affecting chlorophyll synthesis or seedling development. The results obtained are presented in Table 2.

In the table each group of mutants has been subdivided, by means of a chi-square test, into two subgroups, i.e., those fitting a 3:1 ratio, presumably point mutants, and those with a significant shortage of segregating mutants, presumably small intercalary deletions. This subdivision shows that, while both endosperm and seedling mutants are variously distributed among the two subgroups, the majority of chlorophyll mutants fit the 3:1 segregation. Furthermore, as in the case of the mutagenesis at specific loci (Table 1), the stage most sensitive to EMS treatment appears to be the seed, followed by seedling, while later treatments seem to be ineffective.

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## 2. A test of the response of some chlorophyll mutants to different temperature and nutrients.

Mutants with identified blocks in the synthesis of an essential metabolite are very rare in higher plants (cf. Nelson, 1967 and Redei, 1970 for a review). Maize offers a large series of mutants well characterized genetically but not yet investigated from the point of view of their metabolic effect. These mutants, being involved in the control of essential functions like chlorophyll and chloroplast synthesis, plant development, and morphogenesis, represent good material for the analysis of the chain of events linking the gene to its phenotypic effect.

Among all those available, we chose the "chlorophyll mutants", i.e., mutants characterized by a more or less strong reduction in chlorophyll content or by its complete absence. We made a test of the response of these mutants to exogenous sources of nutrients and to different temperature. The test consisted in growing excised mutant embryos under sterile conditions in testtubes containing either mineral or supplemented media at two temperature levels (20 and 30° C) in a growth chamber under continuous light (approximately 300 foot candles). The composition of the mineral medium (M.M.) in mg/l bidistilled water is as follows: $NH_4NO_3$ 600; $MgSO_4 \cdot 7H_2O$ 400; $CaH_4(PO_4)_2 \cdot H_2O$ 400; $KH_2PO_4$ 400; $K_2HPO_4$ 160; $FeC_6H_5O_7 \cdot 3H_2O$ 6.

The "complete medium" (C.M.) was obtained by adding casein hydrolysate, yeast extract, yeast hydrolysate (100, 20, 20 mg/l, respectively) and 4% coconut milk to the mineral medium. Both media were supplemented with 2% saccharose to provide better growth and solidified with 8% agar.

For a quantitative estimate of the response of the mutants to the different growth conditions, their pigment concentration at a given developmental stage (i.e., first two leaves fully extended, the third not yet unfolded) was calculated. Pigments were extracted by grinding leaf material in aqueous 80% (V/V) acetone with a homogenizer. Following centrigugation, pigment concentration was determined spectrophotometrically using the formulas given by Arnon (1949) for chlorophylls and by Wettstein (1957) for carotenoids.

The mutants so far analyzed have the following origin: Albino and luteus were kindly provided by Dr. Robertson, Sienna was from Dr. Anderson, those with a subscript letter have been isolated in our laboratory following E.M.S. treatment of a W22 inbred line and all the others were furnished by Dr. Lambert, Maize Genetics Cooperative. They can be grouped in the two categories of lethals and nonlethals. Results referring to each of these two groups will be presented separately.

Lethal mutants

The following mutants were tested:

$\underline{w}_1$, $\underline{w}_2$, $\underline{w}_3$, $\underline{w}_{7752}$, $\underline{w}_{7748}$, $\underline{w}_{8657}$, $\underline{w}_{8896}$, $\underline{w}_{wisc.2}$, $\underline{w}_{mumml}$, $\underline{w}_{it.1}$, $\underline{w}_{turk}$, $\underline{cl}_1$, $\underline{lw}_1$, $\underline{l}_3$, $\underline{l}_4$, $\underline{l}_{10}$, $\underline{l}_{4120}$, $\underline{l}_{4932}$, $\underline{l}_{42\text{-}4106}$, $\underline{l}_{60\text{-}1106}$, $\underline{l}_{62\text{-}4117}$, $\underline{l}_{blandy\ 1}$, $\underline{l}_{blandy\ 2}$, $\underline{l}_{blandy\ 4}$, $\underline{l}_{brown\ 1}$, $\underline{yd}$, $\underline{l}_a$, $\underline{l}_b$, $\underline{py}_a$, $\underline{py}_d$, $\underline{Sienna}_{7748}$.

They were grown as whole seeds in soil or as excised embryos in testtubes on both mineral and complete medium, in a greenhouse ($t=25\pm 4°$ C). No difference in their pigment content was visible after growth in the two media or in soil except for Sienna. Homozygous Sienna seedlings grown in soil are pale green while those grown in the testtube turn green quickly. In either case, necrotic areas are formed on the leaf tissues as soon as the third leaf unfolds.

