Theor Appl Genet (1995) 91:915–920

B.-y. Lin · H.-j. Yu

Inheritance of a striped-leaf mutant is associated with the cytoplasmic genome in maize

Received: 15 March 1995 / Accepted: 21 April 1995

Abstract A striped-leaf mutant has been identified in a Taiwanese maize line, Tainan White. The striped area is pale green at the seedling stage, and turns yellow and white at maturity, extending from the leaf blade to the sheath and the internode below the sheath. The mutant does not breed true and following self-pollination, produces three different types of progeny: green, striped and completely affected. The green type permanently loses the mutant character, while the completely affected type is lethal, without any green tissue. Only the striped type transmits the mutant phenotype to progeny, and it has a clonal distribution on the ear. Its phenotype is inherited maternally and its expression is unaffected by the paternal genotype. It cannot be inherited through the paternal parent. This pattern of inheritance, which persists after three cycles of backcrossing with the affected types as the recurrent pistillate parent, suggests that the responsible gene is located in the cytoplamic genome.

Key words *Maize-maternal inheritance* · Cytoplasmic genome · Leaf-striped mutant · Nonchromosomal mutant

Introduction

Many mutations are inherited maternally in maize (Newton et al. 1989), some of which, designated as NCS mutants, condition leaf-striping phenotypes. The principal features of this group is the appearance of striped leaves. The stripes, pale green or yellow or else necrotic striations, extend from the leave blades to the sheath and the internode below the sheath (Shumway and Bauman 1967; Coe 1983; Newton et al. 1989). Kernels are aborted in some mutants and absent in the affected areas of the ear in others (Newton and Coe 1986). The striping phenotype is variable, from the slight form that does not effect plant growth to extreme forms that affect plant height, vigor and yield (Newton and Coe 1986).

Some mutants of this group have been characterized and are associated with rearrangements in the mitochondrial genome. NCS2 and NCS3 are associated with a rearrangement on mitochondrial genes whose functions are not understood (Newton and Coe 1986). NCS5 and NCS6 have a partial deletion, as well as a rearrangement, of the *cox2* gene (Newton et al. 1990; Gu et al. 1993). Two of these mutants have been studied by electron microscopy. Both have abnormal structures of the mitochondria and chloroplasts. NCS2 and NCS6 have mitochondria with less inner membranes and intermembrane space than their normal counterparts. Their chloroplasts contain no starch grains, a smaller amount of thylakoid membranes and mesophyll cells with less grana (Roussell et al. 1991; Gu et al. 1993).

Several other previously studied mutants have striped leaves, but the associated cytoplasmic genes have not been identified. Anderson (1923) described a maternally inherited variant that is different from the NCS variants in that the striped plants produce completely pale-green seedlings in addition to green and striped progeny. This difference was interpreted by Coe (1983) as a possible result of the fact that, for NCS variants, the cell lineages with a completely defective expression fail to produce progeny. Such a difference, however, may also be indicative of a fundamental genetic difference between the two classes of mutants. Demerec (1927) also observed a similar striped variant that generated pure pale-green offspring. Rhoades (1946) isolated a mutant, called *iojap (ij)*, located on chromosome 7, that conditions a striping phenotype in the homozygous state. The striping phenotype so generated is transmitted maternally to progeny even after the nuclear *ij*/*ij* genotype is replaced by either a +/*ij* or a +/+ genotype. Stroup (1970) reported a similar mutant, but the responsible nuclear gene is *cm*, which is located on chromosome 10. While the *iojap*-affected

---

Communicated by D. R. Pring

B.-y. Lin (✉) · H.-j. Yu
Institute of Molecular Biology, National Chung Hsing University, Taichung 402, Taiwan, ROC

chloroplasts contain no ribosomes, the *cm*-affected chloroplasts do (Walbot and Coe 1979; Walbot et al. 1980).

The present paper describes a new striped variant that is maternally inherited. This variant, discovered in a Taiwanese maize, resembles Anderson's pale-green mutant.

Materials and methods

Variant origin

The variant stock originated from a unique plant among a group of over 1000 Tainan White, a local Taiwanese maize race of unknown origin. This race was originally imported from Japan and has been cultivated on the island of Taiwan for over 80 years. It has general characteristics resembling Hickory King, including dent kernels, large grains, white endosperm, small ear-row number (8–10), tall stand and slow maturity (60–75 days). The total SDS-protein gel electrophoretic pattern of this race is closer to Hickory King than any other early local cultivated maizes such as White Flint, Hickory King, Large Yellow, Longfellow and Chiachow (Chang 1993).

