

Conference Paper

Using genetic Potential of Teosinte from Collection of N.I.Vavilov Research Institute of Plant Industry to Expand Polymorphism of Prolific Maize (*Zea mays* L.)

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Abstract

A threat of genetic erosion of maize stimulate search for new sources to extend its polymorphism. Teosinte (*Zea mays* ssp. *mexicana*), being wild relative of maize, is easily intercrossed with maize and is characterized with a large potential of variability with respect to economically viable attributes, among them being prolific that is having more than one ear. Results are given of long-term selection of maize lines for the attribute of being prolific among the progeny of a hybrid between teosinte (int-350969) and maize. Selection was conducted against the background of soil preparation ideal for maize in a number of attributes that are congruent with prolificacy and synchronous blooming of all or at least majority of ears on a stalk. A wide diversity in the prolificacy of maize was obtained, with the prolificacy coefficient of up to $k_{pr} = 3.3$ with a complicated mode of inheritance in hybrid progeny. Results of topcrossing with a single-ear sterile tester showed a specific reaction of the lines, provisionally dividing them into 4 groups by the nature of manifestation of the prolificacy in the hybrid progeny. It has been established that the prolific lines, which in hybridization with the single-ear tester are capable of forming more than one ear in the hybrid plant are of higher selective value. At that, it is important to take into account simultaneity of blooming of all the ears at a male plant. Observation of this principle leads to preservation of structure and evenness of ears in upper and lower layer, thus providing their productivity independent of layer on the stalk. Valuable prolific lines have been established for creation of prolific maize hybrids.

Keywords: prolific maize, teosinte, simultaneous blooming, maize yield, VIR collection.

1. Introduction

Maize (*Zea mays* L.) is the third most important crop among cereal crops, taking up at least 30% of the global cereal balance [1]. The global production of maize grain reached 1,112 million tonnes, while in Russia it is 13,235.7 thousand tonnes. The demand for maize

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grain is still growing [2, 3]. Such a result has been largely attained by selection of new highly productive maize hybrids that after prolonged selection starting from teosinte finally led to the modern genotype [4--6]. Use of related maize lines in hybrids creates a threat of its genetic erosion, thus search for new genetic sources of valuable traits and using them to create new highly heterotic varieties is currently very important for selection. The trait of prolificacy has been attracting plant breeders for a long time as it is one of reserves for improving productivity. Unfortunately, traditionally many plant breeders preferred single-ear type of plant in their selection of maize lines. This decision was grounded in convenience and processability of the hybrids, while non-simultaneous blooming of many prolific lines reduced to nothing all the results of hybridization. Hybrids from such lines were characterized with non-simultaneous blooming and uneven ripening of upper and lower ears. It created additional inconvenience in ripening, harvesting and calibration, as the lower ears were always less productive and underperformed in ripeness of both ear and grain. With time, thanks to efforts of plant breeders, most of modern inbred lines of maize and its hybrids moved to a single-ear type, losing the genes of prolificacy from wild ancestors of maize either partially or completely [7]. Such approach provoked an abrupt narrowing of maize polymorphism and led to a threat of its genetic erosion as a crop. Search for new sources of genetic diversity led scientists to pay attention to exotic varieties and wild relatives of maize, such as teosinte and gama grass as possible donors for expanding maize's diversity, including improving its resistance to *Helminthosporium* blight [8--10]. Teosinte has the same set of chromosomes as maize and is easily crossed with it, with the exception of specimens carrying Ga1-s Ga1-s genes of gametophytic incompatibility, as described by Kermicle, J. L.; Allen, J. P. At that, the researchers note that it is preferable to use maize as a maternal form and teosinte as a paternal form in cross-breeding [11]. During the first experiments in breeding involving exotic races and maize's wild relatives, plant breeders encountered a complicated structure of inheritance of many maize traits, such as strong and neutral reaction to photoperiod, prolificacy with primitive ear structure, weak resistance against overcrowding and lodging, cold endurance and high susceptibility to local varieties of fungal diseases when introducing the modified lines or their hybrids to different regions and continents [12, 13]. Despite these complications, selection work aimed at prolificacy with synchronous blooming stimulates accumulation of genes that facilitate pollination of maximum possible number of styles of kernels in upper and lower layer situated on the same stalk. Pollen productivity of male flower head (tassel) is effective during the first 5 days after start of blooming and anther going out. That is why it is important to plant breeders to select genotypes that manage to get the styles out on all the

kernels during these 5 days. Such genotypes set more seeds in the ears and their ear structure has no significant difference depending on its location (layer) on the stalk than genotypes with non-simultaneous blooming do. Establishing lines and hybrids of prolific maize with simultaneous blooming of ears is relevant and is highly promising for increasing maize productivity while significantly expanding polymorphism of the plant.

