ON THE OCCURRENCE OF "CRINKLED DWARF" IN *GOSSYPIUM HIRSUTUM* L.

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(With Plates XIII and XIV)

CONTENTS

I. INTRODUCTION

THE Crinkled Dwarf mutant of Sea Island cotton (*G. barbadense*) was described by Harland (1918), and its genetic behaviour was worked out by him and reported in five papers (1918, 1932, 1933, 1935 and 1936). Harland showed that:

(1) Crinkled Dwarf is a single factor mutant behaving as a complete recessive in crosses with its parent strain.

(2) The mutant of Egyptian cotton (*G. barbadense*) known as "Wrinkled Leaf" is identical with Crinkled Dwarf, but in an F_1 with Sea Island normal, dominance was not quite complete and there were slight signs of crinkling.

(3) In crosses between Crinkled Dwarf and various unrelated strains of *G. barbadense* dominance was not complete, traces of crinkling occurring on some F_1 plants.

(4) In interspecific crosses with Upland cotton (*G. hirsutum*) the dominance mechanism was broken down, giving an intermediate F_1 and an unclassifiable series in F_2.

(5) Repeated back-crossing to Upland strains resulted either in the re-establishment of the dominance of normal (back-crosses to Triumph and Virescent Yellow) or in the establishment of a strain in which there was no dominance and the heterozygotes were intermediate between, and readily separable from, both homozygotes (back-crosses to Meade). This

latter result was ascribed to the presence in Meade of a small component of the *G. barbadense* genotype.

(6) In crosses between the original strain of Crinkled and that extracted from the "no dominance" strain (developed by repeated back-crossing to Meade), it was possible to select strains varying in their manifestation of crinkling from pseudo-normal to super-crinkled.

Harland was unable to find any evidence of the occurrence of Crinkled in Upland, and concluded that in that species the Crinkled locus was stable, or at most mutated very much more rarely than in Sea Island. His data clearly gave strong support to Fisher's (1928) theory that dominance is not an attribute of the gene itself, but is the result of the interaction of the heterozygote with the rest of the genotype in which it occurs. The behaviour of the interspecific crosses showed that dominance has been built up in both species, but in entirely different ways. His conclusion that Crinkled does not occur in Upland, however, prevented Harland from accepting Fisher's theory, that in response to recurrent mutation, dominance is built up by the selection of genes which modify the heterozygote in the direction of normality. He suggested that modifiers of dominance are of value to the normal type, and are therefore subject to selection on their own account. The demonstration that it is possible to build up a pseudo-normal, homozygous for the Crinkled gene, is of great importance, but unfortunately it was not accompanied by any investigation of the effects on the heterozygote of the modifiers of the homozygote. From the description of the "no dominance" strain (Harland, 1933), it appears that the removal of dominance factors had the effect of greatly reducing the vigour of the homozygous Crinkled under field conditions, but there is no evidence as to the nature of their effect (if any) on the normal homozygote.

II. ORIGIN AND BEHAVIOUR OF INDORE CRINKLED

The history of Upland cotton (*G. hirsutum*) in Malwa (Central India) has been given elsewhere (Hutchinson & Ghose, 1937). From the Malwa Upland component of the local crop, selections were made in the 1924 season. In 1925 single plant selections were made from the resulting material grown at Indore. For four generations self-fertilization and single plant selection were practised. In 1930 the produce of the best plants from a single plant progeny of the 1929 season was bulked together and sown in Jaipur State (Rajputana) under the name "Indore 1". It was further multiplied there in the two following seasons. There is no Upland component in the local Jaipur crop, and there were no other strains of American cotton (either *G. hirsutum* or *G. barbadense*) under cultivation on the Jaipur farm during these years. In 1933 seed was returned to Indore for use in varietal and agronomic trials.

At flowering time it was noticed that a number of plants were suffering from a malformation very similar to the Crinkled Dwarf of Sea Island. A count in all plots of Indore 1 growing at Indore from Jaipur seed gave the following:

4025 normal : 135 crinkled

or 3·2 per cent crinkled. No other Upland strain gave any crinkled.

Indore 1 single plant selections descended from the progeny from which seed was supplied to Jaipur were maintained at Indore continuously, and in the 1933 progeny rows there were 103 plants, all of which were normal.

