NONCHROMOSOMAL STRIPE OF MAIZE[1]

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EVIDENCE that nonchromosomal inheritance is important in total heredity has been accumulating with increasing frequency in recent years. Comprehensive literature reviews are available in CASPARI (1948), GIBOR and GRANICK (1964) and JINKS (1964).

Cytochemical, chemical and electron miscroscope studies have shown that chloroplasts and mitochondria contain DNA (KISLEV, SWIFT and BOGORAD 1965; GIBOR and GRANICK 1965; NASS and NASS 1963). These studies, when combined with investigations of mode of inheritance, indicate that some cell organelles exhibit partial self control in inheritance and development and do not arise *de novo*. Nonchromosomal inheritance in maize was first reported in 1923 (ANDERSON 1923). In 1946, RHOADES reported that four instances of nonchromosomally inherited chlorophyll variegation had been described in maize.

This paper presents studies on a phenotype of maize that is characterized by longitudinal light-green striping of the leaves and reduced plant height and vigor. The name nonchromosomal stripe (NCS) is proposed. The NCS phenotype, first observed by DR. MERLE T. JENKINS in 1959, has been studied by the authors for nine generations. These studies included mode of inheritance, mesocotyl grafting, heat treatment and oxygen uptake.

The NCS phenotype ranges in expression from very small plants 18 inches or less in height at maturity to plants normal in height and with various degrees of leaf striping. There is no close relationship between amount of striping and plant vigor. Plants may be small with little striping or nearly normal size with considerable striping.

MATERIALS AND METHODS

The NCS phenotype was studied in the maize (*Zea mays* L.) inbreds H49T, Wf9T, Wf9, H49 and in crosses involving them. The mode of inheritance was determined by studying progeny from self-pollination, reciprocal crosses, and backcrosses, and by use of ear maps. Numerical ratings (1 to 5 with 5 the most abnormal) were given to the plants on the basis of degree of expression of the NCS phenotype to study variation within and between generations. During the years 1962 to 1964, covering five of the nine generations studied, at least 200 ears and 10,000 plants with NCS parentage were observed.

Grafts were formed by removing tissue slices from mesocotyls of 4 to 5 day-old dark-grown plants and tieing the cut surfaces of two mesocotyls together after the manner of KATSUMA (1964).

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We have found that P^{32} was transmitted from the roots of one member of the graft pair to the leaves of the other. After grafting seedlings from NCS plants with seedlings of inbreds Wf9, Mo11225, B8 or B14 the pairs were transplanted to the field and grown to maturity. The initial graft generation as well as the two subsequent generations were observed for appearance of the NCS phenotype.

Tests for heat inactivation of the factor responsible for the NCS phenotype were conducted by germinating 1,000 seeds from abnormal plants at 25°C for 24 hr and then immersing them in water at 57°C for 15 min. Hot water treatment is known to "cure" virus infections in several plants (KASSANIS 1957). Immediately following treatment these seeds and controls were planted in the field.

The reduced growth usually associated with leaf striping was observed also during germination. The possibility that respiration was impaired was tested by measuring the oxygen uptake of leaf tissue and germinating seeds of normal and NCS plants. Plants from tested kernels were grown to maturity in the greenhouse and the phenotype observed to determine if oxygen uptake of a seed was related to the subsequent phenotype. Oxygen uptake was measured in Warburg flasks in a constant temperature bath at 30°C. Leaf tissue giving a net weight of 0.7 to 1.0 g was used. One seed, germinated at 21°C for 24 hr, was used per flask. Calculations of oxygen uptake were based on dry weight of leaves and fresh weight of seeds.

RESULTS

Mode of inheritance: The NCS phenotype is inherited in a manner indicating that the causal factor is not on the chromosomes (Table 1). Thirty-one ears from NCS plants that had been self-pollinated gave 63% NCS plants, 90 ears from NCS plants that had been used as pistillate parents in outcrosses produced 54% NCS plants. When NCS plants were used as pollen parents in crosses, the NCS phenotype did not appear in the F_1, F_2, or F_3 progeny, indicating that the causal factor is not transmitted by pollen. Normal meiotic figures and normal pollen rule out the chance that NCS is due to a chromosome aberration inherited only through the egg.

