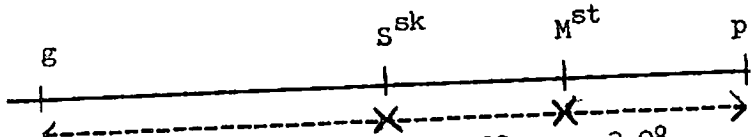
Linkage map:




Recombination values:

The main results of this analysis can be summarized as follows:

1. When P $S^{var}$ / p s females, carrying a nonparental chromosome ("case 2" strand) derived from a p $S^{var}$ / P s parent, are mated to p s males, P - S recombinants are observed in the progeny with a frequency as much as 200 times greater than the frequency of the original event producing the P $S^{var}$ nonparental strand.

2. Linkage relations of the P component of the "case 2" strand have been determined in testcrosses involving the S component of $R^{sk}$, $M^{st}$ and $G_1$. The recombination values obtained are best interpreted by assuming that the P component of the "case 2" strand is removed from its standard position and is relocated at a new position about 5 recombination units to the right of the R locus.

3. The strand with P in its new position shows a decrease of recombination in the g - $S^{sk}$ and $S^{sk}$ - $M^{st}$ intervals. There is some indication that the dislocation of P is associated with physiological and morphogenetic effects, to be reported.

An interpretation of similar effects as well as a tentative hypothesis on the origin of the "case 2" strand is postponed to the time when the analysis of the other three strands will be completed. This analysis is presently underway.

G. Gavazzi
G. Galli

5. A new mutant affecting amino acid metabolism.

The problem of isolating auxotrophic mutants in eucaryotic organisms to use as experimental tools in dissecting the metabolic control processes has been faced by geneticists for some time (Nelson, 1967). Nevertheless, the success of their isolation in higher plants has been very limited when compared to the many results obtained in procaryotes, algae and fungi. Different hypotheses have been offered to explain this failure (Li and Rédei, 1969; Neuffer, 1974). However,

more experiments are needed to evaluate if this failure is a consequence of the difficulties encountered in analyzing complex organisms such as green plants. We have been working on this problem for some time. Most of the mutants we tested are not auxotrophic but temperature sensitive (Gavazzi, et al., 1973). Recently, we found a mutant that affects amino acid metabolism. The mutant is of spontaneous origin. It first appeared in our W22 stocks and it behaves as a monogenic recessive endosperm mutant, exhibiting a reduced and irregular growth of the endosperm. Germination is very poor. The coleoptile is regularly formed but the seedling becomes necrotic and dies before the emergence of the first leaf. Embryo cultures allow growth of the mutant up to about 50 days.

During this period, growth on either mineral or enriched medium (for media composition see Gavazzi, et al., 1973) proceeds slowly, in an irregular manner and with a very limited extent of shoot morphogenesis.

However, embryos cultured on enriched medium (CM) develop a far more abundant root apparatus than those grown on mineral medium (MM). The average fresh weight of the root apparatus together with the attached embryos of 48 day old mutants is 161 mg on MM, while on CM it is 304 mg. To identify the component of the enriched medium that promotes root growth in the mutant, excised roots were cultured on either basic Heller medium or on basic media with single additions of the organic components of the CM. Each medium contains 20 mg/l of sucrose. Root tips of the primary root, 5-10 mm long, were excised, one day after germination, and transferred aseptically to a 250 ml Erlenmeyer flask filled with 50 ml of liquid medium. The flasks, each with three root tips, were left on a shaker (80 rpm) for 12 days at 23 $\pm$ 2°C and 14 hrs/day of light. At the end of this period, root growth was measured as length of primary root, number of laterals, fresh and dry weight. For each weight determination, three roots were used. The average growth values $\pm$ standard error of wild type and mutant sibs are reported in Table 1. It is clear from the results that the only addition promoting growth of the mutant roots is casein hydrolysate; normal roots, on the other hand, are not stimulated in their growth by casein or the other additions but are inhibited by coconut milk. The effect of increasing doses of casein hydrolysate was then measured. It can be seen from the results, reported

Table 1

Effect of different supplements on growth of excised roots of the wild type and endosperm mutant.

| Supplement | No. roots | | LA | | NL | | FW | | DW | |
|---|---|---|---|---|---|---|---|---|---|---|
| | + | m | + | m | + | m | + | m | + | m |
| Control (H) | 12 | 9 | 89±4 | 15± 2 | 11±3 | 3±1 | 52±3 | 14±1 | 3.0±0.3 | 0.4±0.1 |
| Casein hydr. (50 mg/l) | 12 | 6 | 81±6 | 47±12 | 13±3 | 12±4 | 54±2 | 35±5 | 3.2±0.3 | 3.5±0.5 |
| Yeast hydr. (10 mg/l) | 6 | 9 | 71±3 | 12± 1 | 22±3 | 2±1 | 53±7 | 11±2 | 4.0±0.5 | 1.0±0.4 |
| Yeast extract (10 mg/l) | 3 | 3 | 78±7 | 13± 1 | 15±6 | 2±1 | 52 | 13 | 2.6 | 1.0 |
| Coconut milk (2.5%) | 9 | 9 | 20±1 | 10± 1 | 4±1 | 0 | 23±1 | 12±2 | 2.4±0.4 | 1.4±0.3 |

Root growth determined after 12 days of liquid culture.
LA = Length of primary root (mm/root)
NL = Number of lateral roots
FW = Fresh weight (mg/root)
DW = Dry weight (mg/root)

in Table 2, that the first two doses of 50 and 100 mg/l are equally effective in promoting growth and that the same is true for the two succeeding doses (200 and 400 mg/l) that account for a further increase in growth.