The Upland Crinkled was first observed when the plants were full grown, but it will be convenient to describe their appearance from the seedling stage onwards. When grown in the field, seedlings of Indore Crinkled appear normal until the development of about the fourth or fifth leaf. The first evidence of abnormality is usually a slight yellow mottling of the young leaf. Subsequent leaves develop very abnormally. The balance between growth of the main leaf veins and the lamina appears to be upset, and the leaf becomes crinkled and remains small. Growth is very slow, and the plants remain very small and stunted, flowering only rarely, and only very occasionally yielding a ripe boll see (Pl. XIII *d*). When grown under good conditions in pots, the plants develop considerably better, and usually yield a small crop, but the development of the crumpling is the same.

In order to hasten the study of its genetics, Indore Crinkled and the parental type were grown side by side in a greenhouse in the winters of 1933–4, 1934–5 and 1935–6. The normal type grew exceedingly well and the conditions appeared to be optimum for both vegetative growth and reproduction. The Crinkled type usually began to show mottling on the fifth or sixth leaf and crinkling on the leaves immediately following. Crinkling was strongly marked at about the eighth leaf, but was not so severe as under field conditions (see Pl. XIV). Growth proceeded satisfactorily, but not so vigorously as in the normal. Later the crinkling was lost, and by the end of February, when the earlier crinkled leaves had been shed, the plants were quite normal in appearance, and remained so until the bolls were bursting. In mid-June crinkled leaves were again produced, and by the end of June the tops of the plants presented the typical crinkled appearance. Crinkled plants in pots outside the greenhouse were typically crinkled throughout the period. Under greenhouse conditions, Egyptian "Wrinkled Leaf" remained fully crinkled throughout its life history.

The Jaipur crop of Indore 1 and a crop grown under similar conditions from the same seed in Jodhpur were examined in September 1933. Crinkled plants were observed in about the same proportion as at Indore, but the appearance of the plants differed considerably. They were well grown (about equal to normals in height), had cropped fairly satisfactorily, and were very much less crinkled than in the field at Indore. Several plants were observed which showed only slight evidences of crinkling, and would have passed for normal except under careful scrutiny.

III. GENETICS OF INDORE CRINKLED

Crosses were made between Indore Crinkled and four normal Upland strains: the parental strain (Indore 1), another Malwa Upland selection (Indore 25), Buri, and Punjab 289 F. F_1's were completely normal in both field and greenhouse, except in Crinkled × Buri. In the Crinkled × Buri F_1 there were 115 plants in the field, all completely normal, and thirteen in the greenhouse, twelve completely normal and one showing slight crinkling on some leaves.

In F_2 there was clear segregation into normal and Crinkled. Apart from a small percentage of plants suffering badly from red leaf and leaf roll, classification was very easy. No special search was made for traces of crinkling on normal plants, but if any were present they must have been extremely slight. Data for the four crosses are given below.

Cross	No. of F_2 families	Normal	Crinkled	Total	P (3 : 1)
Crinkled × Indore 1	49	1003	310	1313	0·40
Crinkled × Indore 25	10	415	122	537	0·25
Crinkled × Buri	59	4270	1381	5651	0·45
Crinkled × P 289 F	4	148	40	188	0·25
Total	122	5836	1853	7689	0·07
Exp.		5766·75	1922·25		

The data from the four crosses agree well together. In every cross there was a deficiency of the crinkled class below what was to be expected on a single factor theory, but in no case did the deficiency even approach significance. On the total, the deficiency of Crinkled is large enough to be regarded as highly suggestive, though it does not reach the level customarily regarded as significant. In view of the extremely poor performance of Crinkled in the field, the deficiency can be confidently ascribed to low viability.

In four small back-crosses between F_1's and Crinkled, there were obtained:

	Normal	Crinkled	Total
Obs.	42	36	78
Exp.	39	39	78

a close approach to 1 : 1.

Thirty-eight progenies were grown from normal F_2 plants in the Crinkled × Buri cross. Twelve gave normal plants only in F_3, 780 in all, and 26 segregated, giving

	Normal	Crinkled	Total
Obs.	829	271	1100
Exp.	825	275	1100

a very close approach to 3 : 1. Agreement between individual families and the totals was good in both F_2 and F_3. The single factor relationship between Indore Crinkled and normal is therefore satisfactorily established.

In order to establish the identity of Indore Crinkled with the mutants described by Harland, it was crossed with the Egyptian "Wrinkled Leaf". In the field the F_1 plants were strongly crinkled throughout their life history, but grew better than the Indore Crinkled parent. In the greenhouse the F_1 plants followed the same cycle of behaviour as the Upland Crinkled, but the crinkling in the early stages was less marked and more rapidly lost.

