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Plastid Inheritance in Job's Tears, *Coix lacryma-jobi* L.

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Summary. The yellow striping in Job's tears showed nonchromosomal maternal inheritance of the trait producing green, yellow lethal and striped seedlings in the offspring in widely different ratios whenever the striped plant was used as female parent.

Plastids in yellow regions of the striped plant were of various sizes and colours ranging from normal to minute size and light yellow or pale green to transparent ones. They were even absent in some cells. The plastids in the leaves of the yellow lethals were usually pale green.

While the histological studies indicate the probability that the genetic determinants affecting plastids were in the cytoplasm, the possibility that all the abnormal plastid types could be different expressions of the same original mutation that occurred in the plastids, together with the extensive data on the breeding behaviour of the striped plant, is strongly in favour of the concepts of plastid mutation and autonomy.

Introduction

Extranuclear genes, called plasmagenes, show non-segregation at meiosis and non-Mendelian inheritance in that crosses between contrasting parents give rise to progeny exclusively or predominantly of one parental type. The frequent appearance in many plant species of white, yellow, yellow-green, and striped or variegated seedlings is known to be the consequence of phenotypic changes in the plastids. The pattern of inheritance of plastids has been the subject of considerable study since the beginning of this century because of their relatively large size and the ease with which any gross failures of function can be assessed visually by the absence of chlorophyll. Although many of the chlorophyll mutants that have been described in diverse plant species are known to follow Mendelian inheritance, there are instances of extranuclear extrachromosomal inheritance of chlorophyll mutations in a large number of plants (de Haan, 1933; Jinks, 1964; Williams, 1964; von Wettstein and Eriksson, 1965; Hagemann, 1965; Kirk and Tilney-Bassett, 1967; Walles, 1971; Sager, 1972).

The difference between green and white is in most species (*Mirabilis, Antirrhinum* etc.) inherited through the female parent only: uniparental maternal inheritance (= "status albomaculatus"). In a few taxa there is biparental inheritance of non-Mendelian variegation (= "status paralbomaculatus"), reported first by Baur (1909) in *Pelargonium zonale* and later by others in *Oenothera* and *Hypericum* (see Hagemann 1965). The extrachromosomal variegation was explained by Baur (1909) in that the variegated plants develop from zygotes having two genetically distinct kinds of plastids — green and mutant — the green plastids by multiplication giving rise to green ones, and mutant ones to mutant plastids; the two types are sorted out by somatic segregation during development into different sectors of green and white tissue with some cells having both types of plastid. This hypothesis has received considerable support from Winge (1919), Renner (1934, 1936), Hagemann (1965) and others. However, Correns (1922, 1937), Noack (1932), Rhoades (1946, 1955), Gustafsson and von Wettstein (1957) and Sager and Ryan (1961) considered also the possibility that the genetic determinants of variegation are in the cytoplasm rather than in plastids.

In the present study, the breeding behaviour and plastid phenotypes of a yellow-striped mutant located in Job's tears (*Coix lacryma-jobi* L.), a cereal of potentially great economic importance (Schaaffhausen, 1952), were investigated and the results obtained are reported here.

Materials and Methods

During 1967, one of the plants, raised from seeds originally collected from *C. lacryma-jobi* that ran wild in the University Campus, showed yellow (sometimes white) stripes prominently on leaf sheats, blades and capsular spathes. With the exception of striping, all other characters of this plant were similar to those of the Campus Wild strain from which it arose. The mode of origin of the striped plant in the first instance is not known.

Striped and green plants, or some tillers from each, were crossed reciprocally by enclosing the two parents in large cloth pollination bags, with removal of the male parts in the female parent so that crossed seed were formed in the female parent and selfed seed in the male parent. For selfing, entire plants or a few tillers were enclosed in cloth bags.

Since the striped phenotype could be observed even in young seedlings, large progenies could be raised and scored in germinator trays or seed beds.

Plastid phenotypes in striped and yellow leaves were studied from unstained transverse sections.

Results

The striped plant located had three tillers, of which one was allowed to open-pollinate and two to self-pollinate, the latter also being used as male in crosses with green sibs. Under both open and self-pollinations, the offspring showed green, striped and yellow lethal (which die at the two leaf stage) seedlings in random frequencies (Table 1, items 1 and 2). When striped tillers were used as male in crosses with green sibs, only green seedlings appeared in the progeny (Table 1, item 3). These crosses were repeated using the striped plants in the progeny. Consistently green, striped and yellow lethal seedlings occurred in the progenies in widely different ratios whenever the striped plant was used as female (Table 1, items 6 and 12), while only green seedlings resulted from the reciprocal cross (Table 1, item 3).

