COMBINED S1-TC-RRS WITH CONSIDERATION OF CMS AND DIHAPLOIDS IN MAIZE

Jelena VANČETOVIĆ, Dragana IGNJATOVIĆ-MICIĆ, Sofija BOŽINOVIĆ, Nenad DELIĆ and Zoran ČAMDŽIJA

Maize Research Institute “Zemun Polje”, Belgrade, Serbia


Herein, we present the combined S1-HS-RRS method using inbred testers (S1-TC-RRS) as a long-term maize breeding program, which increases the frequency of favorable alleles and maintains genetic variability in two genetically opposite populations. The method improves two different genetic sources simultaneously, where S1 families, developed by selfing phenotypically superior plants from both breeding populations are crossed with opposite inbred testers for specific combining ability selection, accompanied by selection of S1 families per se. A certain percentage of the evaluated S1 families is used for the next TC-RRS selection cycle. Maternal haploids from the selected S1 lines of each cycle of S1-TC-RRS can serve to produce elite 100% homozygous inbred lines (dihaploids) in a short time, which decreases the time and expenses of the selection cycle and influence the efficiency of seed production, as well as,

Corresponding author: Jelena Vančetović, Maize Research Institute, Belgrade, Serbia +381-11-3756-704, fax +381-11-3756-707 e-mail: vjelena@mrizp.rs
variety protection rights. This elite lines than can be converted to cms versions (paternal haploids), for the seed production, which lowers the costs of it.

Key words: cytoplasmic male sterility, dihaploids, maize, S1-TC-RRS

RECURRENT SELECTION

Recurrent selection (RS) is a long-term selection programme that increases the frequency of favourable alleles in the selected population, while maintains the population genetic variability. There are several methods for both intra- and interpopulation improvement in maize.

TC-RS and S1-S2-RS are the two frequently applied and mutually compared methods. TC-RS (the use of an inbred line as a tester) for the improvement of a specific combining ability of the populations is based on assumption that superdominance has the greatest importance in inheritance of the grain yield in maize. Both combining abilities, specific (SCA) with a used tester, and general (GCA), can be improved by TC-RS (ZAMBEZI et al., 1986). For the commercial selection programmes, it is best to use the opposite elite inbred line as a tester (HALLAUER and LOPEZ-PEREZ, 1979).

Combined S1-RS is superior over TC-RS because of the putative masking effects of dominant alleles of testers (HARRIS et al., 1972) and greater variance among S1 families compared to TC families.

It was considered that S1-RS would also improve combining ability of populations to the extent to which the yield of S1 family was correlated with the yield of its testcross, and especially GCA, since S1-RS was mainly based on the additive genetic variance. However, a greater number of studies showed that correlations between S1 lines and their testcrosses, i.e. between traits of inbred lines and their hybrids, were mainly low.

Because of advantages and disadvantages of certain methods, as well as, for more complete utilisation of the genetic variability within the population, combined RS methods were proposed (HALLAUER and MIRANDA, 1988). Combined selection makes use of two or more selection methods within the same programme. In a broad sense, most breeding methods can be considered as combined selection.

Combined S1-TC selection is the most appropriate for the improvement of both populations per se and their combining abilities (VANČETOVIĆ, 1994). HALLAUER (1989) suggested the combination of S1-S2 with TC selection, with visual selection within and among S1 families, their simultaneous crossing to a tester and recombination of the best families selected based on testcrosses.

A predicted genetic gain from selection is important for a certain RS method preference. The following factors affect the genetic gain from RS: existing variability within the population, heritability of the trait under selection, selection intensity and the number of generations necessary for one cycle (EBERHART, 1970). LAMKEY and HALLAUER (1987) summarized results of 121 experiments within seven RS programmes. Based on variances among observed progenies it was shown that
heritability for grain yield ranged from 53.3% (TC-RS with the use of inbred testers) to 79.8% (S1-RS).