Ear-mapping

The germination system employed essentially follows that outlined by Anderson (1923). Briefly, corn kernels were planted in a plastic germination plate with 12 × 16 holes in such a way that the kernel position on the plate corresponded to its position on the ear. The seedling phenotype was recorded 1 week after germination together with its position on the plate. After collecting the data, seedlings were transplanted to the field by pulling them out of the plate without damage to the root system. The same plate was used multiple times.

Results

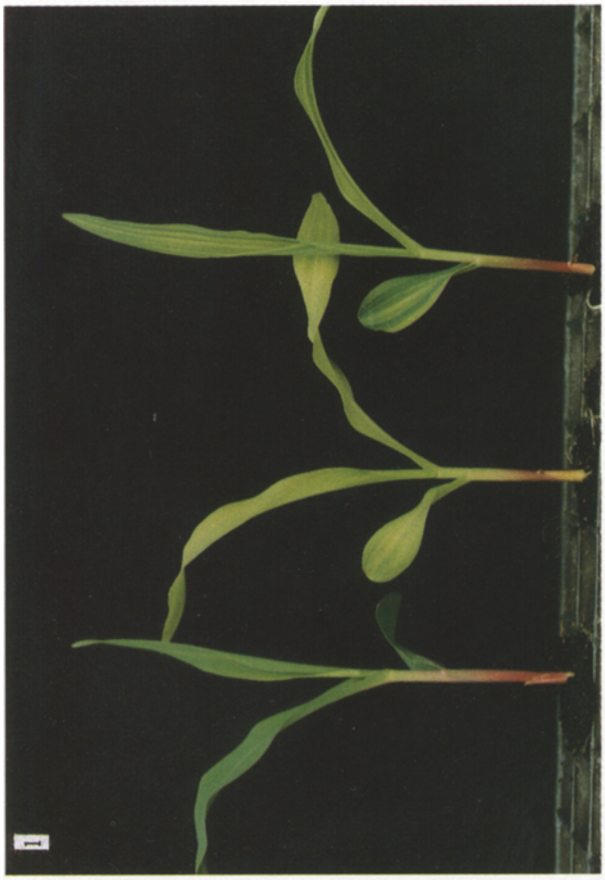
A single initial variant plant, identified in the fall of 1992, and its derived plants, have variable degrees of leaf striping. The affected leaf area is pale green at the seedling stage (Fig. 1) but turns first yellowish and then white at maturity. No necrotic tissue was observed in the affected area. Frequently, a single leaf showed a gradient of coloration, with pale green at the base but yellow at the distal half (Fig. 2). The stripes range in size from fine streaks to an entire half leaf on one side of the midrib. The area affected extends from the leaf blade to the sheath and to the internode below the sheath. In those leaves with only one side of the midrib affected, either with multiple stripes of different sizes or total absence of green tissue, no displacement of the midrib from the leaf center is evident (Fig. 2). Some large striped areas contain small narrow green islands of variable length. Mostly, the border of the stripes with the normal green tissues are sharp, but there is occasionally a gradient of transition from pale green or yellow to green. The extent of leaf areas affected is variable, ranging from under 10% to over 50%. For ease of reference, the affected plants were classified into three groups according to the extent of striped areas: light striping (> 10% of leaf area), medium striping (10–50%) and extreme striping (> 50%). Plants with light and medium striping were viable and produced pollen and relatively healthy ears, while those with extreme striping had substantially reduced vigor and height, and, usually, did not produce any ear at maturity. Irrespective of their leaf phenotypes, ear shoots of the affected plants are normal in size and shape, without any abortive or miniature kernels (data not shown).

