lines for kernel set likely is caused by two recessive mutations of A619 line preventing outlet of silk from ear. In the A619 line these mutations do not act in full as they are compensated by several (5-7) semi dominant suppressors. In a recombinant progeny this compensatory gene complex breaks down.-

COLUMBIA, MISSOURI
University of Missouri

More about curious mottling in highly paramutant R1 kernels

— Coe, E

In a previous note I asked, "Mottling expression is curious, and so is blotching — what is responsible?" This note does not answer the question but gives more information.

Typical mottling of r/r/R kernels shows colored aleurone cells in irregular, scattered distributions that are inconsistent with the morphogenesis of the aleurone layer. Tantalizing clues appear with highly paramutant *R1-iv* or *R1-v* (four or five times paramutagenized), in which pigmented cells are greatly reduced in frequency. The distributions suggest some systematic process is at play. Photographs at high magnification have been added to the database (Figs. 1A-G).

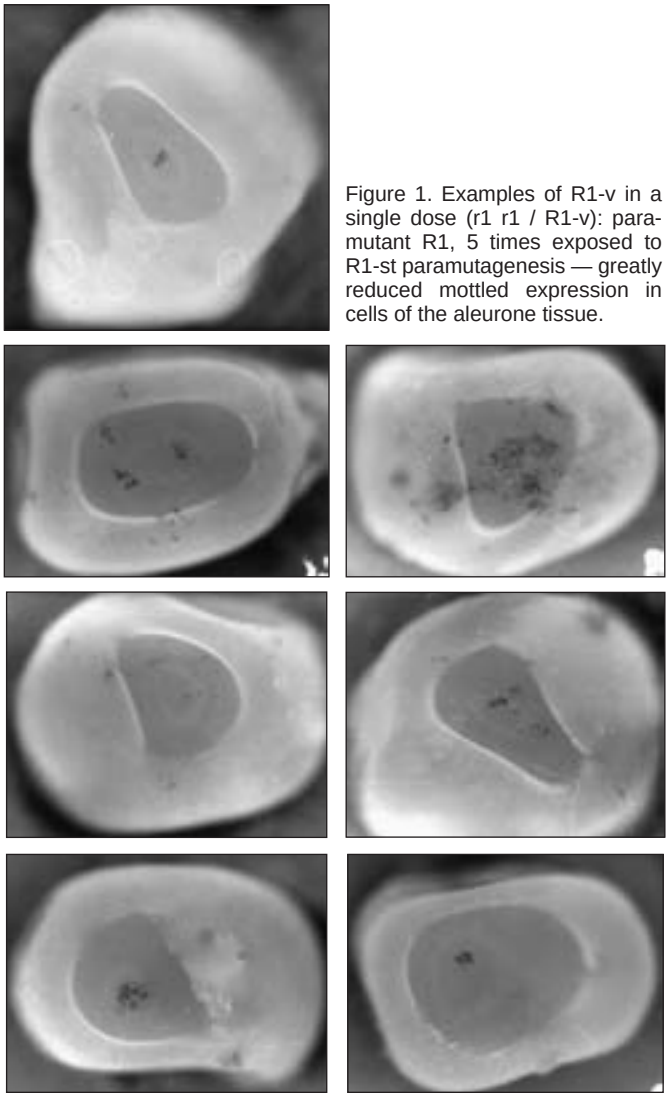

Figure 1. Examples of R1-v in a single dose (r1 r1 / R1-v): paramutant R1, 5 times exposed to R1-st paramutagenesis — greatly reduced mottled expression in cells of the aleurone tissue.

In highly paramutant genotypes there may be as few as 10-20 colored cells in an entire aleurone tissue of some 160,000 cells. If each cell makes an independent decision to be "on" or "off," a binomial or Poisson distribution would result in a random display of single colored cells. However, colored cells occur not in single, independently pigmented cells, but in irregular, very localized clusters, as seen in the images. _

What do the clusters suggest?