The higher selection intensity (decrease of % of selected families for the succeeding cycle) the higher genetic gain from selection is, but also the risk from genetic drift and narrowing genetic variability within the population, which results in the reduction of achieved gain from selection in the later cycles. The number of superior families as families per se and in testcrosses also increases with the intensity decrease of individual methods in combined selection (VANČETOVIĆ, 1994). HALLAUER (1988) states that 25-35 selected families per cycle are sufficient for maintenance of population genetic variability and provides a long-term success of the selection programme. The use of winter nursery, that is, the reduction of the number of years per cycle of RS is another method to increase the gain from RS per year. Furthermore, the estimated heritability within RS programmes with the progeny tested in one year also contains genotype x year interaction, which affects the increase of expected genetic gain.

One of the combined RS methods is S1-testcross (TC) reciprocal recurrent selection, which simultaneously improves two genetically opposite populations. Herein we propose such a method, considering the need of using cms in the seed production and a possibility to use maternal and paternal dihaploids for shortening the complete process of commercial selection.

**CYTOPLASMIC MALE STERILITY**

Utilisation of male sterile versions of a female component in maize eliminates a requirement for detasseling, reduces the number of workers necessary for control and super control, significantly improves quality of the seed production and considerably reduces costs and accompanying risks, and finally, in such a way the seed production becomes very attractive for growers. Nowadays, a new approach in using CMS in commercial production is being examined (WEINGARTNER et al., 2002; VANČETOVIĆ et al., 2009; BOŽINOVIĆ et al., 2010).

There are three known types of cytoplasmic male sterility in maize: Texas-type (cms-T), USDA-type (cms-S) (also known as Moldavian or S-type) and C-type (cms-C). Today, only cms-S and cms-C are commercially used.

In Maize Research Institute Zemun Polje all commercial lines are at the same time converted to four versions: cmsC; cmsC-RfC/RfC; cmsS and cmsS-RfS/RfS, and the more appropriate type of cms is used for each hybrid combination (less late break of sterility, lack of natural restorers in mother lines, etc.)

Conversion to cms maternal versions is relatively easy, performed by a simple backcrossing method and every multiplication of the female component is at the same time another backcross (BC). The problem occurs in the conversion of the male component to Rf component, because six backcrosses and two to three self-pollinations are required (eight to nine generations, i.e. four to five years if winter nursery is used) (VANČETOVIĆ et al., 2006). On the other hand, cms and Rf conversion could be timely accomplished by marker-assisted backcrossing, when only 2-3 backcrosses are sufficient. This is the more expensive approach, though.
Based on everything stated, we consider that it is the best if initial breeding material already contains appropriate \textit{cms} and \textit{Rf} genes necessary for the seed production based on \textit{cms}.

\textbf{IN VIVO HAPLOID INDUCTION IN MAIZE}

Two methods of \textit{in vivo} haploid induction are known in maize, leading to maternal and paternal haploids. The genomes of maternal haploids originate exclusively from the seed-parent plant. A pollinator parent (COE, 1959) in this case causes the haploid induction. The opposite applies to the induction of paternal haploids, where the pollinator serves as a genome donor and the female as an inducer (KERMICLE, 1969).

\textit{The role of paternal haploids in maize}

Paternal haploids can be used in development of maize hybrids (BELICAUS \textit{et al.}, 2007) and rapid conversion of female components to \textit{cms}. The embryogenic development of sperm nuclei in maternal cytoplasm results in the formation of androgenetic haploids. Androgenetic haploid is produced when the maternal nucleus is eliminated or inactivated subsequent to fertilisation of the egg cell. The presence of the \textit{ig} gene (indeterminate gametophyte) increases the occurrence of paternal haploids from the natural spontaneous frequency of about one per 80,000 to 1 to 3\% of maize plants observed. Even 9\% of the occurrence of paternal haploids was reported (KINDIGER, 1993). This mutation can be used to produce paternal haploids whose chromosomes then can be doubled. The procedure is very easy, that is to plant haploid seed, and cross the succeeding sterile haploid plants as a mother with original line as the father. Derived diploid plants contain the cytoplasm of the female parent. Therefore, this mutation is potentially useful for placing a given nuclear germplasm in a different cytoplasm. This is of interest to the seed industry because it would make it possible to place a nuclear genome in a sterile cytoplasm in far fewer generations than by conventional backcrossing. This method has some disadvantages, though, as the low productivity of inducer genotypes, due to small ears with large quantity of defective kernels (ZABIROVA \textit{et al.}, 1999).