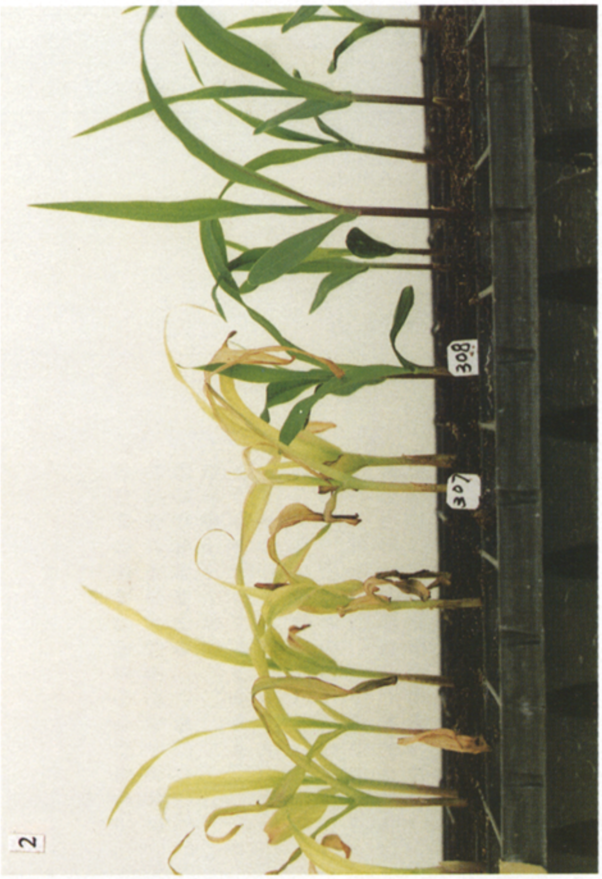
Figs. 1–3 **1** The seedling phenotypes of three different progeny produced by the new cytoplasmic variant. Left, green; middle, completely affected; right, striped. **2** Different leaf-striping phenotypes of eight striped plants. Note that the position of the midrib is at the central portion of the leaf blade. **3** Seedlings of reciprocal crosses between a green and a striped plant (plants no. 1 and 2, Table 2), showing the maternal inheritance of the striped variant. Left (*307*), the striped plant as the pistillate plant; right (*308*), the striped plant as the pollen plant

Additionally, there was an extremely affected group that appeared in the subsequent selfed or outcrossed progeny. These affected plants carried no normal green tissue (Figs. 1 and 2). Their leaves were pale green shortly after germination, turned yellow green at the seedling stage, and then grey before withering. These plants die after the endosperm is consumed (Fig. 2).

The original striped plant was self-pollinated to produce a healthy ear carrying about 200 kernels, a sample of which were germinated to give rise to normal green, striped, and completely affected progeny. Eleven green progenies were selfed and a sample of kernels from each ear (200–300 kernels) was germinated in the green house for seedling classification. All seedlings were green, and 20 of these from each ear were transplanted to the field to be selfed for the production of the second-generation progeny. Nearly all of these (5549 seedlings in total, 300–400 seedlings from each ear) were green, resembling their parents, while 40 were white seedlings from a single ear. The exceptional white characteristic, lethal at the seedling stage, was proven in later generations to be an unrelated recessive chromosomal mutant.

The inheritance of the striped progeny, however, is different. Five medium-striped plants from the original ear were selfed or crossed with the pollen of the F_1 hybrid between W22 and W23 inbreds (or W22/23). One selfed and two outcrossed ears were obtained. Seedlings from the three ears were either green, striped, or completely affected, but the number of each type was not recorded. The medium and extremely striped seedlings were again transplanted to the field and selfed or crossed at maturity. Despite poor field conditions, four ears from medium-striped plants were harvested; three of them segregated green, striped, and completely affected progeny like their parents, with the green progeny being the major class on two ears but the minor class on the last ear (plants no. 1, 2 and 6, Table 1). The fourth ear produced from an extremely striped plant was small, with 14 germinable kernels which turned out to be striped or completely affected (plant no. 7, Table 1). No green seedling was observed on this ear. Some of those striped plants were again selfed and outcrossed in the