Procedures to improve Stock 6

— Chang, MT; Coe, E

Stock 6 was named by Coe in his genetic collection. Ed recognized its high haploid induction rate in 1952. He then converted Stock 6 in 1960 to carry homozygous *A B Pl C R-nj* anthocyanin genes, expressing purple plant color, purple plumule and purple seed crown for easy identification of haploid seeds. The induction rate of Stock 6 is about 2% to 3% with poor agronomic traits such as poor stalk, poor roots, easy to lodge and ear rot. These poor traits were improved by Chang with advice from Coe according to the following procedures, and the rate of haploid induction was increased by selection.

A green plant, yellow seed unfixed material (*AA cc rr bb plpl*) that had shown it was producing haploids in the field was used as female and crossed by purple plant, purple seed crown and purple plumule Stock 6 (*AA CC R-njR-nj BB PlPl*) pollen.

20 F1 seeds (*A/a C/c R-nj/r B/b Pl/pl*) were planted and selfed for F2 seeds.

The F2 seeds segregated nine colored crown and plumule seeds vs. seven colorless seeds. Selected and planted 200 F2 seeds of the most dark-colored crown and plumule to enhance the probability of homozygous *AA CC R-njR-nj* plants.

Selfed F2 plants with dark purple leaves. Ears should either segregate purple crown and colorless seeds or all homozygous colored seeds. Selected the homozygous ears that showed seed color fixation (*AA CC R-njR-nj*).

Planted 50 F3 seeds from each ear with seed color fixed. Identified the most dark purple leaf, sheath, and tassel plants and selfed. These plants were supposed to be homozygous for *AA BB PlPl* genes.

Planted 20 ear-to-row F4 seeds from each selfed F3 ear. Selected the rows showing all purple plants to confirm plant color fixation, and selfed all ears.

The genetic make-up of F5 seeds was fixed for *AA CC R-njR-nj BB PlPl*. The phenotypic expression was all purple plants and purple crown and plumule. Planted 200 F5 seeds and also 500 hybrid seeds as tester.

Selfed each F5 plant and carried pollen to cross onto two hybrid ears. Harvested all selfed F5 ears with ear number -1, -2, -3 and so forth. Also harvested all crossed ears with pollen source X-1,

X-2, X-3 and so forth.

Screened all crossed ears for haploid seeds production. For example, there should be two ears crossed by -1 F5 plants or X-1. If one ear showed 6% haploids and the other ear showed 4% haploids, then the -1 F5 plant had a 5% induction rate. If one ear showed 9% and the other ear 4%, the induction rate was too far apart, so the lower 4% was assigned to define the F5 plant induction rate. Screened all the ears and sorted induction rate from low to high. A range of induction rate from almost 0% to 8% was obtained.

Selected the highest rate F5 ears and planted 20 F6 seeds ear-to-row from 10 ears to form a 200-seed population. Also planted 500 hybrid seeds as tester, using the above procedures again to determine induction rate of each plant. The induction rate was increased in the range of 1% to 9% in response to selection.

Selected the highest induction rate F6 ears and planted 20 F7 seeds ear-to-row from 10 ears to form a 200-seed population. Also planted 500 hybrid seeds as tester. Repeated the above procedures. Also planted 200 F7 seeds with highest induction rate, selfed the haploid plants to form a new pure Stock 6 line with higher induction rate and better phenotypic traits.

Repeated the above procedures for a couple of more generations. The F10 seeds from higher induction F9 ears were sibbed to retain plant vigor and progeny were sibbed again and again. From the progenies, several high induction lines were produced to form new Stock 6 lines. The average haploid induction rate is 6% to 8%.

Seeds of this improved Stock 6 are being sent to the Maize Genetics Cooperation Stock Center.