An F_2 was grown in the field in 1936. The seedlings were at first normal in appearance. Crinkling began to develop when the plants were about a month old. At 5 weeks old and again at 10 weeks old the F_2 was classified, according to intensity of crinkling, as below:

Date	Normal	Very slight	Slight	Medium	Heavy	Very heavy	Total
31 July	165	155	135	64	46	18	583
2 September	27	44	156	126	107	117	577

In Pl. XIII are reproduced photographs of slight, medium, and very heavy types. In the "very heavy" class were included plants ranging from typical Wrinkled Leaf (Pl. XIII *c*) to typical Indore Crinkled (Pl. XIII *d*) in appearance. It will be seen from the frequency arrays that the intensity of crinkling increased greatly during August. It further increased in later months, but the growth of *barbadense* hybrids in black cotton soil is very poor and almost all plants suffered heavily from leaf roll and jassid attack. No further attempt was made at complete classification, but the behaviour of the normals was watched. On 7 December all save five had shown definite evidence of crinkling.

IV. Discussion

The cross between Indore Crinkled and "Wrinkled Leaf" behaved in the same way as Harland's (1935) cross between Upland Crinkled and Sea Island Crinkled. The few normals that remained at the last count correspond to Harland's pseudo-normals. It may be concluded that Indore Crinkled is due to the same gene as Sea Island Crinkled and Egyptian "Wrinkled Leaf", and that the remaining normals in F_2 were in fact homozygous for the Crinkled gene. In the four crosses within the species *G. hirsutum*, Indore Crinkled behaved as a complete recessive, except for the manifestation of slight crinkling on a single F_1 plant in the Buri cross. Harland's (1933) suggestion that complete dominance may be the normal condition in *G. hirsutum* is thus strongly supported.

The bearing of the genetics of Crinkled Dwarf on Fisher's (1928, 1931) theory of dominance may now be considered. Harland (1936) endeavoured to show that there exist at least three normal alleles which differ in their degree of dominance over Crinkled. His data must be regarded as inconclusive, since the results he presented can be equally easily explained on the behaviour of dominance modifiers under the back-crossing systems which he used. The possible role of normal alleles having a "margin of safety" may be put aside until more critical data are available.

It is clear that the main cause of dominance is groups of modifiers present in the rest of the genotype, and is independent of differences at the Crinkled locus. Harland believes that these modifier groups are part of the normal genetic complement of the species, as developed in response to selection among normals. Fisher considers that they are an addition to the normal genotype resulting from the action of selection on Crinkled heterozygotes. There appears to be no positive evidence in favour of Harland's theory, and it was put forward as an alternative to Fisher's in view of two objections which Harland raised to the theory of selection among heterozygotes. The occurrence of Crinkled as a mutant in an Upland strain removes one of these objections. His other is that the heterozygote in his "no dominance" strain is not, in fact, at a disadvantage against the normal, and therefore there is no basis for Fisher's selective effect. In considering the validity of Harland's estimate of the vigour of the heterozygote, it must be remembered that on Fisher's theory, modifiers improving vigour would be those most intensively selected, those for normal appearance only being selected in so far as they improve survival. Similarly, in the back-crossing series, in the absence of adequate precautions to ensure a random sample of the heterozygous class being used as parents, the selection in favour of vigorous heterozygotes will be maintained, while that for normality will be reversed. The final result, therefore, is probably due more to the selective forces at work during the experiment than to the nature of the Crinkled factor. This interpretation is supported by the fact that the variance of the heterozygous class in bolls per plant is nearly double that of the homozygous normal class (Harland's Table VI, 1933):