2. Methods and Materials

The studies were conducted at the territory of Nartan experimental farm under Kabardino-Balkar Agricultural Research Institute from 2006 to 2018. The selection plot is located within the boundaries of North Caucasus piedmont zone, at a watershed between Urvan and Nalchik rivers. The soil is largely meadow chernozem. Humus content in the topsoil does not exceed 2.64 %, reaction of soil solution is medium alkaline throughout the soil profile (pH=8.1), with average cation exchange capacity in the topsoil (32 meq per 100 g of soil), gradually diminishing with depth. Carbonate content in the topsoil varies from medium (6.7 %) on the surface to high (13.6--14.7 %) at depth. Availability of labile phosphorus is very low (0.4 mg per 100 g of soil), while labile potassium is very high (8 g/100 g).

The climate in the area is characterized as moderately hot, with the sum of active temperatures of 3000--3200 °C and moderate humidity (the humidity factor is 0.5--0.9), the hydrothermic factor is 0.9--1.2.

Altogether, during the period of research, vegetation periods through the years show that growth and development of maize underwent under conditions of excess heat and deficient moisture. The lines were tested at a crop density of 60 thousand plants / ha, in two-fold repeatability, that of test-crosses - in three-fold repeatability. Phenological observations, measurements and counts were conducted on 10 plants and 10 ears in two-fold repeatability. The selected prolific lines were tested in top-crosses to determine their combining ability, urea cycle reaction and heredity pattern of the prolificacy train in F_1 progeny. In our research, we determined the prolificacy factor (k_{pr}) to evaluate the obtained specimens for their prolificacy. The value of the prolificacy factor is indicative of an average number of ears on a single plant. For that end, the number of plant in a plot and the number of full-scale ears from the plot were counted. The prolificacy factor was determined by dividing the sum of the ears by the number of the plants. Single-ear sterile line GK26M bred at Krasnodar Agricultural Research Institute named after P.P. Lukianenko was used as a tester; a specimen of teosinte in VIR collection (int. no 350969) was used as a source of prolificacy. Crossing with teosinte happened in

field conditions under parchment insulators. Blooming phase of teosinte was stimulated by shortening the photoperiod to 10 hours. For that end, the teosinte plants were covered with photoinsulators from 18:00 to 8:00 starting from the 3rd leaf phase for 30-40 days. After artificial stimulation of teosinte blooming with shortened photoperiod, hybridization was performed by transferring teosinte pollen onto maize ears. In the next generation, 2 back-crosses (BC_1 , BC_2) were performed with maize to influence the negative reaction of the progeny to photoperiod and improve the primitive structure of the ear. Subsequently, all the back-crosses were subjected to prolonged inbreeding, where each reproduction involved culling genotypes prone to photoperiod reaction, with non-simultaneous blooming and primitive ear structure, multiple kernels in leaf sheath, unstable under biotic and abiotic environmental factors.

3. Results

The first attempts at crossing teosinte with maize started in 2006. Its results revealed that stimulating teosinte blooming by photoperiod regulation is a complicated task in the context of Kabardino-Balkar peidmonte. Despite complications in selection of parental pair for the ripeness group, the research succeeded in obtaining first hybrid kernels in sufficient numbers already in the first year of experiments. Teosinte's tassles showed abundant flowering for sufficient time. Development of hybrid plants did not require additional effort for creation of a shortened photoperiod and successfully bloomed in the end of the summer. That year also saw the BC_1 back-cross with hybrid maize pollen onto hybrid ears. The ears were of primitive structure and were largely represented by a double-row spire with large cariopses in rigid scales and were easily broken into two-cariopses articles, however, the cariopses themselves were solidly stuck between the scales and were hard to remove. BC_1 hybrid plants were characterized with early bloom and 4-row ear. Scales on the ear were still rigid, but cariopsis was easier to remove than in hybrid progeny; division into articles of 4 cariopses was well-pronounced. The BC_2 progeny was characterized with even larger shift towards early blooming in comparison with flowering dates of F_1 hybrid and BC_1 . The ear had softer floral glume and 8-row structure. Division into articles was more pronounced in the top part of the ear. At that, an elongation of ear shank was noted, as well as increased number of cariopses on the ear. During the subsequent stages of the research, back-crosses were stopped and substituted with inbreeding, thus allowing obtaining 10- and 12-row ears, largely similar to regular maize ears (Fig. 1, Fig. 2).