DNIPROPETROVSK, UKRAINE

Agricultural Steppe Zone Institute of the National Academy of Agrarian Sciences of Ukraine[1]

Ukrainian State University of Chemical Technology[2]

L-proline amount in callus tissues of Lancaster maize inbred lines under chloride load

— Derkach, KV[1]; Abraimova, OE[1]; Dzubetskij, BV[1]; Cherchel, VJu[1]; Sitalo, MJu[2]; Konoschuk, JuV[2]; Satarova, TM[1]

Salinity of soil and soil waters are actual problems of land utilization. Chloride salinity is the most common kind; in Ukraine it is dominated by sulphate and carbonate forms. Chloride salinity has a super-negative effect on the maize plant. Growth inhibition is observed already at 0.1% salt content in soil, but the salt level of 0.3-0.4% provokes the wilting and plant death. Numerous metabolic cell abnormalities occur under the salt influence: inhibition of enzyme activities, photosynthesis, protein synthesis, and disorders of respiratory processes (Dolgyh, Ph.D. Dissertation, 2005).

Protective plant response to the negative effect of abiotic factors is induced by a lot of cell systems. One of the responses to the stress factors (salinity, drought, and low temperatures) is the accumulation of free L-proline in the cells. The precursor of proline synthesis is glutamate or ornithine. Under the stress proline content increases due to the regulation of two opposite processes: the intensification of its biosynthesis and the inhibition of its catabolism.

Proline is an organic compound of low molecular weight that lightly resolves in water and forms colloidal polymer structures. Free proline and proline in the protein molecules are the required components of any plant cell. This aminoacid is a component of the antioxidant protective system; it stabilizes the subcellular structures and macromolecules, regulates redox potential, and participates in the modification of functions of mitochondria. Proline is a part of the signal transmission systems that control gene expression in response to stress (Anjum et al., Afr J Agric Res 6(9):2026-2032, 2011).

Ions Na^+ and Cl^- from the nutrient medium overcome the cell wall and enter the cell through anionic and cationic channels, penetrate through protein hydrate coverage and affect the noncovalent bonds that maintain the structure of the protein molecules. Proline does not penetrate through hydrate coverage and does not enter into direct contact with the proteins, but creates obstacles for the hydrate coverage destruction and the protein denaturation by ions (Alyohina et al., Physiology of plant: 636, 2006). Proline is an important cell osmoprotector. It protects proteins from denaturation and forwards their native conformation, interacting with them during stress. Additionally, it helps to achieve the osmotic balance of cytosol with vacuoles and other cell organelles.

The subject of our work includes the determination of proline amount in maize callus tissues under chloride load and, after its removal, the characterization of influence of sodium chloride on the regeneration potential of callus tissues. Research material was represented by five inbreds of maize commercially valuable Lancaster germplasm (DK633/266, DK633/325, DK236, DK3070, DK6080) and one inbred of Polish germplasm (PLS61). Primary explants for induction of callus tissues were immature embryos, 1.5 mm in length. Callus tissue was initiated within 30 days on N6 medium (Chu et al., Sci Sinica 18:659-668, 1975) modified with 690 mg/l L-proline, 100 mg/l inositol, 100 mg/l casein hydrolyzate, 1 mg/l 2,4-D, 0.1 mg/l abscisic acid and two levels of sucrose - 30 g/l or 60 g/l. Chloride load in vitro was simulated by adding into the medium for subcultivation sodium chloride in concentrations of 6, 30, and 60 g/l. The content of L-proline was determined for 330-day stabilized maize callus tissues obtained in two different ways. In the first version the callus tissue was subcultivated on the N6 medium with 0 (control), 6, 30, or 60 g/l sodium chloride for 300 days right after the induction period. In the second version the callus tissue was subcultivated on the N6 medium with 0 (control), 6, 30, and 60 g/l sodium chloride during 210 days right after the induction period and maintained during the following 90 days on hormone-free regeneration medium MS (Murashige et al., Physiol Plant 15:473-497, 1962) without sodium chloride.

Determination of the proline amount was performed by a modified method (Bates et al., Plant soil 39:205-207, 1963). Callus tissue sample (approximately 1 g) was poured by boiling distilled water (10 ml) and placed for 10 minutes in a boiling water bath. 2 ml of glacial acetic acid and 2 ml of ninhidryn reagent was placed into the clean test tube. Then 2 ml of extract was flowed to the same