SOME ATTEMPTS TO MODIFY THE GERM PLASM OF *PHASEOLUS VULGARIS*¹

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INTRODUCTION

Several investigators have called attention to various chromatin and other nuclear abnormalities in cells of *Vicia faba* with the application of chloral hydrate. However, the extent to which heritable variations may be produced by such treatment has not been reported as far as the writer could learn. This should be of considerable scientific value. Further experiments, therefore, on attempts to modify the germ plasm with chloral hydrate applications and then to determine to what extent heritable variations may thus be induced is fully warranted.

NĚMEC (1903) calls attention to the occurrence of true heterotypic divisions of treated root tips. SAKAMURA (1920) states that various chromatin abnormalities may be produced and KEMP (1910) points out that any of the following may take place: An arrested movement of the split chromosomes to the opposite poles; the refusion of split chromosomes and the formation of a single large nucleus; double nucleated cells; amoeboid or "bridge" nuclei; and abnormally small or abnormally large nuclei. KEMP also found what seemed to look like heterotypic divisions in plant tissue which ordinarily does not produce germ cells. He states that these are merely the results of some physico-chemical effect of the chloral hydrate and are not true heterotypic divisions. Experiments with this drug are described by HOTTES (1924). He points out the striking effects upon the chromatin content of cells treated with either of these chemicals. His cytological studies revealed some marked departures in

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the migration of the chromosomes and particularly in the arrangement and structure of the chromatin material. Cells passing into the resting stage after having been treated with these drugs showed chromatin more or less irregularly broken up often into more than a dozen separate parts, each one of which rounded off into separate nuclei devoid of spindle fibres. At other times unequal division and subsequent refusion resulted in the formation of either abnormally small or abnormally large nuclei.

DESCRIPTION OF MATERIAL AND METHODS

The investigations reported in this paper were made with a strain of Navy bean, *Phaseolus vulgaris* which had been selected for genetic purity. The Navy bean was chosen, first, because of its cosmopolitan nature and wide adaptability, second, because of its high degree of constant uniformity and its comparative freedom of departures from type, and third, because it is self-fertilized and only rarely, if at all, cross-fertilized. A carefully selected strain of Navy bean was therefore thought to be good material to work with from the standpoint of studying any heritable variations that might be induced through artificial means.

Since HARRIS, et al (1916, 1920) show that abnormalities occur in the Navy bean to the extent of at least one in fifty-seven and that a heritable departure from type has been found, it was, therefore, very essential, before subjecting a strain of the Navy beans to any special treatment, to determine the nature and extent of the departures from type which might occur without treatment. Otherwise the occurrence of a modification might have been credited to a treatment when it should have been attributed to something else.

Although variations were found in the selected strains of the Navy bean used for experimentation by the writer, these variations did not quite correspond to those described by HARRIS and his co-workers. Certain departures from type were observed in plantings made at Nebraska, Kentucky, Indiana, and Illinois, covering a period from 1910 to 1924. Out of 194,654 plants grown to maturity and 18,071 through the seedling stage, only 424 showed deviations from type. The deviations found were, therefore, approximately one in five hundred. This is a much lower percentage of deviates than HARRIS and his associates found. This low percentage found by the writer is perhaps due to the fact that the lines were selected for uniformity. The deviates that were found in this selected strain were either fasciations or showed an opposite leaf insertion instead of the normal alternate arrangement. Others had more than two cotyledons. In some instances, types were found with fasciated seed leaves

which inhibited further growth of the plant. In no case, in the progeny of the different deviates described, were deviates found occurring in greater frequency than in the progeny of normal types. Thus these were not heritable variations.

FIGURE 1.—Number 1, a normal untreated plant. Number 2, a plant treated with .75 percent chloral hydrate solution before the plant commenced to vine. Number 3, a plant treated with .75 percent chloral hydrate solution after the plant had vined.