Nonlethal mutants

They were grown on both mineral and complete medium at 20 and 30° C. Their pigment content values are reported in Table 1. Each value is the average of three determinations based on the pigment extract of six seedlings.

Table 1

Pigment concentration in mg per gram fresh weight of normal and mutant seedlings grown from excised embryo on mineral and complete medium.

| Mutant | 30°C | | | |
|---|---|---|---|---|
| | C.M. | | M.M. | |
| | chlorophylls | carotenoids | chlorophylls | carotenoids |
| wild type ($v_8^+$) | 1.476 ± .099 | .228 ± .005 | 1.389 ± .086 | .223 ± .004 |
| wild type (W22) | 1.335 ± .061 | .236 ± .007 | 1.461 ± .107 | .245 ± .012 |
| $v_1$ | .806 ± .014 | .111 ± .007 | .819 ± .096 | .108 ± .015 |
| $v_2$ | .928 ± .024 | .215 ± .014 | 1.048 ± .117 | .249 ± .017 |
| $v_4$ | 1.042 ± .069 | .153 ± .010 | 1.019 ± .073 | .149 ± .010 |
| $v_8$ | .712 ± .111 | .133 ± .017 | 1.029 ± .025 | .182 ± .003 |
| $v_{12}$ | 1.095 ± .057 | .222 ± .007 | .920 ± .079 | .193 ± .015 |
| $v_{16}$ | 1.156 ± .094 | .220 ± .007 | 1.116 ± .096 | .282 ± .079 |
| $v_{18}$ | .957 ± .087 | .174 ± .011 | 1.037 ± .054 | .188 ± .016 |
| wt | 1.046 ± .068 | .188 ± .000 | .883 ± .038 | .162 ± .000 |
| $ws_3$ | .545 ± .023 | .104 ± .005 | .532 ± .048 | .093 ± .008 |
| $yg_1$ | .584 ± .032 | .158 ± .007 | .627 ± .049 | .177 ± .005 |
| $py_c$ (W22) | .627 ± .040 | .140 ± .000 | .512 ± .014 | .139 ± .000 |
| $g_1$ (W22) | .672 ± .016 | .172 ± .008 | .635 ± .031 | .140 ± .004 |
| et | .622 ± .037 | .147 ± .006 | .708 ± .004 | .163 ± .004 |
| $pg_{11}pg_{12}$ | .662 ± .002 | .129 ± .005 | .612 ± .000 | .125 ± .005 |

Table 1 (continued)

| Mutant | 20°C | | | |
|---|---|---|---|---|
| | C.M. | | M.M. | |
| | chlorophylls | carotenoids | chlorophylls | carotenoids |
| wild type ($v_8^+$) | 1.745 ± .040 | .302 ± .010 | 2.005 ± .020 | .327 ± .033 |
| wild type (W22) | 1.636 ± .160 | .270 ± .018 | 1.987 ± .209 | .304 ± .027 |
| $v_1$ | .725 ± .011 | .157 ± .000 | .777 ± .043 | .157 ± .002 |
| $v_2$ | .517 ± .005 | .160 ± .007 | .704 ± .046 | .188 ± .004 |
| $v_4$ | .672 ± .051 | .148 ± .006 | .638 ± .051 | .135 ± .011 |
| $v_8$ | .095 ± .027 | .038 ± .010 | .037 ± .013 | .016 ± .003 |
| $v_{12}$ | .551 ± .040 | .189 ± .006 | .536 ± .005 | .184 ± .002 |
| $v_{16}$ | .318 ± .014 | .107 ± .003 | .243 ± .050 | .097 ± .017 |
| $v_{18}$ | .781 ± .020 | .181 ± .005 | .893 ± .081 | .187 ± .014 |
| wt | .963 ± .141 | .226 ± .017 | .895 ± .069 | .222 ± .010 |
| $ws_3$ | .491 ± .044 | .110 ± .008 | .581 ± .032 | .124 ± .001 |
| $yg_1$ | .579 ± .048 | .151 ± .007 | .660 ± .034 | .145 ± .006 |
| $py_c$ (W22) | .445 ± .026 | .133 ± .000 | .250 ± .040 | .093 ± .010 |
| $g_1$ (W22) | .964 ± .125 | .199 ± .008 | .996 ± .072 | .208 ± .011 |
| et | 1.156 ± .074 | .258 ± .013 | 1.026 ± .050 | .246 ± .003 |
| $pg_{11}pg_{12}$ | .670 ± .034 | .209 ± .010 | .743 ± .062 | .191 ± .004 |

In Table 2 the pigment content of the mutant analyzed is expressed as mutant/normal ratio while in Table 3 the chlorophyll a/b ratio is reported.