To test the possibility that NCS plants with the most abnormal phenotype would produce the more abnormal progeny, plants of various degrees of NCS expression, but which came from the same ear, were outcrossed with pollen from the same inbred line. In the following generation there was a general relationship between the degree of expression in the pistillate parent and the progeny. This is

TABLE 1

Inheritance of the nonchromosomal stripe phenotype

Number of ears represented	Number of normal plants	Number of NCS plants
Progeny from NCS plants self-pollinated		
31	367	628
Progeny from NCS plants crossed as pistillate parents		
90	2,865	3,318
Progeny from NCS plants crossed as pollen parents		
F_1 30	1,219	0
F_2 32	1,909	0
F_3 1	127	0

illustrated by the observation that 15 ears from "Normal" plants (rating of 1) giving abnormal progeny did so in proportions of 35%, 33%, 15%, 8% and 8% (ratings 1 through 5 respectively). Fifteen ears from plants rated 3 (most abnormal type still able to produce seed) gave progeny in proportions of 23%, 44%, 20%, 9% and 4% (ratings 1 to 5 respectively). However, there were many exceptions and the parental phenotype was not always a good indication of the average type of progeny to be expected. More consistently, it was observed that seed which germinated slowly and had a low percent of germination was the same seed among which the average expression of the NCS phenotype of the survivors was most severe. There were no definite segregation ratios observed for the various degrees of expression of the NCS phenotype. Height measurement showed that each rating class (1 to 5) had very distinct averages but overlapping ranges. One ear for example giving 249 progeny had averages of 154, 129, 91, 69 and 36 cm and ranges 110–170, 95–145, 60–95, 40–70, 25–45 cm for ratings 1 to 5 respectively. This probably indicates that a continuum and not distinct classes is present.

Several aleurone and plant color genes (*B*, *pl*, r^{ch}, bm_4, A_1, A_2, *C*, and *R*) and several genes for leaf variegation (sr_1, zb_4, f_1, and *og*) were crossed into NCS stocks to determine if their expression might be altered in the NCS cytoplasm. However, aleurone and plant colors were normal and the leaves showed a superimposition of the marker and NCS phenotypes rather than an interaction to give a third phenotype.

Although the NCS phenotype was inherited only through the pistillate parent, it was possible that some genotype might alter its expression. To test this, 20 inbred lines and ten different genetic stocks were crossed to NCS plants. There was no modification in expression or frequency of the NCS phenotype in the progeny that could be attributed to the change in genetic background. Furthermore, some NCS lines were outcrossed as pistillate parents up to six times and still the NCS expression remained unaltered in what was largely a different genetic (chromosomal) background.

Normal appearing plants, arising from ears giving NCS plants, are of special interest since the occurrence or nonoccurrence of NCS plants gives some indication of the expressivity and intraplant distribution of the NCS factor. Thirty-four normal appearing plants arising from NCS pistillate parents were either selfed or outcrossed and then progeny tested. About one half (19) of these plants gave only normal progeny while the remainder (15) produced both normal and abnormal progeny. Thus, in some plants the normal phenotype may indicate a loss of the NCS factor, but in other plants the NCS factor was present but not expressed.

While normal appearing plants with NCS background may produce NCS progeny, the reverse was also occasionally true. Eight NCS plants (5 selfed, 3 outcrossed) produced 695 progeny all of which were apparently normal. These eight plants came from seven different families.

This erratic inheritance pattern can be partly explained on the basis of results obtained from ear maps. Ear maps were established by planting seeds in the field

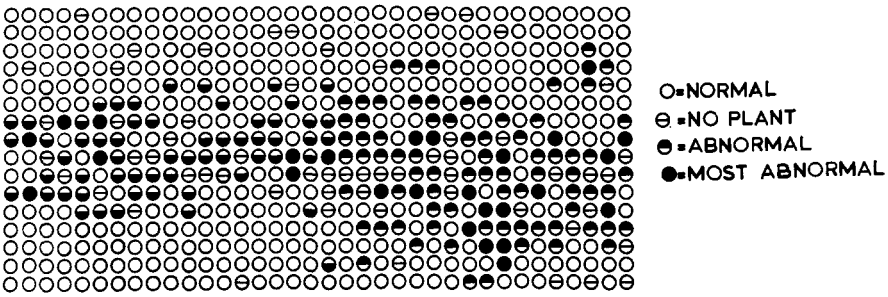

FIGURE 1.—Ear map showing that nonchromosomal stripe plants (abnormal and most abnormal) did not come from random locations on the ear.