However, it is possible to apply the conventional method of backcrossing for the development of \textit{cms} maternal versions. Using the winter nursery this can be accomplished in two years, since after the fourth conversion material can be given to the foundation seeds for multiplication. In this way, development of the commercially multiplied sterile mother is finished after two years of multiplications (which equals backcrossing).

\textit{Developing of maize inbred lines by using maternal haploids}

In crosses with inbred line Stock 6 as a pollinator parent, Coe (1959) observed 2.3\% maternal haploids. Segregation studies (DEMLING \textit{et al.}, 1997) showed that \textit{in vivo} haploid induction of maternal haploids in maize is a quantitative trait under the control of an unknown number of loci. Stock 6 subsequently led, by intensive selection, to the creation of a few inducers of maternal haploids, and the newest are KEMS (SHATSKAYA \textit{et al.}, 1994), MHI (CHALYK, 1999), RWS (ROBER \textit{et al.}, 2005), PHI (ROTARENCO \textit{et al.}, 2010). These lines can have even more than 10\%
of the maternal haploid induction rate, depending on the environment and the genetic material used as a mother parent (SHATSKAYA et al., 1994; ROBER et al., 2005).

The method of developing maize inbred lines in the course of two generation by the dihaploid production is a new, widely used method in maize selection, and is described in detail by CHALYK (1999) and ROBER et al. (2005).

The system functions based on reduced germination of pollen grains of paternal haploid inducer. Pollen grains germinate on stigma without reaching the egg cell and performing fertilisation, but stimulate the egg cell to divide and form a haploid embryo (germ).

THE PROPOSAL OF COMBINED S1-TC-RRS WITH CONSIDERATION OF CMS AND DIHAPLOIDS IN MAIZE

Considering a comprehensive male sterility programme at the Maize Research Institute Zemun Polje it is possible to select lines from two opposite genetic pools (for deriving two synthetics), with good traits per se and good general and/or specific combining ability. One source should have a genetic constitution \( \text{cmsC-RfC/RfC, nrS/nrS} \) (homozygous restorer for \( \text{cmsC} \) in \( \text{C} \) cytoplasm, and non-restorer for \( \text{cmsS} \)), while the other should have the opposite one: \( \text{cmsS-RfS/RfS, nrC/nrC} \) (homozygous restorer for \( \text{cmsS} \) in \( \text{S} \) cytoplasm, and non-restorer for \( \text{cmsC} \)). For the convenience, the first, i.e. second synthetic could be named synthetic C, i.e. synthetic S, respectively. Selected lines should be good as female, as well as, male components in the seed production (high yield per se, large number of kernels per ear, kernel of a smaller size, to be good pollinators). Moreover, they should not exhibit the late break of sterility for the \( \text{cms} \) type for which they are non-restorers. Satisfactory genetic variability within newly derived synthetics should be achieved with five to six lines per each source.

The review of proposed selection method is shown in Table 1. Duration of the scheme is six years, with the utilisation of winter nursery. Within this time, three cycles of RRS could be completed, with the simultaneous development of elite commercial lines from C0 that would be converted to a system of seed production based on \( \text{cms} \). These elite inbreds shall be used for new commercial hybrid production. During the seventh year, foundation seed department could multiply derived \( \text{cms} \) counterparts of mother lines and \( \text{Rf} \) counterparts of father lines.

The selection scheme is consisted of the following steps:

During the first year (1), self-pollination of as many phenotypically superior plants from C0 of both synthetics as possible, and selection of the best ones at harvest.

In the following generation, winter nursery (1/2), S1 families should be crossed, in spatial isolations, to opposite inbred testers (the most elite inbred line that was used to form an opposite synthetic).