Various concentrations of chloral hydrate were tried at first to find out the maximum concentration that could be used to produce modification without killing the treated seed or growing plant. Seventy-five hundredths of one percent gave the best results. The solution was applied throughout the entire cycle of development from seed to seed in one of the following

ways: (a) Germination of seeds between blotters kept moistened with chloral hydrate solution and then planted and allowed to grow into maturity with or without further treatment with this drug; (b) like (a) except that chloral hydrate solution was added to the base of the growing plant so long as there was no danger of killing it; (c) application of chloral hydrate solution to the base of growing plants in various stages of development from the time that the seedling emerged from the soil through to the production of seed; and (d) provision for the penetration of the solution through and into the axil of the leaf, by means of a fine thread the opposite ends of which were brought in contact with absorbent cotton kept moist with the chloral hydrate solution.

Seeds germinated in chloral hydrate and allowed to grow without further treatment produced at first abnormal growth. Later, however, normal growth developed without the recurrence of any of the initial modified growth. In the end such a plant had lost all of its former abnormal appearance and became normal in appearance. Only by occasional repeated applications of the chloral hydrate solutions to the base of the plant stem could modified growth be maintained (See figure 1, number 2). Treatment confined to certain parts of the plant caused the portion above the point of application to become modified (See figure 1, number 3). If the plant had been treated after it had made a certain amount of normal growth, all subsequent leaf development showed various degrees of modification until further treatment was again discontinued. Then the new growth was of a normal character. The range of leaf variability caused by chloral hydrate treatment is illustrated in figure 2. A comparison of figure 2 with figure 1, number 1 brings out the contrast between the normal trifoliate and the modified leaf shapes.

By way of summary, it may be stated that the modifications caused by chloral hydrate were as follows: (1) Marked constriction and blunting of the radicle; (2) marked constriction of the hypocotyl, stem and petioles, giving the seedling, the plantlet and plant, if the solution was occasionally applied, a very much dwarfed and constricted appearance; (3) a pale yellowish-green greasy appearance and a blistered mosaic pattern very much like that found in plants infected with virus diseases; (4) fasciation of the stem, petioles and leaves; (5) modification of the characteristic tri-foliate leaf symmetry; and (6) the production of various abnormal leaf shapes and sizes (See figure 2).

INHERITANCE OF MODIFICATIONS

In the first generation of the chloral hydrated progeny, 146 out of 200

plants were aberrant types. Among these, the first leaf whorl was altogether different from the normal trifoliolate of the controls. The nature of the modification of the primordial leaf is shown in figure 3. Instead of the normal trifoliolate leaf type others were found which varied in shape from single or unifoliolate unilobed to five leaved or pentafoliolate types. The pentafoliolate types are illustrated in number 18 of figure 3. Although modifications from the normal trifoliolate type were found in other bean varieties, no modifications, such as have been shown to occur as a result

FIGURE 2.—Types of leaves found after plants were treated with .75 percent chloral hydrate solution. Compare with leaves of untreated plant shown in figure 1, number 1.

of initial chloral hydrate treatments, were in any instance found in the strain of Navy bean from which the seed material for treatment was derived. When compared, the type of aberrancy produced by the first generation progeny of the chloral hydrated parents was found to be entirely different from any type departures observed in 194,645 plants grown to maturity and 18,017 seedlings where no treatment was used. Furthermore, the proportion of aberrants to normals in the progeny of the treated parents was 1.37 to 1 as compared with one abnormal to five hundred normals in the first generation of the untreated plants. These

results indicate that chloral hydrate was an effective means of modifying the parent and of causing a high percentage of new aberrant types to be produced.

The most extreme of the 146 modifications found in the first generation, were grown to maturity and seed was harvested from them to make up a series composed of 200 seedlings for the second generation. Likewise, in each succeeding generation, seed was gathered from the most extreme aberrants to provide for a series composed of 200 seedlings for the next

FIGURE 3.—Aberrant leaves found in first generation descendants of a parent initially modified with chloral hydrate. Number 19 is designated as fasciated, number 18 as pentafoliate, and numbers 13 and 14 as quadrifoliate.

generation. (See figures 4 and 5). The number and percentage of aberrants in 200 such seedlings for each generation is shown in table 1.