1


2

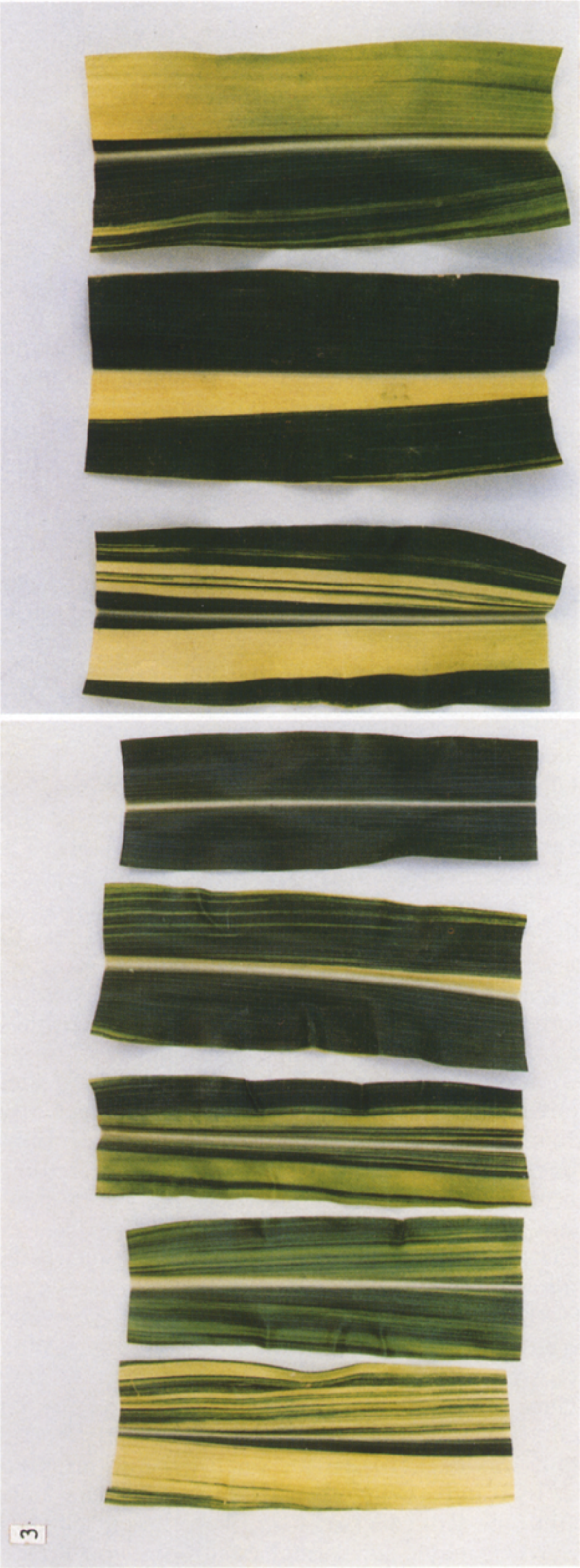

3

Table 1 Progeny of plants with various degrees of leaf striping

Plant no.	Stripe type	Pollen source	Progeny		
			Green	Striped	Completely affected
1	Medium	Self	89	69	38
2	Medium	Self	90	15	4
3	Medium	Self	180	0	0
4	Light	Self	72	68	60
5	Light	Self	0	0	125
6	Medium	B 73	3	8	5
7	Extreme	B 73	0	8	6
8	Medium	B 73	78	0	0
9	Medium	Mo 17	30	50	125

third season, resulting in a number of ears; some of them were germinated for progeny characterization. Two of these were from lightly striped plants; three were from medium, and the last one was from an extremely striped plant. One of the lightly striped plants produced green, striped, and completely affected progeny with the green progeny as the major class (plant no. 4, Table 1), and the other lightly striped plant gave only completely affected progeny without green or striped progeny (plant no. 5, Table 1). Among the three ears from medium-striped parents, only one had green, striped, and completely affected progeny with the completely affected progeny as the major class (plant no. 9, Table 1); the other two ears bore only green progeny (plants no. 3 and 8, Table 1). These data indicate that the parental phenotype bears no apparent relationship with the progeny phenotype; plants with a similar striped phenotype can produce entirely different progeny distributions.

Before each planting, kernels from some of these ears were analyzed by ear-mapping. Three ears showed clear clustering of green and completely affected plants in different areas of the ear, and the striped plants appeared in the merging zone between them. An example of the results of ear mapping is shown in Fig. 4 A, where the green seedlings are clustered at one end of the ear and the completely affected striped seedlings are clustered at the other. The striped seedlings appear between these two classes, indicating a clonal distribution of mutant variants on the ear with the striped and completely affected seedlings resulting from somatic sorting. Not all ear-maps exhibited clonal distribution; one exceptional ear had the striped and the completely affected seedlings mixed with the green seedlings without any clear clonal pattern (Fig. 4 B).

