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.

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

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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