The other qualitative characters of the striped plants in the progeny were the same as in the Campus Wild strain, but when crossed reciprocally to green plants belonging to another strain from Anantagiri having contrasting characters (such as green seedling base, white style and hairy leaf compared with the purple seedling base, purple style and glabrous leaf of Campus Wild strain) it produced, in F_1, green, striped and yellow lethal seedlings when the striped parent was used as female (Table 1, item 11), and only green seedlings when it was used as male (Table 1, item 10), while the other characters followed the usual Mendelian segregations in F_2, irrespective of whether the striped parent was used as male or female.

When one of the striped F_1 plants, chosen at random, was used as female in backcrosses to the green parent, the progeny gave green, striped and yellow lethal seedlings. Using a randomly chosen striped plant as female in each of the backcrosses, 4 backcross generations were raised, in each of which characteristically random segregation of green, striped and yellow lethal seedlings occurred (Table 1, item 14). Similarly, when a randomly taken green plant from each of the F_1 and subsequent backcrosses was crossed to a striped plant, using the latter as the recurrent male parent, green offspring occurred in each of 5 successive backcross generations (Table 1, item 13). In spite of the fact that, in a series of 5 backcrosses, nearly 98,4% of genes were transferred from a striped (as male) to a green plant, or 96,9% of genes from a green (as male) to striped plant in another series of

Table 1. *Breeding behaviour of striped plant in Job's tears*

S. No.	Particulars of pollinations		Seedling progeny			Total	Remarks
			Green	Striped	Yellow lethal		
1.	One tiller from striped plant allowed to open pollinate		19	2	10	31	
2.	Two tillers from striped plant self pollinated		45	5	22	72	
3.	Green plant × Striped plant	i)	120	—	—	120	F_1
		ii)	145	—	—	145	
4.	Three green plants, chosen at random from F_1 above, self pollinated	i)	272	—	—	272	F_2
		ii)	166	—	—	166	
		iii)	144	—	—	144	
5.	Three green plants, chosen at random from F_2 above, self pollinated	i)	196	—	—	196	F_3
		ii)	279	—	—	279	
		iii)	174	—	—	174	
6.	Striped plant × Green plant		38	2	14	54	F_1
7.	Striped plant × Green tiller from the same plant		56	4	56	116	
8.	Green tiller × Striped tiller from the same plant		29	—	—	29	
9.	Green tiller from the Striped plant self pollinated		41	—	—	41	
10.	Green plant (Anantagiri strain) × Striped plant (Campus Wild strain)		242	—	—	242	
11.	Striped plant (Campus Wild strain) × Green plant (Anantagiri strain)		91	3	42	136	
12.	Open pollination of several striped plants		348	18	142	508	
13.	F_1 green plant (from item 3 above) × Striped plant as recurrent male parent	i)	188	—	—	188	BC_1
		ii)	147	—	—	147	BC_2
		iii)	122	—	—	122	BC_3
		iv)	127	—	—	127	BC_4
		v)	169	—	—	169	BC_5
14.	F_1 striped plant (from item 6 above) × Green plant as recurrent male parent	i)	145	3	121	269	BC_1
		ii)	184	5	102	291	BC_2
		iii)	68	1	32	101	BC_3
		iv)	241	4	115	360	BC_4

4 backcrosses, the inheritance pattern of the striped character remained unchanged.

Three of the F_1 green plants (from crosses of striped × green), taken at random, when selfed gave a population of all green plants in F_2, and the F_3 progenies raised from three F_2 greens also showed only green seedlings (Table 1, items 3, 4 and 5).

All the striped plants studied cytologically showed normal meiosis with pollen fertility and seed set similar to those of green sibs. Most of the striped individuals, however, were generally weak compared with green plants and some of them even died 4 to 10 weeks after planting.

In general, there appeared to be some relationship between the proportion of yellow lethals obtained in the offspring and the extent of yellow tissue in the striped parent, the greater the extent the higher would be the proportion of yellow lethals.

C. lacryma-jobi being perennial, a few of the striped plants could be maintained in pots for a couple of years. Some of the tillers that came up from these were green; yellow tillers, however, were not observed. These green tillers, on selfing, and when used as female in crosses with the striped tiller in the same plant, gave only green offspring (Table 1, items 9 and 8); when crossed as male to striped tillers in the same plant, the progenies showed the usual three types of seedling (Table 1, item 7).

Histological studies of the striped and yellow leaves were made to determine the plastid phenotypes. In the striped leaves, the green areas showed large green plastids, and in the yellow areas the plastids were of all sizes, ranging from normal to minute ones. The colour of these also varied from light yellow or pale green to a colourless or almost transparent condition. The colourless plastids were less numerous and some of the cells in the yellow area farther removed from the green tissue were completely devoid of plastids. Pale green plastids were as large as the normal chloroplasts, whereas the transparent ones varied from normal to minute size. It was difficult to decide whether any of the cells in the yellow regions adjoining the green sectors had plastids of both types, normal and pale green, as the difference between the two was not always critical. It was rather difficult too to ascertain whether any of the cells containing either green or pale green plastids also contained colourless ones, as some of the latter were so minute that their presence, if any, could easily escape attention. The yellow and green areas on the plant appeared to the naked eye as distinctly demarcated, but in some small areas a clear transition zone, light green in colour, could be seen.