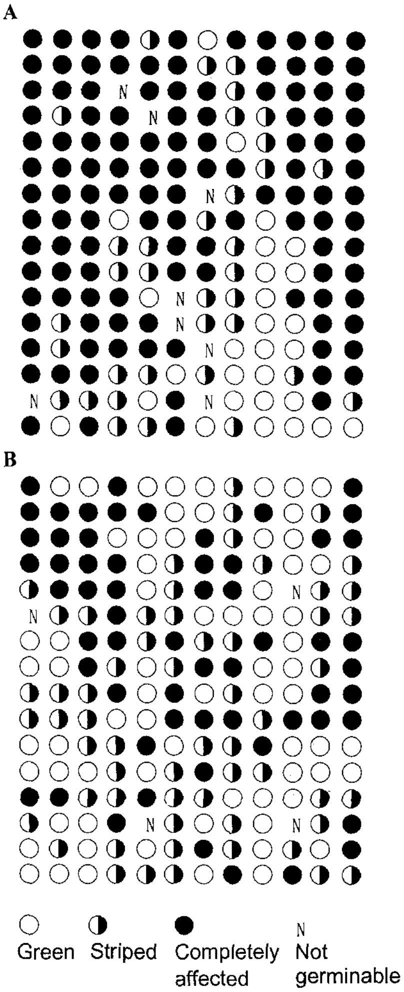


Fig. 4 A, B Ear mapping of two medium striped plants; **A** shows that green and completely affected seedlings clustered in different regions of the ear and striped seedlings are located between these two seedling classes; **B** shows a random distribution of the three seedling classes

The striped and the completely affected characters exhibit maternal inheritance. When outcrossed as females, the striped plants gave striped and completely affected progeny irrespective of the pollen source. When outcrossed as males, however, they gave only green progeny, again irrespective of the source of the pistillate parent. These inheritance patterns persisted after the mutant was backcrossed for three generations, using W22, B73 and Mo17 inbreds as the recurrent pollen parent. One striped plant from the original selfed ear was grown under subnormal conditions and crossed reciprocally with W22/W23. It produced a small ear with only 80 kernels, 73 of which were germinable. Of these, 70 were of the completely affected type with three others of the striped type. There were no green progeny on this ear (Fig. 2; plant no. 1, Table 2). The ear obtained from the reverse cross using the same striped plant as

Table 2 Progeny distribution from reciprocal crosses between green and striped plants

Plant no.	Female parent	Male parent	Stripe type	Progeny		
				Green	Striped	Completely affected
1	Striped-1	W22/23[a]	Medium (female)	0	3	70
2	W22/23	Striped-1	Medium (male)	60	0	0
3	Striped-2	Mo 17	Medium (female)	30	35	123
4	Mo 17	Striped-2	Medium (male)	94	0	0

[a] Hybrid between inbred W22 and inbred 23

male gave a small healthy ear, of which 80 kernels were germinated and all seedlings were green (Fig. 2; plant no. 2, Table 2). In the subsequent plantings, five striped plants were reciprocally crossed with B73 and Mo17. Without exception, the striped and the completely affected traits were transmitted only by the pistillate parent (plants no. 3 and 4, Table 2). The striped phenotype is apparently inherited in a way which suggests that the gene responsible for the character is located in the cytoplasm.

Discussion

This paper reports a character which is transmitted to progeny only by the maternal parent. Three lines of evidences support this conclusion. (1) The character does not breed true. The striped plants produce green, striped, and completely affected progeny whenever it is self-pollinated. The green progeny are genetically stable; they never produced striped or completely affected progeny despite an intensive search for such plants. In other words, the striped character is not a recessive trait. (2) The affected plants are clustered on the ear, suggesting a clonal distribution resulting from a somatic-sorting mechanism. (3) The reciprocal mating of the striped plants with unrelated maize gives different results; the striped and the completely affected progeny appear only when the maternal parent is a striped plant. The progeny produced by the reverse cross and subsequent self-pollination are always green.

The current variant is different from the NCS mutants which are located in the mitochondrial genome (Newton et al. 1989). First, there is no displacement of the central midrib on the affected leaves, even when only one side of the midrib is partially or totally affected (Fig. 3), while NCS mutants have an unexpanded affected leaf area (Coe 1983; Newton et al. 1989). Secondly, the striped ears and kernels are normal (data not shown); there are neither aborted kernels nor an unexpanded affected area on the ear, abnormalities which are frequently associated with NCS mutants (Coe 1983; Newton et al. 1989). Thirdly, there are completely affected plants which carry no green tissues on either leaves or stems; such plants have not been reported to be associated with NCS mutants.