in positions relative to that occupied on the ear (ANDERSON 1923). Ear maps for a chromosomal gene mapped simultaneously with NCS show a random distribution of the gene. This is not true for NCS. The NCS plants (abnormal in Figure 1) tend to be grouped on the ear. It was also observed that fewer well developed seeds were present in the areas giving NCS plants. The germination percent for even full kernels in these areas was also less than in areas from the same ear which gave largely normal plants. Of the ten ears mapped, the results shown for one (Figure 1) are typical, except for cases when the progeny were either nearly all normal, or nearly all NCS. In such cases there was a tendency for the minority plant type to come from scattered regions on the ear, and a clearly defined pattern such as shown in Figure 1 was not readily apparent.

Grafting: The results indicate that the factor causing the NCS phenotype is not transmissible by the type of graft used. In the initial graft generation 110 inbred plants grafted with NCS plants were observed. Sixty-eight of these were sib- or self-pollinated, and 1,362 progeny were grown. Six of these second generation plants were self-pollinated, and 181 progeny were grown. There was no evidence for graft transmission of the factor causing the NCS phenotype. Mechanical transmission was unsuccessful. These results indicate that the causal factor may not be a virus.

Heat treatment: Only 32% of the 1,000 seeds treated with hot water germinated, while 87% of the 252 control seeds germinated, but neither the phenotype nor the proportion of NCS plants among the survivors differed from the control.

Oxygen uptake: In each test the oxygen uptake was higher for normal leaf tissue than for NCS leaf tissue, but the differences were not statistically significant at the 5% level. On the average, 21 seeds giving normal plants used 108 μl of oxygen per hour per gram fresh weight, while 21 seeds giving NCS plants used 123 μl. This difference was not significant at the 5% level. The rate of oxygen uptake of a germinating seed was not related to the degree of expression of the NCS phenotype of the plant which developed. Some seeds with rapid oxygen uptake gave normal plants, some also gave NCS plants.

DISCUSSION

Nonrandom ear distribution of seeds giving rise to NCS plants is interpreted to be the result of unequal distribution of the causal factor during mitosis. Thus the eight NCS plants (out of over 120 studied) which produced only normal progeny had presumably developed ears from cell lineages in which the factor was no longer present. That normal plants may come from an ear giving mostly NCS plants suggests that a large number of cell divisions is not necessary to produce a cell which is without the NCS causal factor. This may indicate that there are relatively few factors per cell and that they are particulate. Outcrossed NCS plants tend to have fewer NCS progeny than selfed NCS plant (54% and 63%, respectively). This may be a result of greater vigor in the outcrossed progeny and a resulting higher frequency of NCS factor loss.

The causal factor is not transmitted by the pollen, but is transmitted through the egg. At present not enough is known about pollen transmission to say what, if anything, besides their chromosomes plays a part in zygote formation and development. There is evidence that chloroplasts are transmitted by pollen of Oenothera (RENNER 1936). However, in NCS, plant development was affected at various stages, including seed development and germination, when one would not expect chloroplasts to affect plant growth processes drastically. Some plants which were very retarded in growth were nearly normal green and it was apparent that something besides chloroplasts was affected.

SUMMARY

A new maize phenotype, nonchromosomal stripe (NCS), is inherited in a nonchromosomal manner and is characterized by longitudinal leaf stripes of light-green tissue and reduced vigor. Although the presence of a virus can explain reduced growth, negative results from mechanical and graft transmission and heat treatment tests gave no indication that a virus is present. Nonrandom ear distribution, loss during plant development in some plants, and continuity for nine generations in others indicate that the causal factor is a reproducing, particulate entity which affects several stages of plant growth.

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