During the inbreeding, plants with lower tillering factor and branching of stalk were preferred in selection.

Stalk branching was accompanied with formation of many ears with non-simultaneous blooming and was further complicated by the fact that even from weak gusts of wind they were broken at the base of plant during the seed-filling and ripening (Fig. 3). It should be noted that the majority of the first generation of BC₂ were highly susceptible to local varieties of fungal diseases (*Ustilago maydis*, *Fusarium moniliforme*).



Figure 1: Consequent improvement of ear structure from 1-row teosinte spike to 2-, 4-, 8- and 12-row maize ear (left to right) .

The process of culling genotypes prone to branching and formation of multiple ears in a single leaf sheath turned out to be one of the most tedious and painstaking. During the selective breeding, preference was given to genotypes prone to forming a single ear in a single leaf sheath. All the other genotypes that showed more than one ear in a single leaf sheath or tended to branching were strictly culled (Fig. 4).

An alternative variant for selection of prolific maize from progeny of culled genotypes with multiple ears in a single joint is creating double or more ears with synchronous development (Fig. 5); this method was described in [14].

During the selection, a control was established for simultaneous blooming of ears being formed in all the leaf sheaths and capable of attaining flowering stage of development. At that, duration of tassel flowering was taken into account together with synchronousness of shooting out styles in all the ears on the same plant. The variation range of this trait varied from single-ear to 5-ear genotypes that formed full-scale ear with good grains/ear count under the conditions of long photoperiod characteristic of Kabardino-Balkar piedmont. At this stage the selection was limited to accumulation of inbred homozygotic alleles of genes facilitating simultaneous blooming of ears in combination with the genes of maize prolificacy. The results of the experiments have shown that blooming of prolific maize starts from the top one or two ears and gradually goes down to the lowest ears. In case of non-simultaneous blooming, only the ears



Figure 2: Spikes of the first back-cross BC₁ (left side) and the second back-cross BC₂ (right side) of maize onto teosinte hybrid.



Figure 3: Branching of a 9-row ear plant of BC₂ progeny, maize to teosinte hybrid in its natural (left) and prepared (right) view.

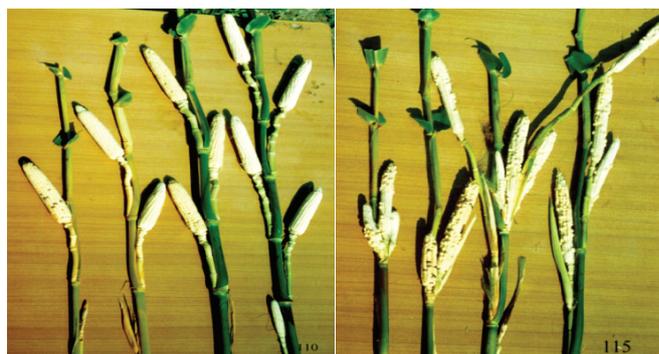


Figure 4: An example of genotype selection with a preference for one ear inside a single leaf sheath (left) and genotypes of culled plants that form more than one ear in a leaf sheath or demonstrate branding.

that shoot out their styles during the first 3-4 days of flowering may attain quality

grains/ear count. Structural differences between top and bottom ears are insignificant in the simultaneously blooming plants (Fig. 6).



Figure 5: Development of double ear with separate cornhusks and a leg in a single joint.



Figure 6: Examples of simultaneous (left) and non-simultaneous (right) blooming of ear and their results after ripening on a dissected stalk (center).

TABLE 1: Ear structure values of prolific maize with simultaneous and non-simultaneous blooming.

Traits	Simultaneous genotype	Non-Simultaneous genotype
Number of ears per plant, ea	5.0±0.0	5.0±0.0
Difference in blooming between the 1st (top) and 5th (bottom) ear, days	3.5±0.2	7.2±0.3
Duration of male flower (tassel) blooming, days	7.4±0.3	7.5±0.3
Difference in length between the 1st and the 5th ear, cm	6.6±0.2	10.8±0.2
Difference in the number of seed rows between the 1st and the 5th ear	0.2±0.0	4.5±0.1
Difference in the number of seeds per row between the 1st and the 5th ear	18.1±1.1	31.3±1.3
Difference in grains/ear count between the 1st and the 5th ear	206.2±2.6	421.7±3.8



Figure 7: Grains/ear count of ears formed on the same plant with non-simultaneous (a) and simultaneous (b) blooming.