The current variant is close to the variant reported by Anderson (1923). His variant was transmitted to the progeny only by the maternal parent and produced green, striped, and pale-green progeny (Anderson 1923). Anderson did not describe the exact phenotype of the pale-green progeny, but the fact that it is lethal was consistent with the completely affected individuals of the current variant. In addition, the affected area of Anderson's variant did not cause the midrib to displace from the center of the leaf blade. Although Anderson did not state this fact, his picture clearly indicates this to be the case. He also did not report on the effect of his variant on kernel development, while the current variant has no such effect. Given that the Anderson variant is lost, a critical comparison between these two cytoplasmic variants is not possible.

The origin of the current variant is apparently not related to that of Anderson. His variant was identified as a single plant from an outcrossed family of 121 individuals with Sanford's White as the pistillate parent. The current variant was similarly observed as the only plant out of more than 1000 individuals. Its parent is Tainan White, a local maize line cultivated in Taiwan, whose exact origin is impossible to trace (see Materials and methods). The general characteristics of this line somewhat resemble Hickory King which has no apparent pedigree relationship with Sanford's White or Wf9 (Gerdes et al. 1993). The latter has a nuclear genes(s) capable of inducing NCS mutants.

The nature of the cytoplasmic genome that carries the factor responsible for the current variant phenotype has not been determined conclusively at this point. A preliminary light-microscopic observation revealed a reduction of chloroplast number in affected mesophyll cells (Lin et al. 1993). The result of chloroplast extraction is consistent with this observation. A critical electron microscopic and genomic DNA analysis of cytoplasmic organelles will answer this question.

Acknowledgements The authors wish to acknowledge their indebtedness to Dr. L. L. Darrah for his information on the origin of Hickory King, Wf9 and Sanford's White.

References

Anderson EG (1923) Maternal inheritance of chlorophyll in maize. Bot Gaz 76:411–418

Chang Shin-chi (1993) Local white maize – Tainan White. Know You News Lett 157:76–83

Coe EH Jr (1983) Maternally inherited abnormal plant types in maize. Maydica 20:151–157

Demerec M (1927) A second case of maternal inheritance of chlorophyll in maize. Bot Gaz 84:139–155

Gerdes JT, Behr CF, Coors JG, Tracy WF (1993) Compilation of North American maize breeding germplasm. Crop Science Society of America, Madison, Wisconsin, ISBN 0-89118-536-4

Gu J, Miles D, Newton KJ (1993) Analysis of leaf sectors in the NCS6 mitochondrial mutant of maize. Plant Cell 5:963–971

Lin B-Y, Yu H-J (1993) A new type of non-chromosomal stripe from Taiwanese maize. Maize Genet. News Lett. 68:100–101

Newton KJ, Coe EH Jr (1986) Mitochondrial DNA changes in abnormal growth (nonchromosomal stripe) mutants in maize. Proc Natl Acad Sci USA 83:7363–7366

Newton KJ, Coe EH Jr Gabay-Laughnan S, Laughnan JR (1989) Abnormal growth phenotypes and mitochondrial mutations in maize. Maydica 34:291–296

Newton KJ, Knudsen C, Gabay-Laughnan S, Laughnan JR (1990) An abnormal growth mutant in maize has a defective mitochondrial cytochrome oxidase gene. Plant Cell 2: 107–113

Rhoades MM (1946) Plastid mutation. Cold Spring Harbor Symp Quant Biol 11:202–207

Roussell DL, Thompson DL, Pallardy SG, Miles D, Newton KJ (1991) Chloroplast structure and function is altered in the NCS2 maize mitochondrial mutant. Plant Physiol 96:232–238

Shumway LE, Bauman LF (1967) Nonchromosomal stripe of maize. Genetics 55:33–38

Stroup D (1970) Genic induction and maternal transmission of variegation in *Zea mays* J. Hered. 61:139–141

Walbot V, Coe EH Jr (1979) Nuclear gene *iojap* conditions a programmed change to ribosome-less plastids in *Zea mays*. Proc Natl Acad Sci USA 76:2760–2764

Walbot V, Thompson D, Veith GM, Coe EH Jr (1980) Nuclear genes controlling chloroplast development. In: Leaver CJ (ed). Genome organization and expression in plants. Plenum Publishing Corp, New York, pp 381–399