Genotype	Variance
Normal	12·77
Heterozygote	19·86

That the intermediate heterozygote is equal in vigour to the normal must therefore be regarded as not proven. Fisher's theory, which is supported by a large and increasing array of evidence on the susceptibility of both heterozygotes and homozygotes to modification by selection (Fisher, 1928, 1931, 1932, 1935; Ford, 1930; Hutchinson, 1931), is of value in the present instance in making predictions concerning the probable reaction to Crinkled of types so far very little studied. The fundamental difference between the dominance complexes of *G. barbadense* and *G. hirsutum* shows that the development of dominance must have taken place since the separation of the species (cf. Fisher, 1931). The partial breakdown of dominance in Sea Island Crinkled × Egyptian normal (Harland, 1932) suggests that the last stages of the evolution of dominance of normal have taken place in the last century, since the separation of Egyptian from the Perennial × Sea Island hybrid population from which it was selected (Balls, 1919). Since Crinkled occurs in both *G. barbadense* and *G. hirsutum* the mutation is probably older than the specific distinction (Fisher, 1928, 1931). On the other hand, the development of dominance of normal may be recent for two reasons. First, Fisher (1928) showed that if the initial viability of the heterozygote was low, improvement would be extremely slow at first, becoming progressively more rapid as the difference in viability between heterozygote and normal decreased. Secondly, the rate of improvement must have been further accelerated in recent times by the change in conditions resulting from modern cultivation methods. The cultivation of the primitive perennial forms of *G. barbadense* by the South American Indians was very crude, and reproduction was usually from a cluster of seeds. Under such conditions there must have been intense competition among seedlings, and the survival value of a crinkled heterozygote intermediate in vigour between normal and homozygous Crinkled would be very low, and its rate of improvement correspondingly slow. Under modern cultivation, with a low seed rate and thinning of seedlings at an early stage, there is a much greater chance of a Crinkled heterozygote becoming established with room enough to develop without serious competition from its neighbours. In addition, the development of the annual habit has increased the rate of improvement measured in time by compressing four or five generations into the period formerly occupied by one. This provides a possible explanation of the comparatively recent development of dominance, which is susceptible of verification. Of the crosses reported by Harland, only that with Trinidad Red Kidney was with a type taken from primitive cultivation conditions, and no back-crosses of heterozygotes to the Kidney parent were made. If the above deduction is true, such back-crosses should result in the breakdown of dominance. Annual types have been cultivated for a much longer time in *G. hirsutum* than in *G. barbadense*, and it might therefore be expected that the improvement of Crinkled would have proceeded further in Upland than in Sea Island or Egyptian. This expectation is strikingly realized in Indore Crinkled. Not only is the dominance of normal complete, but, as predicted by Fisher (1928), the homozygote also has been considerably improved. In pot culture it is considerably less abnormal than Egyptian "Wrinkled Leaf". (Comparison under field conditions at Indore is prevented by the extremely poor development of all Egyptians in black cotton soil.) Under greenhouse conditions it is completely normal for nearly three quarters of the life cycle, and in parts of Rajputana it grew sufficiently well to increase until it comprised 3 per cent of the crop.

In conclusion it may be said that all the available evidence agrees strikingly with expectation on the selection theory, and while not inconsistent with Harland's theory of selection of modifiers on their own account, provides no support for it.

V. SUMMARY

The published accounts of the behaviour and genetics of Crinkled Dwarf in *G. barbadense* are summarized.

The origin and genetic behaviour of a Crinkled in *G. hirsutum* are reported. It is shown that it arose as a mutant at the same locus as Crinkled Dwarf in *G. barbadense*, and behaves as a complete recessive in crosses with normal strains of Upland.

In an F_2 of a cross between Indore Crinkled and the Egyptian "Wrinkled Leaf" (identical with Sea Island Crinkled Dwarf) all grades between fully normal and extreme Crinkled were obtained, an exactly

parallel behaviour to that found by Harland in crosses between Sea Island Crinkled and Crinkled transferred to the Upland genotype.

The bearing of the data on Fisher's theory of the origin of dominance by selection among heterozygotes is discussed, and it is shown that the occurrence of Crinkled by mutation in *G. hirsutum* disposes of one of Harland's main objections to the selection theory. His other objection is that the intermediate heterozygote is not actually at a disadvantage compared with the normal. His data are re-examined, and it is shown that it was to be expected that the vigour of the heterozygote would be maintained by unconscious selection in the back-crossing series.

The available evidence on the history of the mutant and its dominance modifiers is reviewed, and it is concluded that, while the mutation has been recurrent since before the separation of the species, dominance of normal is probably a comparatively recent development. From this it is predicted that dominance should be broken down if Crinkled is transferred from the cultivated annual forms of *G. barbadense* to perennial types of the same species recently brought on to the research farm from the indigenous bush or from the more or less spontaneous shrubs to be found in peasant gardens. Further, it is shown that it is reasonable to expect that the modification of Crinkled will have gone farther in *G. hirsutum*, annual types of which have long been cultivated, than in *G. barbadense*, in which the annual habit is of late origin, and to which field cultivation, with its accompanying low seed rate and seedling thinning, have only recently been applied. It is shown that the behaviour of Indore Crinkled provides very good evidence of the correctness of this expectation. Not only is normal completely dominant, but the Crinkled homozygote has also been considerably improved (as predicted also by Fisher, 1928). Indeed it is practically normal for the greater part of its life cycle under greenhouse conditions at Indore, and only slightly malformed in the field in parts of Rajputana.