During the second year (2), S1 families should be tested for traits per se in two locations, if possible in high density (approximately 120,000 plants ha\(^{-1}\), which serves as the additional test for drought tolerance, resistance to stalk lodging and breakage, diseases and pests). If necessary, artificial inoculation of S1 families could
be performed for prevailing maize diseases and/or pests for a certain growing region, for a better evaluation of their resistance.

Table 1: Scheme of combined TC-RRS with the use of cms and dihaploids in maize

<table>
<thead>
<tr>
<th>Year</th>
<th>Winter nurses</th>
<th>Stage of RRS</th>
<th>Stage of dihaploid breeding</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Selfing of C0 within two synthetics – production of S1 families.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/2</td>
<td>Crossing of selected S1 families in spatial isolations with opposite elite inbred testers.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Test-trials with testcrosses (S1 x inbred testers) and with S1 <em>per se</em>; selection of the best S1s.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2/3</td>
<td>Recombination of the chosen S1 families – formation of C1.</td>
<td>Chosen S1 x maternal haploid inducer.</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Selfing of C1 of two synthetics – production of S1 families.</td>
<td>Selfing and selection of D0 plants – production of D1 progeny.</td>
<td></td>
</tr>
<tr>
<td>3/4</td>
<td>Crossing of selected S1 families of C1 in spatial isolations with opposite elite inbred testers.</td>
<td>Seling of D1 (multiplication – production of D2) and their crossing with opposite inbred testers (spatial isolations).</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Test-trials with testcrosses (S1 of C1 x inbred testers) and with S1 of C1 <em>per se</em>; selection of the best S1s.</td>
<td>Test-trials with D1 x inbred testers. Selection of the best performing D2 <em>per se</em> and in testcrosses of D1. Multiplication of D3 progeny.</td>
<td></td>
</tr>
<tr>
<td>4/5</td>
<td>Recombination of the chosen S1 families – formation of C2.</td>
<td>Paternal cms haploid inducer x chosen D3 lines. Multiplication of D3 lines = D4 production.</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Selfing of C2 of two synthetics – production of S1 families.</td>
<td>cmsD3(n)xD4 lines = derivation of new cms lines. Further multiplication of D5 lines. Crossing of D4 (S synth.) x D4 (C synth.) and vice versa = making commercial hybrid combinations.</td>
<td></td>
</tr>
<tr>
<td>5/6</td>
<td>Crossing of selected S1 families of C2 in spatial isolations with opposite elite inbred testers.</td>
<td>cmsD3 (2n) x D5 lines = multiplication of new cms lines. Further multiplication of D6 lines.</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Test-trials with testcrosses (S1 x inbred testers) and with S1 of C2 <em>per se</em>; selection of the best S1s.</td>
<td>Test-trials with commercial hybrid combinations; selection of the best ones.</td>
<td></td>
</tr>
<tr>
<td>6/7</td>
<td>Recombination of the chosen S1 families – formation of C3.</td>
<td>Multiplication of the chosen cmsC D5 lines from synth. S, and D7 R/C lines from sythn. C, and vice versa.</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Further RRS from C3 cycle.</td>
<td>Proceeding cms and Rf D5 and D7 commercial lines to the foundation seed.</td>
<td></td>
</tr>
</tbody>
</table>
Beside grain yield, other maize traits are also important (ear height, lodging, etc.), hence parallel selection for two or more traits are performed. Furthermore, depending on the available amount of seeds of S1 families, conventional test-trails for grain yield could be performed. Testcrosses from the winter nursery would be included into test-trials for grain yield (i.e. combining ability). Beside selection for grain yield, selection for some other important traits (e.g. percent of moisture at harvest, lodged and broken plants, etc.) could be carried out in these trials based on some of selection indices.