Table 2

Chlorophyll and carotenoid content of mutant seedlings expressed as mutant to normal ratio.

| Mutant | 30°C | | | | 20°C | | | |
|---|---|---|---|---|---|---|---|---|
| | C.M. | | M.M. | | C.M. | | M.M. | |
| | chlor. | carot. | chlor. | carot. | chlor. | carot. | chlor. | carot. |
| v$_1$ | 0.54 | 0.48 | 0.58 | 0.48 | 0.41 | 0.51 | 0.38 | 0.48 |
| v$_2$ | 0.62 | 0.94 | 0.75 | 1.11 | 0.29 | 0.52 | 0.35 | 0.57 |
| v$_4$ | 0.70 | 0.67 | 0.73 | 0.66 | 0.38 | 0.49 | 0.31 | 0.41 |
| v$_8$ | 0.48 | 0.58 | 0.74 | 0.81 | 0.05 | 0.12 | 0.01 | 0.04 |
| v$_{12}$ | 0.74 | 0.97 | 0.66 | 0.86 | 0.31 | 0.62 | 0.26 | 0.56 |
| v$_{16}$ | 0.78 | 0.96 | 0.80 | 1.26 | 0.18 | 0.35 | 0.12 | 0.29 |
| v$_{18}$ | 0.64 | 0.76 | 0.74 | 0.84 | 0.44 | 0.59 | 0.44 | 0.57 |
| wt | 0.70 | 0.82 | 0.63 | 0.72 | 0.55 | 0.74 | 0.44 | 0.67 |
| ws$_3$ | 0.36 | 0.45 | 0.38 | 0.41 | 0.28 | 0.36 | 0.28 | 0.37 |
| yg$_1$ | 0.39 | 0.69 | 0.45 | 0.79 | 0.33 | 0.50 | 0.29 | 0.44 |
| py$_c$ | 0.46 | 0.59 | 0.35 | 0.56 | 0.27 | 0.49 | 0.12 | 0.30 |
| g$_1$ | 0.50 | 0.72 | 0.43 | 0.57 | 0.58 | 0.73 | 0.50 | 0.68 |
| et | 0.42 | 0.64 | 0.50 | 0.73 | 0.66 | 0.85 | 0.51 | 0.75 |
| pg$_{11}$pg$_{12}$ | 0.44 | 0.56 | 0.44 | 0.56 | 0.38 | 0.69 | 0.37 | 0.58 |

Table 3

Chlorophyll a/b ratio in normal and mutant seedlings grown from excised embryos.

| Mutant | 30°C | | 20°C | |
|---|---|---|---|---|
| | C.M. | M.M. | C.M. | M.M. |
| wild type ($v_8^+$) | 3.82 | 3.82 | 3.62 | 3.69 |
| wild type (W22) | 4.62 | 4.81 | 4.41 | 4.17 |
| $\underline{v}_1$ | 4.48 | 4.46 | 5.61 | 5.77 |
| $\underline{v}_2$ | 3.76 | 3.64 | 3.69 | 3.48 |
| $\underline{v}_4$ | 3.89 | 3.97 | 3.59 | 3.46 |
| $\underline{v}_8$ | 4.03 | 4.27 | 2.59 | 1.53 |
| $\underline{v}_{12}$ | 2.92 | 2.87 | 3.93 | 3.53 |
| $\underline{v}_{16}$ | 4.40 | 4.44 | 3.88 | 3.95 |
| $\underline{v}_{18}$ | 4.46 | 4.34 | 4.76 | 4.48 |
| $\underline{wt}$ | 4.68 | 4.75 | 5.22 | 4.44 |
| $\underline{ws}_3$ | 3.95 | 3.68 | 3.78 | 3.71 |
| $\underline{yg}_1$ | 3.49 | 3.67 | 3.59 | 3.13 |
| $\underline{py}_c$ | 3.90 | 3.57 | 3.66 | 3.75 |
| $\underline{g}_1$ | 4.86 | 3.87 | 4.14 | 4.39 |
| $\underline{et}$ | 5.37 | 4.88 | 4.95 | 4.71 |
| $\underline{pg}_{11}\underline{pg}_{12}$ | 3.59 | 3.56 | 5.44 | 4.71 |

Non mutant (wild type) values are those of green seedlings in the progeny of a selfed $+/\underline{v}_8$ ear or in the W22 inbred line. The latter are used as reference values for mutants in the W22 genetic background, while the former furnish reference values for the other mutants tested.