It is concluded that the whole of the evidence now available is in close agreement with expectation on Fisher's theory of the origin of dominance by the selective improvement of the heterozygote.

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EXPLANATION OF PLATES XIII AND XIV

PLATE XIII

Plant apices from Indore Crinkled × Wrinkled Leaf F_2.

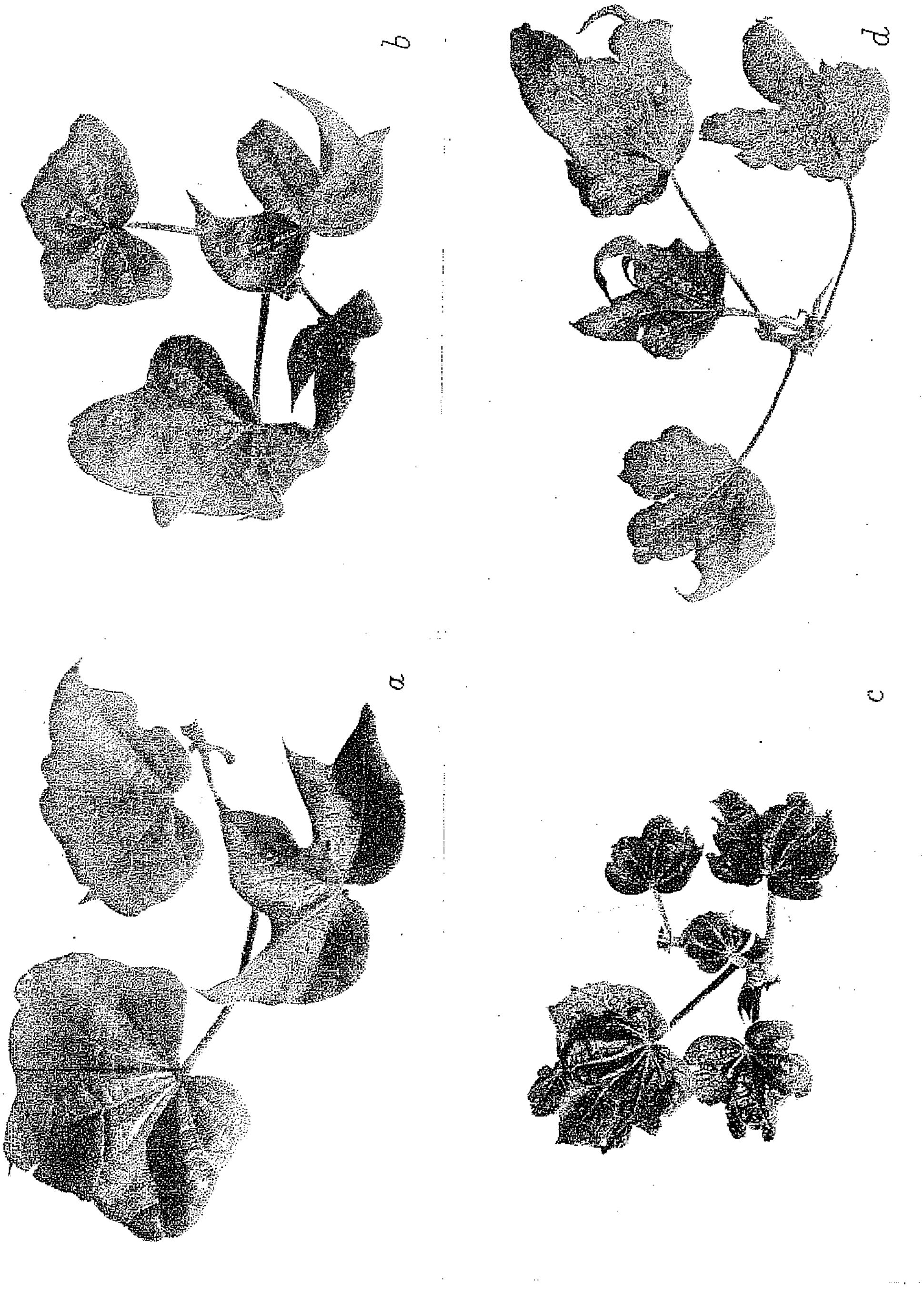
a. Slight.
b. Medium.
c. Very heavy (like Wrinkled Leaf).
d. Very heavy (like Indore Crinkled).

