ data resulting from assays of 441 seeds are summarized in Table 2. Each of the progenies fits a 3 (high) to 1 (low) ratio with the probability of each of the chi-square values exceeding the 0.01 level.

<table>
<thead>
<tr>
<th>F₁ Progeny* from Cross</th>
<th>Reaction Category</th>
<th>Chi-square</th>
<th>Probability Exceeds</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&quot;High&quot;*</td>
<td>&quot;Low&quot;*</td>
<td>3 High to 1 Low</td>
</tr>
<tr>
<td>Fordhook 242 × Cowey</td>
<td>86</td>
<td>74</td>
<td>31</td>
</tr>
<tr>
<td>Cowey × Fordhook 242</td>
<td>29</td>
<td>88</td>
<td>4</td>
</tr>
<tr>
<td>Fordhook 242 × L-121</td>
<td>155</td>
<td>76</td>
<td>48</td>
</tr>
<tr>
<td>L-121 × Fordhook 242</td>
<td>75</td>
<td>85</td>
<td>13</td>
</tr>
</tbody>
</table>

* Female parent given first.
† Seeds giving extracts active at dilutions of 1:10 or higher.
‡ Seeds giving inactive extracts or extracts active only when undiluted.

There were differences in the segregation of the progenies of reciprocal crosses. When the low parent, Fordhook 242, was used as the female parent in crosses with each of the high parents, the ratio in the F₂ was nearly a perfect 3:1 as is indicated by the percentages in each reaction category and the low chi-square values (Table 2). However, when Cowey, one of the "high" parents, was used as the male in the crosses with the "low" parent, Fordhook 242, the ratio deviated somewhat from 3:1. An even poorer fit was obtained when the other "high" parent, L-121, was used as the female in crosses with Fordhook 242. The F₂ progenies derived from crosses between a "high" female and a "low" male were thus skewed to the high side.

A re-examination of the F₁ progeny data (Table 1) revealed that the mean progeny values of reciprocal crosses were similarly skewed but the differences, though suggestive, were not significant.

**Discussion and Summary.**—The inheritance in lima beans of the agglutinin for human erythrocytes of blood group A₁ was investigated by making crosses between a line with low agglutinating activity (Fordhook 242) and two with high activity (Cowey and L-121). The reciprocal crosses also were made. The F₁ progeny all showed high activity, and the F₂'s resulting from selfing these segregated approximately in the ratio of 3 "highs" to 1 "low," although the ratios in the F₂ progenies from the high female by low male cross were skewed to the "high" side.

These results suggest that the agglutinating activity for A₁ erythrocytes is genetically controlled in the three varieties of lima beans tested, but that the genetic ratios among the offspring are also influenced by other factors. The uniformly high activity of the F₁ progeny and the approximate 3:1 segregation in the F₂ progenies indicate that one locus is involved and that the gene for "high" agglutinating activity is dominant.

When the "high" parent was used as the female, the F₂ generation resulting from selfing the F₁'s showed an excess of "high" progeny, suggesting an extragenic influence on the ratios. There are a number of possible explanations for the skewness
of the ratio to the high side: (1) Ovule or embryo abortion occurred as a result of an effect of the cytoplasm of the "high" parent. (2) Selective fertilization occurred due to an incompatibility associated with the cytoplasm of the "high" parent. (3) Cytoplasmic inheritance of agglutinating activity was manifested in at least some of the homozygous recessive progeny.

The "low" lines, apparently homozygous recessives, were not completely devoid of activity, and the difference between "high" and "low" lines seems to be merely a difference in the concentration of agglutinin present, or alternatively, in the concentration of one or more agglutinin-inhibiting substances. We have not yet attempted to purify the agglutinin from plants of different activities.

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**THE HUMAN CHROMOSOMAL SATELLITES IN NORMAL PERSONS AND IN TWO PATIENTS WITH MARFAN'S SYNDROME***

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In 1958, quantitative characterizations of all the human chromosomes were published in which measurements of total length and centromere position of each member were presented.¹ These measurements were shown to be sufficiently reproducible to permit unequivocal identification of each chromosome (including the sex chromosomes), provided that a sufficiently large number of clearly defined and well separated mitotic figures is available for analysis, a feature characteristic of the methodology employed by us, since it permits indefinite cell multiplication in vitro without change in chromosome constitution.² ³ Two pairs of satellited chromosomes were described and characterized, listed as number 18 and 21 in Figure 1. Subsequently two other laboratories also published quantitative morphologic analyses of the entire human karyotype which agreed with ours