Ears that bloom on the 5th and subsequent days show significant drawbacks in qualitative indicators from the top ears, having smaller ear size, number of rows, number or seeds per row and grains/ear count (Table 1, Fig. 7). Besides, ripening of such ears also happens non-simultaneously, complicating their simultaneous harvesting and calibration.

After creating a foundation of collection in a form of 3-, 4- and 5-ear lines, the subsequent selection work was limited to leveling of the traits and simultaneousness of blooming. The results of the experiment has shown that with accumulation of ratio of homozygotes in the genome of prolific lines, the value of prolificacy factor decreases (Table 2). In the I6 progeny, the value of the prolificacy factor was $k_{pr}=3.92$, while in the progeny of I10 it reduced to $k_{pr}=2.98$ on average between 5 lines. Thus, after reaching a certain value of homozygoteness, all the lines were further supported by cross-pollination within the line (sibs method). Such a method for preservation of prolificacy trait from elimination somewhat reduced the rate of reduction in prolificacy factor among the selected lines.

TABLE 2: Value of simultaneousness of blooming and dynamics of prolificacy factor (k) in prolific lines of maize.

Name	k_{pr}		Duration of blooming, days	
	I ₆	I ₁₀	♂	♀
MP-0110	4.4±0.16	3.2±0.11	5.9	5.6
MP-0135	3.9±0.22	3.3±0.14	7.3	5.4
MP-2207	3.5±0.21	2.5±0.16	7.0	4.5
MP-2388	4.0±0.11	3.1±0.09	6.5	4.9
MP-2392	3.8±0.18	2.8±0.10	5.8	4.8
Average	3.92±0.17	2.98±0.12	6.50	5.04

An important criterion for evaluation of inbred lines is determining their abilities to transmit the prolificacy trait to their hybrid progeny [15, 16]. Testing of 7 lines of prolific maize with a factor of $k_{pr} = 2.0$ in top-crosses with single-ear sterile line GK26M (k_{pr}

=1,0) has shown that the heredity patterns of the prolificacy trait are different. Evaluation of the hybrid progeny has shown that manifestation of prolificacy trait in hybrid plants produced by top-crossing may be divided into 4 groups:

Group 1 -- hybrids with k_{pr} = maternal type (no more than 1.0 ear)

Group 2 -- hybrids with k_{pr} = intermediate type (from 1.5 to 1.5 ears)

Group 3 -- hybrids with k_{pr} = paternal type (from 1.6 to 2.0 ears)

Group 4 -- hybrids with k_{pr} = heterosis type (more than 2.0 ears)

Of those groups, only groups 3 and 4 are of selective value, as they demonstrate prolificacy traits in hybrid progeny. It is possible that using different testers with different genetic foundation and ancestry will allow obtaining more comprehensive data on heredity patterns of such a complex trait as prolificacy of maize. From the data obtained in our experiment it follows that the prolificacy trait may be a manifestation of either hereditary transmission of quantitative traits, or effect of heterozygosity in hybridization. Overlapping of both of these factors is also possible. A spectacular example of promising nature of maize selection in this direction may be seen in inter-linear hybrid combinations of prolific maize (Fig. 8).

4. Conclusion

Expansion of genetic diversity of prolific maize by involving teosinte-sourced prolificacy genes into selection process is effective. Selection material obtained as a result of introducing into maize genome the gene sources that control the prolificacy trait is characterized with a high potential productivity due to formation of more than one ear on a stalk and simultaneous blooming with the male flower. The process of selection of the prolific maize included several stages with a complex system of evaluation and culling of selection material against the agricultural background optimal for maize. Inbreeding depression has a negative effect on phenotypic manifestation of the prolificacy trait, reducing the value of the prolificacy factor at each subsequent state of inbreeding. Phenotypic manifestation of the prolificacy trait in hybrid progeny follows 4 types that are either hereditary or heterotic and depends on parental pair genotypes.

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Figure 8: Hybrids of maize with simultaneous blooming of 2 ears (top), 3 ears (middle) and 4 ears (bottom).

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