The best 10-30% of S1 lines (depending on desired selection intensity), that performed satisfactory *per se* as well as in testcrosses, should be recombined in the following cycle of S1-TC-RRS in the subsequent generation, i.e. winter nursery (2/3). In this way, C1 of these two synthetics will be derived. The selection intensity could be increased (10% of chosen S1 families) if the faster progress is desired, but that would increase the risk of a faster loss of genetic variability in synthetics and vice versa (Hallauer and Miranda, 1988). Progress per a selection year is increased with the reduced number of years per cycle - if winter nursery is used. One cycle of such RS lasts two years, which increases gain per year. This scheme presents a combination of conventional test-cross RS for the intrapopulation improvement and RRS, since testers are from opposite synthetics, and therefore an increase of interpopulation heterosis, i.e. heterosis among lines derived from two improved synthetics, is expected. S1-RS, either in the form of actual test-trials with S1 families or as phenotypic selection of S1 families in high density, is also included.

Maternal dihaploids and thereby RfC or RfS lines (depending on the synthetic) can be obtained from seeds of selected S1 families of both synthetics from the remnant seed. Using paternal dihaploids these lines could be placed into the opposite type of *cms* (RfC lines into *cmsS*, and vice versa), so the inbred lines ready for the seed production based on *cms* are obtained. After the final testing of derived lines for *per se* traits and combining abilities (first with inbred line testers used for RRS, and then the best performing selected lines between themselves), the best performing lines are selected for the commercial use. In the course of time, with the increased number of RRS cycles, inbred testers could be changed, that is the best newly developed opposite lines should be used.

Selected S1 families (remnant seed) from both sources would be crossed in winter nursery (2/3) to male inducers of maternal haploids. During the third season (3) selfing of C1 and selection of new S1 families should be done. At the same time, D0 (derived dihaploids) would be selfed (multiplication – production of D1 generation).

In the succeeding generation, i.e. winter nursery (3/4), S1 families from C1 would be crossed, as well as D1 families, to opposite testers in spatial isolations, with simultaneous multiplication of D1 lines (production of D2 generation).

During the fourth season (4), test-trails with S1 and TC families should be carried out, and 10-30% of the best S1 families should be selected from C1. Moreover, test-trails with D1 x inbred testers should be performed, while D2 would
be further multiplied (these lines represent \textit{RfC} lines from the synthetic C, i.e. \textit{RfS} lines form the synthetic S), and the best combining D2 lines would be selected.

In winter nursery (4/5), recombination of selected S1 families from C1 should be done – formation of C2 of these two synthetics. The best D3 lines should be multiplied (production of D4) and as a male component crossed to \textit{cms} inducers of paternal haploids for the development of their sterile counterparts.

During the fifth year (5), C2 would be selfed for the production of S1 families. At the same time, \textit{cms} D3 (haploid plants) would be crossed to appropriate D4 lines (the same line) and \textit{cms} versions of these selected D3 lines would be derived. Further, derived D4 lines would be multiplied. Hybrid combinations of selected D3/4 lines between these two opposite sources should be developed.

In winter nursery (5/6), S1 families from C2 should be crossed to opposite testers and \textit{cms} D3 lines would be further multiplied.

During the sixth season (6), trails with S1 and TC families from C2 (combined RRS) and test-trials with new commercial hybrid combinations should be carried out. Based on the results, the selection of new commercial D4/5 lines, as well as selection of the best 10-30\% of S1 families from C2 of both synthetics for recombination would be done.

In winter nursery (6/7), recombination of S1 families from C2 would be performed - C3 of these two synthetics would be formed. Simultaneously, \textit{cmsC} D4 and \textit{RfS} D5 lines from the synthetic S and \textit{cmsS} and \textit{RfC} lines form the synthetic C would be multiplied.

Combined RRS would be further performed after the seventh year (7), while D4 \textit{cms} and D5 \textit{Rf} commercial lines would be proceeded to the seed production.

From the selected S1 families from each succeeding cycle of selection (C1, C2, Cn) female dihaploids would be derived. Hence, in practice, it would mean that new commercial lines converted to male sterility would be derived every second year from this selection scheme. A small number of usually obtained maternal dihaploids is compensated by the selection already done in the S1 generation, so 8-10 dihaploid plants per selected S1 family is supposed to be enough to retain the combining ability and superior \textit{per se} performance of the line.