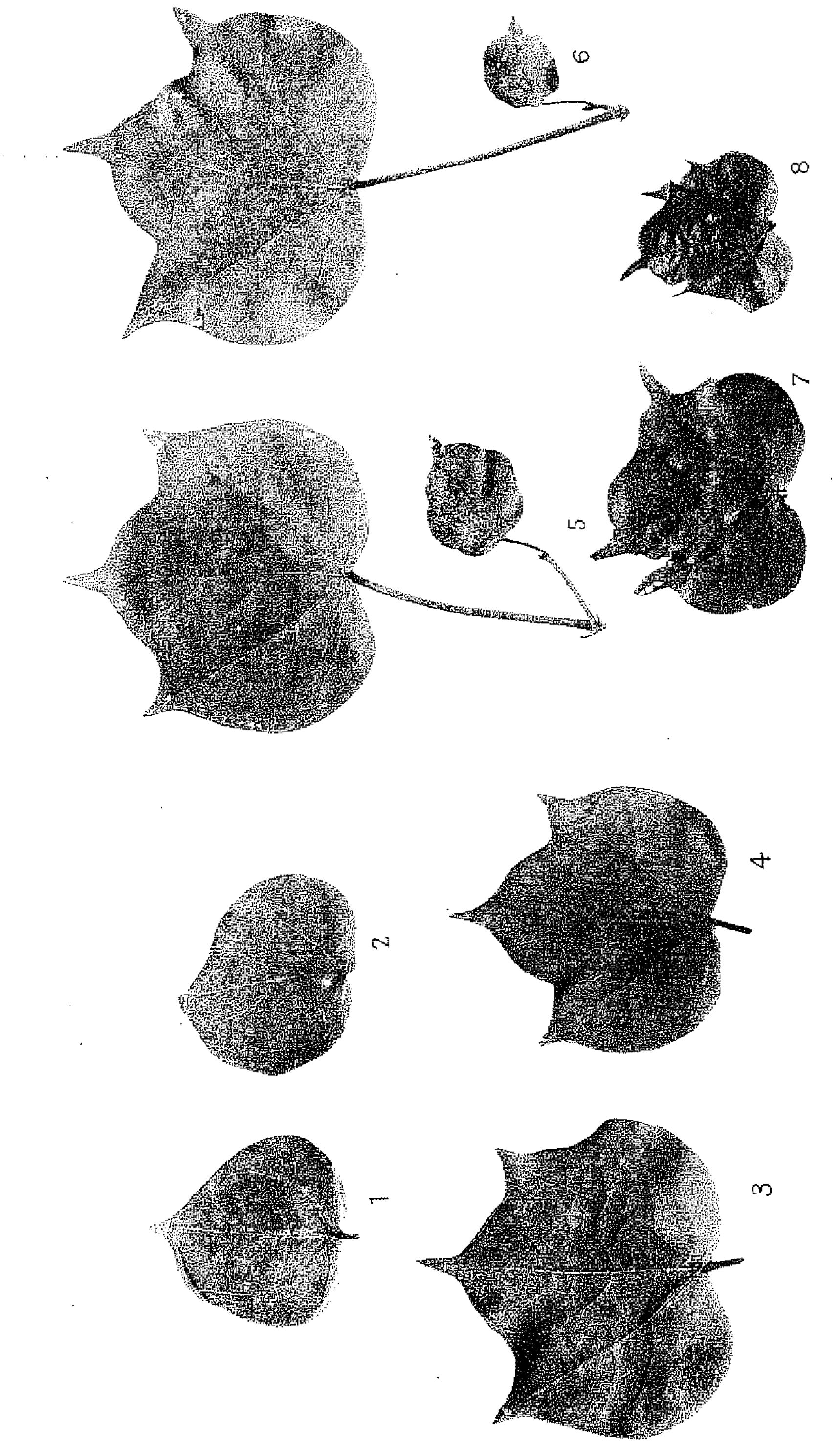
PLATE XIV

Indore Crinkled. First eight leaves of greenhouse-grown plant.

1–4. Fully normal.
5 and 6. Fully normal, but subtending branchlets with crinkled leaves.
7 and 8. Crinkled.

PLATE XIII

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