A decrease in the number of abnormalities is shown up to the fourth generation when a slight increase is noticed. However, the degree of modification found among the 103 plants recorded in the fourth generation was in the aggregate much less than that found among the 94 third generation plants. Thus from the standpoint of total modifying effect, there was a general falling off of aberrancy in each generation. To determine this, observations were made of the amount of variation carried

FIGURE 4.—Aberrant leaves found in second generation descendants of a parent initially modified by chloral hydrate. This is the first generation of aberrancy.

FIGURE 5.—Aberrant leaves found in the seventh generation descendants of a parent initially modified by chloral hydrate. This is the sixth generation of aberrancy.

over into succeeding generations, when certain types of aberrants were selected as parents (See table 2). Two pentafoolate types 76 A (14) and 84 B (3) produced 67.6 and 41.1 percent of aberrancy respectively. However, although 76 A (14) produced a higher percent of aberrant types than 84 B (3), the latter produced 13.5 percent quadrifoliate aberrants while 76 A (14) produced only 9.6 percent. The progenies of two quadrifoliate and two unifoliate aberrations behaved somewhat similarly in this respect to the pentafoolate types. The two quadrifoliate types produced respectively 64.9 per cent aberrancy with 3.2 percent quadrifoliate, and 70.6 percent aberrancy with 0.0 percent quadrifoliate. The differences between the unifoliate was 1.9 percent aberrancy and 0.0 aberrancy. From these data it would seem that although the same types of modifications produce different types of aberrants in their progeny, the aberrancy from a quantitative standpoint is about the same.

Judging from the decrease of modifications in each succeeding generation, it would appear that the modifications produced as a result of the initial chloral hydrate treatment were not due to any alteration of chromatin matter. This was verified in crosses made by pollinating normal plants with the pollen of plants treated and modified by chloral hydrate. The progeny of such a cross showed no variation from normal plants nor did the backcrosses of this progeny with chloral hydrated plants. Thus the persistency of the modifications into the sixth generation must have been due to some cause other than an alteration of the chromatin material.

TABLE 1
Number and percent of modifications in each generation due to an initial treatment with .75 percent chloral hydrate.

GENERATION Number	NORMALS		ABNORMALS		TOTAL
	Number	Percent	Number	Percent	
1	54	27	146	73	200
2	66	33	134	67	"
3	106	53	94	47	"
4	97	48.5	103	51.5	"
5	184	92	16	8	"
6	192	96	8	4	"
7	200	100	0	0	"

It seems that there are three ways in which the carry-over effect might have been brought about, namely: (1) The mechanical or actual carry-over of some of the chloral hydrate in either the cytoplasm of the egg or in the tissue of the seed or in both; (2) the carry-over effect of an altered seed tissue that had reacted to chloral hydrate while developing

on the treated parent plant, and (3) the carry-over effect of an altered cytoplasm of the egg cell. In view of the fact that seeds germinated in .75 percent chloral hydrate lost their aberrant nature unless treatment was kept up from time to time, the amount of chloral hydrate that could be carried over mechanically would be insufficient to maintain the degree and persistency of aberrancy secured. For this reason it would seem very

TABLE 2

Kinds of aberrancy in the progeny of selected aberrant parent types from grandparents initially modified by chloral hydrate.

PARENTAGE		NUMBER OF SEED- LINGS	CLASSIFICATION OF ABERRANTS										NUMBER OF ABER- RANTS	PER- CENT ABER- RANTS
Type	Label Number		Quadrifoliate		Unifoliate						Miscellaneous			
					Trilobed		Bilobed		Unilobed					
			No.	Percent	No.	Percent	No.	Percent	No.	Percent	No.	Percent		
Pentafo- liate	76A (14)	83	8	9.6	11	14.2	7	8.2	8	9.6	22	2.7	57	67.6
	84B (3)	102	14	13.5	3	2.9	3	2.9	2	1.9	14	13.5	42	41
Quadri- foliate	76A (17)	91	3	3.2	16	17.5	3	3.2	11	12.1	26	28.5	59	64.9
	84B (4)	109	0	0	11	10.1	16	14.6	12	11	37	33.9	76	70.6
Unifoli- ate	76A (21)	104	0	0	0	0	0	0	1	.9	1	.9	2	1.9
	85B (11)	83	0	0	0	0	0	0	0	0	0	0	0	0