The results indicate that the majority of the mutants tested are thermosensitive in the sense that they show an increase in chlorophyll and carotenoid concentration when grown at a high temperature (30°C). This increased pigment concentration at 30°C is observed among the $\underline{v}$ series as well as in other mutants ($\underline{yg}_1$, $\underline{wt}_1$ and $\underline{ws}_3$). Even though all these mutants respond to temperature treatment in the same direction, the mutations do

not affect the pigment levels equally. The capacity of recovering at high temperature in fact ranges from a barely visible effect (see $\underline{v}_1$) to a 28-fold increase (see $\underline{v}_8$).

Three mutants are worth considering in more detail. They are:

1. golden ($\underline{g}_1$). Previous studies (Smith et al., 1956) had shown that the $\underline{g}_1$ chlorophyll deficiency is not the result of insufficient production of chlorophyll precursor, but of an increased chlorophyll destruction. The temperature effect here reported seems to indicate that the rate of chlorophyll destruction is significantly reduced at low temperature. Thin layer chromotography of acetone extracts of golden leaves show that they differ from wild type tissues by the absence of a yellow spot. This mutant is presently under investigation.
2. etched (et). Like $\underline{g}_1$, this mutant shows an increased pigment concentration when grown at low temperature. Contrary to $\underline{g}_1$, however, this effect is observable only if et is grown on complete medium. These are the results expected from a thermosensitive auxotrophic mutant. Further experiments are necessary to confirm this interpretation and to establish the nutritional requirements of et.
3. pale yellow ($\underline{py}_c$). At emergence, this mutant has a green coleoptile and the tip of the first leaf is green, while the rest of the leaf tissues remain white. This pattern is repeated in succeeding leaves. Most plants die before reaching maturity. The same phenotype is observed when embryos are grown at 20°C. In this condition, however, the mutant grows slowly till emergence of the third leaf, while at 30°C it grows normally and appears pale green in phenotype.

The low temperature inhibition is released as soon as the mutant is transferred to high temperature. $\underline{py}_c$ is thus a conditional lethal. It is also clear from Table 1 that the growth medium has an effect on the pigment concentration; this will be further investigated. It might be of interest to recall that of the five EMS induced mutants tested, $\underline{py}_c$ is the only thermosensitive one. This suggests that this mutant is probably a

point mutant as opposed to the more common chromosomal mutations induced by chemical mutagens in maize.

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3. Genetic properties of an atypical chromosome 10.

In previous notes (MNL 45: 115-119 and 46: 120-122) the nonrandom transmission of a chromosome 10 was described. From the crosses made with the trisomic condition, it appeared as though the lower frequency of transmission of that chromosome was the result of an abnormal pairing at meiosis and of male gametophyte competition. It was suggested that an unfavorable chromosomal condition, linked to $\underline{R}^{st}$, the marker used to follow this chromosome, was the cause of the low recovery of two classes ($\underline{R}^{st}$ and $\underline{R}^{nj}\underline{R}^{st}$) in reciprocal crosses involving putative trisomic parents ($\underline{R}^{nj}\underline{R}^{st}\underline{r}$) and a tester ($\underline{rr}$).

The linkage mentioned was confirmed since such crosses produced 41 progenies in 40 of which the situation remained unchanged, while in one, two doses of this abnormal chromosome were apparently present, respectively marked by $\underline{R}^{st}$ and by $\underline{R}^{nj}$. The individual found with two such chromosomes is believed to be the result of recombination between the marker and the chromosomal condition, involving an abnormal and a normal chromosome, followed by the recovery of two abnormal chromosomes in the same spore. The low recombination frequency is probably the result of both physical linkage and nonrandom pairing, similar to that observed by Dr. Rhoades in K10, k10, k10 individuals (Preferential Segregation in Maize, in "Heterosis," 1954). Out of the 41 putative trisomics tested, four gave no transmission of the marker $\underline{R}^{st}$, while two gave no transmission of the marker $\underline{R}^{nj}$. In the first four cases $\underline{R}^{nj}$ was transmitted in typical disomic ratios, while in the other two cases $\underline{R}^{st}$ was transmitted at a much lower