Table 2

Effect of casein hydrolysate at different concentrations on growth of excised roots of the endosperm mutant. For abbreviations see Table 1.

| Supplement | No. roots | LA | NL | FW | DW |
|---|---|---|---|---|---|
| Basic medium (H) | 3 | 12±0 | 4±0 | 10 | 0.7 |
| H + cas. 50 mg/l | 6 | 37±4 | 11±1 | 19±2 | 1.0±0.0 |
| H + cas. 100 mg/l | 5 | 35±3 | 9±2 | 14±1 | 0.9±0.3 |
| H + cas. 200 mg/l | 6 | 63±3 | 16±2 | 27±1 | 1.9±0.1 |
| H + cas. 400 mg/l | 6 | 77±7 | 15±2 | 29±1 | 2.1±0.1 |

Casein hydrolysate (acid) contains all the amino acids present in casein except tryptophan.

Excised roots of the mutant were then grown on Heller medium, either basic or supplemented with groups of amino acids (in the L form) except tryptophan.

The amino acids were grouped according to their biosynthetic relationship. Their concentration in the medium is that corresponding to an addition of 400 mg/l of casein hydrolysate. Of the four groups of amino acids tested (Table 3), the one containing ala, val, leu, ile, pro, arg, lys and glu is clearly the one with growth promoting effect. Further tests (Table 4) indicate that this effect is promoted by glutamic acid, proline and arginine. The experiment to establish whether the effect is due to a synergistic action of the three amino acids or to a single one of them has not yet been performed. However, since proline and arginine have glutamate as precursor, the latter is likely to be the component required for growth. Glutamate auxotrophy would be an interesting mutation for the study of nitrogen metabolism since the most

Table 3

Effect of groups of amino acids on growth of excised roots of the endosperm mutant. For abbreviations see Table 1.

| Growth medium | No. roots | LA | NL | FW | DW |
|---|---|---|---|---|---|
| Basic (H) | 12 | 14± 0 | 4±1 | 16± 1 | 1.2±0.2 |
| H + (1) | 6 | 68± 0 | 17±3 | 42±10 | 3.4±1.1 |
| H + (2) | 9 | 14± 0 | 4±1 | 15± 2 | 0.8±0.1 |
| H + (3) | 11 | 27±11 | 4±1 | 14± 1 | 0.8±0.1 |

(1): ala, val, leu, ile, pro, arg, lys, glu (for concentrations see text)

(2): phe, tyr, his

(3): met, thr, cys, gly, ser

Table 4

Effect of different amino acids on growth of excised roots of the endosperm mutant. For abbreviations see Table 1.

| Growth medium | No. roots | LA | NL | FW | DW |
|---|---|---|---|---|---|
| Basic (H) | 7 | 20±2 | 3±0 | 22±2 | 2.4±0.2 |
| H + pro, arg, glu | 14 | 98±6 | 36±3 | 57±6 | 6.0±0.7 |
| H + val, leu, ile | 8 | 18±1 | 2±1 | 22±1 | 2.5±0.1 |
| H + lys | 2 | 17 | 3 | 23 | 1.5 |

important reaction transforming ammonia into amino acids is a reductive amination that, under the catalytic influence of glutamic dehydrogenase, reduces d-ketoglutarate to L- glutamic acid.

Further experiments will elucidate the amino acid requirement of the excised roots as well as of the entire plant.

References:

Gavazzi, G., Piccardo, C., and Manzoni, L. Maize Genet. Coop. News Letter 47: 114-121, (1973).

Li, S. L., Rédei, G. P., and Gowans, C. S. Molec. Gen. Genetics 100: 77-83, (1967).

Nelson, O. E. Ann. Rev. Genet. 1: 265-268, (1967).

Neuffer, M. G. Maize Genet. Coop. News Letter, (this vol.).

G. Gavazzi
C. Piccardo

UNIVERSITY OF MISSOURI
and
U.S. DEPARTMENT OF AGRICULTURE
Columbia, Missouri

1. New mutants located with A-B translocations.

Continuing previous years' work (MNL 45:144, 46:131 and 47:148), we have located an additional 100 mutants. They are listed below by chromosome arm and can be added to those previously reported.

| 1S | 1L | 2S | 2L | 3S | 3L | 4S | 4L |
|---|---|---|---|---|---|---|---|
| 1 wl | 1 wl | 1 v | 1 Yg | 2 pg | 1 wl | 1 gl | 1 v |
| 1 yg | 1 yg | 1 wt | 1 spotted | 1 v | 1 pg | | 2 nec |
| 6 pg | 1 pg sr | 1 pg | 1 pg | 1 zb | 1 sr | | 1 et |
| 1 nec | 1 ad | 3 d | 1 d | 1 d | 1 gl | | 1 mn |
| 1 leth | 1 rgd | 1 nl | 1 nl | | 1 d | | |
| 1 bleached | 2 d | 1 nec | | | 1 bz | | |
| 1 et | 1 bt | 1 sr | | | | | |
| 1 mn | 1 wrinkled | 1 ys | | | | | |
| | | 1 et | | | | | |