unlikely that the seed, much less the very small amount of cytoplasm in the egg cell, could carry over the amount of chloral hydrate that maintains the aberrancy. There is some possibility that the seed tissue while developing on the treated parent may have reacted and that a changed seed tissue was carried over. But such a carry-over could last only one generation. For a more extended effect lasting into several succeeding progenies a more deep-seated reaction must have taken place. Such an extended effect seems most likely to have occurred as a result of an actual modification of the egg cytoplasm and its inclusions with the resultant carry-over from one generation to another of an altered cytoplasm. With time, the altered cytoplasm became gradually restored to its normal state and as

a consequence aberrancy fell off correspondingly. Of the three explanations the last seems the most plausible.

DISCUSSION

It has been shown in the experiments herein described that plant material which had been carefully selected and grown for genetic purity, when treated initially with .75 percent chloral solution, not only produced some very marked modifications, but produced aberrant types which persisted through six generations. Just how the chloral hydrate solution brought about the effects in the parent which caused it to be the progenitor of a very aberrant progeny, is still unaccounted for by the writer. That these aberrancies are not heritable changes has been shown (1) by a diminution of aberrant types in each succeeding generation; (2) because normal plants pollinated by chloral hydrated plants or by aberrant types selected from the progeny of parents that had been treated and modified by chloral hydrate, produced no aberrant types, and (3) because the hybrids of normals $\times$ chloral hydrate modifications back-crossed with chloral hydrate types showed no modifications. The persistence of the aberrant types were, therefore, not due to a change in chromatin material. If this were so, obviously the aberrancy would have been handed down by the generative nucleus of the pollen grain also. This was not the case, and, therefore, the transmission must have been entirely by means of the egg.

In so far as it has been shown that the chromatin material of the male nucleus does not transmit a factor for aberrant leaf shapes, it could be concluded that the chromatin material is not sufficiently altered. Aside from the chromatin material of the egg, the only material left to carry over the effects, is the cytoplasm and of course its inclusions. An explanation of carry-over effects made upon such a basis may, therefore, be considered in this connection.

There are some reasonable arguments to be made in favor of the carry-over of certain properties by means of the cytoplasm in the egg. It is quite possible that the cytoplasm of the egg had been caused to react in a peculiar manner to the effects of the chloral hydrate solution and thus had become altered. An altered cytoplasm of the egg may from then on have been carried over in the subsequent cell divisions. As long as such modified cytoplasm was handed down, aberrancies were produced accordingly.

The part that cytoplasm plays in regard to the transmission and the initiation or cause of certain developmental processes, is brought out by

MORGAN (1924). It has been pointed out by him, that such relations as right and left, anterior and posterior and the spatial parts of the embryo in relation to the early established plans of symmetry and localization of organs along axial lines, seem to be laid down in the beginning of development. Thus the altered symmetry of leaves in the progeny of the bean plants initially modified by treatment with the chloral hydrate solution may be accounted for on the basis that the cytoplasm was altered by a reaction to this solution. MORGAN states further that it is surprising that so few instances have been found that are referable to the cytoplasm since there is an ever increasing body of information relating to cytoplasmic inclusions.

It appears to the writer that these chloral hydrate results have a certain significance in a study of the after effects of exposure to various conditions of disease, insect injuries, toxicity and too abrupt changes in the temperature as well as many other environmental changes. Such conditions could bring on an initial modification of the cytoplasm. The carry-over of a modified cytoplasm would then be the cause of aberrant types even when the initial cause which brought about a change in the cytoplasm is absent. In this way the after effects of certain factors causing the initial modification may persist for several generations. This may be an explanation for the origin and then the gradual disappearance of some of the so-called bud sports and somatic mutations.

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