In the leaves of yellow lethal seedlings, cells with apparently normal looking plastids but paler in colour were present. Since it was not possible to critically differentiate between green and pale green plastids, it is not certain whether any green plastids were present in these. Even if present, their percentage must have been quite low. The yellow seedlings having largely pale green plastids die at the two leaf stage, as mentioned earlier, and their inviability apparently is not directly concerned with the content of chlorophyll (see Demerec, 1935) but is dependent on photosynthetic efficiency (see Schötz, 1956).

Hitchcock (1950) mentioned the existence of a garden form of *C. lacryma-jobi* with yellow striped blades which is known to gardeners as variety *aurea zebrina*. It is not known whether the breeding behaviour of the *aurea zebrina* striping would be similar to that in the present study.

Discussion

The occurrence of green, striped and yellow lethal seedlings in widely different ratios in the offspring of the striped plant whenever it is used as a seed parent, and non-transmission of the trait through the male parent, indicate that the character is inherited extrachromosomally through the maternal parent. This suggests that ovules borne on the yellow areas of the striped plant give rise to yellow lethal seedlings, those borne on the green areas to green seedlings and those occurring on the adjoining regions of yellow and green tissues produce striped seedlings. Since the plastids or proplastids are located in the cytoplasm and the male gamete contributes no cytoplasm, or very little, in fertilization, the striped character is transmitted through the cytoplasm of the egg. The fact that the green and yellow sectors are restricted to clearly demarcated areas in the plant and some of the basal suckers produced subsequently by the striped plant are green suggests somatic segregation and sorting out of green and yellow cells. Breeding behaviour of striping in the present study can, therefore, be referred to the type "status albomaculatus", uniparental maternal transmission.

The question now is whether the somatic segregation of green and yellow cells is due to the sorting out of green and yellow plastids or some other components of cytoplasm affecting plastid development, that is, whether genetically distinct types of plastids exist or the abnormal plastid types represent degeneration products of the activity of other cytoplasmic components.

Several investigators who studied the albomaculata type of variegation (see Rhoades, 1946; Hagemann, 1965; Kirk and Tilney-Bassett, 1967; Sager, 1972), and also Renner (1934, 1936) working with *Oenothera*, support the conclusion of Baur (1909) that these variegations are due to the segregation of genetically distinct plastids. The critical observation for the acceptance of Baur's hypothesis is of cells containing both normal and mutant plastids. Recently, mixed cells have been reported fairly convincingly in such organisms as *Spirogyra* (see Jinks, 1964), *Nepeta catarea, Antirrhinum majus, Hypericum, Lycopersicon pimpinellifolium, Oenothera* (see Hagemann, 1965), *Epilobium* (see Michaelis, 1959) and in many other cases (see Sager, 1972).

Correns (1922, 1937), however, proposed an alternative hypothesis, that the cytoplasm in embryonic cells of variegated plants exists in an indifferent labile condition which changes fortuitously into a normal state that allows normal development of plastids, or into a diseased condition that prevents normal plastid development. From his studies on *Epilobium*, Michaelis (1959) argued that non-Mendelian genes affecting chloroplast development are not necessarily located within the chloroplast, but may be anywhere outside the nucleus. But others argued that the breeding results and the studies of the patterns of variegation can be interpreted in terms of plastids themselves as the carriers of these genetic determinants. Further, the establishment of the genetic continuity of plastids (see Walles, 1971), and the occurrence of DNA in plastids (see Kirk and Tilney-Bassett, 1967; Walles, 1971; Sager, 1972), suggesting that they are capable of undergoing mutation, are strongly in favour of the latter argument, and indeed the plastid mutation governing the albomaculata variegation cannot easily be denied.

Histological studies in the present investigation did not reveal the presence of mixed cells but the possibility of their occurrence cannot be ruled out. The fact that the plastids in the yellow regions are pale green, colourless, transparent, less numerous, of various sizes and even absent in some cells arouses suspicion that all these abnormal plastids might represent various stages in the process of impaired development or degeneration caused by some cytoplasmic particles, and that the extent of damage to plastids probably depends on the concentration of these particles; the higher the concentration, the greater is the damage, resulting in cells with no plastids at all or with fewer, smaller and colourless, plastids, and lack of these particles might result in cells with normal green plastids. The occurrence of pale green plastids in the area lying between tissues having normal and colourless plastids might be due to the action of metabolites transported across the cell walls from cells with colourless plastids into the otherwise normal cells, impairing the development of plastids there, as in the case of *Pelargonium, Zea* and *Epilobium* (see Hagemann, 1965).

However, all the abnormal plastids met with in this study may be looked upon as different expressions of the same original mutation that occurred in the plastids. This, together with the extensive data obtained on the breeding behaviour of the striped character, is fully in support of the concepts of plastid mutation and autonomy.

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