It is desirable to maintain recombined seeds of each cycle of selection, in order to monitor progress of this RRS method, both in populations \textit{per se} and their test crosses with opposite inbred testers, as well as, in the interpopulation crosses. Progress from C0 to C3 could be observed after first six years of this selection method.

In this breeding scheme, maternal dihaploids are used only for shortening the process of inbreeding. On the other hand, from breeder’s standpoint you can often hear the argument against maternal DH breeding i.e. that it is not enough efficient to produce inbred lines (DHs) without previous selection. Our proposed breeding scheme overcomes this argument.

BERNARDO (2009) concluded, based on a simulation study, that doubled haploids should be induced from F2 rather than from F1 plants, which increased the response from multiple cycles of testcross selection.
The suggested method of selection combines HS-RRS using inbred testers, which is a long-term programme that provides conservation of genetic variability in the populations, with phenotypic (or genotypic – conventional test-trials) selection of S1 families, as well as, rapid methods for the development of completely inbred lines converted to cms system for the seed production. Shortening the length of an RS scheme considerably increases its efficiency. In addition, the success of employing DH lines depends on the choice of an efficient breeding procedure and the optimum allocation of technical and budget resources to the individual breeding steps in order to maximise the genetic gain from selection. From the aspect of commercial selection and maize seed production, the suggested RRS method is much faster for the development of 100% homozygous elite lines (maternal dihaploids). In addition, this method allows fast conversion of the obtained dihaploid lines to male sterility (paternal dihaploids). As for maternal dihaploids, high cost savings are assured due to reduced expenses for the selfing programme, handling of seed batches, and maintenance breeding (ROBER et al., 2005). Furthermore, their development is not accidental as in the conventional scheme of dihaploid development, as they are derived from S1 families with verified good combining abilities, which is especially important if it is emphasised that there is a weak correlation between traits of lines per se and their combining ability (HALLAUER and MIRANDA, 1988).

LONGIN et al. (2007) concluded that maximum selection gain in S1TC-DHTC was 10% higher than in DHTC, indicating the large potential of early testing prior to maternal DH production. These authors also emphasise the current limitations of the DH technique, and a need for substantial increases in haploid induction and chromosome doubling rates (i.e. lowering costs of maternal DH production) that would allow early testing of S1 lines and subsequent production and testing of DH lines. All of this would make more realistic suggested breeding scheme that would combine high selection gain and a short cycle length.

The proposed method is still theoretical and this year we are starting with its application in Maize Research Institute.

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REFERENCES


KOMBINOVANA S1-TC-RRS PRI KORIŠĆENJU CMS-A I DIHAPLOIDA KOD KUKURUZA

Jelena VANČETOVIĆ, Dragana IGNJATOVIĆ-MICIĆ, Sofija BOŽINOVIĆ, Nenad DELIĆ and Zoran ČAMDŽIJA
Institut za kukuruz “Zemun Polje”, Beograd, Srbija

I z v o d

Prestavlja se kombinovani S1-HS-RRS metod uz korišćenje inbred testera (S1-TC-RRS), kao dugoročni program u oplemenjivanju kukuruza, koji povećava frekvenciju poželjnih alela i održava genetičku varijabilnost dve genetički opozitne populacije. Metodom se u isto vreme popravljaju dva genetički opozitna izvora, gde se S1 familije, dobijene samooplodnjom fenotipski superiornih biljaka iz obe populacije, ukrštaju sa opozitnim inbred testerom radi selekcije na posebne kombinacione sposobnosti, uz istovremenu selekciju S1 familija per se. Određen procenat ispitivanih S1 familija se koristi za sledeći TC-RRS ciklus selekcije. Uz pomoć metode majčinskih haploida, iz odabranih S1 linija svakog S1-TC-RRS ciklusa mogu se dobiti 100% homozigotne elitne inbred linije (dihaploidi) za kratko vreme, što smanjuje vreme i troškove seleksionog ciklusa, a utiče na efikasnost semenske proizvodnje i zaštitu oplemenivačkih prava. Ove elitne inbred linije mogu se prevesti na cms verziju (očinski haploidi), što smanjuje troškove u semenskoj proizvodnji.

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