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Heterosis in Alfalfa Breeding

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Summary: The progress of alfalfa breeding has been slow, most notably due to its complex genetic structure (autotetraploidy) and tetrasomic inheritance. Alfalfa breeding programs are based on recurrent phenotypic selection with or without progeny testing. The genetic control of major agronomic traits is determined by both additive gene action (accumulation of frequency of desirable alleles represented by significant general combining ability (GCA) effects, and nonadditive gene action, complementary gene interactions represented by significant specific combining ability (SCA) effects. This type of gene action expression in alfalfa also determines the way in which breeding is carried out and brings about changes in the methods used. It has also given rise to the idea of the semi-hybrid breeding of this crop which involves breeding alfalfas within the population, identification of heterotic germplasm, and the seed production of semi or free hybrid seed. The studies of the relationship between the molecular variability of alfalfa populations and heterosis in their hybrids could contribute to a more precise selection of parental populations to be used for crossing in semi-hybrid alfalfa breeding procedure, aiming to reduce number of necessary crossings and therefore make future alfalfa breeding programs more efficient. Future tasks of alfalfa breeders should be to discover how to translate heterosis from single plants in hybrids planted in dense stand to generate "yield for free" (capture heterosis) in alfalfa semi hybrids.
Key words: alfalfa, crop yield, heterosis, hybrids, plant breeding, semi-hybrids

Introduction

Alfalfa is a highly productive forage species, with great potential for hay production. Main goals in alfalfa breeding are biomass yield (for hay or biofuel), forage quality: protein, neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL) content, leaf to stem ratio (Milić et al. 2011a), but also persistence, adaptability and stability of newly created populations/cultivars (Katić et al. 2008, Veronesi et al. 2010, Milić et al. 2011c). Breeding alfalfa for yield has not been very successful compared to other species (Lamb et al. 2006, Brummer 2008, Veronesi et al. 2010, Li & Brummer 2012). The progress of alfalfa breeding has been slow, most notably due to its complex genetic structure (autotetraploidy), allogamy and tetrasomic inheritance present in alfalfa, and besides this plant architecture, hermaphroditic flowers and meadow conditions (Scotti & Brummer 2010).

Alfalfa breeding programs are based on recurrent phenotypic selection with or without progeny testing, to accumulate desirable alleles at high frequency into a population (Li & Brummer 2012). The tetraploid species usually express severe inbreeding depression. Because of that the process of derivation of homozygous plants is very slow or not possible. So, true inbred lines of alfalfa are not available (Li et al. 2009). Rotili et al. (1999) were the first to understand and propose the effect of heterosis to be partially used for development of free-hybrids by crossing lines obtained from 2-3 generations of selfing. The idea on partial utilization of heterosis in alfalfa that emerged in the USA proposed the development of semi-

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hybrids obtained by crossing genetically divergent germplasms and identifying heterotic groups (Brummer 1999). However, genetic diversity *per se* is insufficient for heterosis expression (Kidwell et al. 1999, Riday et al. 2003), divergent populations must be ”good combiners” and testing for this ability has to be included in the breeding programs for heterosis in alfalfa (Scotti & Brummer 2010).

Present and previous research clearly shows existence of heterosis in alfalfa (Riday & Brummer 2002, Bhandari et al. 2007, Madril et al. 2008, Katić et al. 2010, Al Lawati et al. 2011, Milić et al. 2010, Tucak et al. 2012). The genetic control of major agronomic traits is determined by both additive gene action (accumulation of frequency of desirable alleles represented by significant GCA effects) and nonadditive gene action (complementary gene interactions represented by significant SCA effects). This type of gene action expression in alfalfa also determines the way in which breeding is carried out and brings about changes in the methods used.

It has also given rise to the idea of the semi-hybrid breeding of this crop which involves: breeding alfalfas within the population, identification of heterotic germplasm, and the production of hybrid.

A) <u>Breeding alfalfas within populations</u>

Intrapopulation breeding of alfalfa leads to the accumulation of desirable alleles, i.e. to additive gene action, and helps improvement of the performances of populations with a narrow genetic base. Used germplasm should be genetically divergent, but its potential maintenance can be carried out more easily and more safely in geographically separate centers, which is why genetic divergence often coincides with different geographic origin (Milic et al. 2011).

Important topic in this phase is number of plants that have to be chosen, for the next improved generation of advanced population. Our opinion is that it should not be less than 200 plants because of inbreeding depression which can appear in multiplication of seed in space isolation. More efficient approach should be that selection intensity (index selection) has to be 10-20% selected plants upon the number of plants in initial population. In this stage, applications of molecular markers can be very helpful in order to obtain more unique population such as RAPD/SSR DNA markers as it is proposed by Nagl et al. (2011) and Taški et al. (2013).

Creation of improved, more homogenous populations means moving into a new phase of breeding scheme:

B) <u>Identification of heterotic groups</u>

Until recently (Sakiroglu et al. 2007, Madril et al. 2008, Al Lawati et al. 2010, Katic et al. 2010, Milic et al. 2011, Tucak et al. 2012), little was known about other heterotic groups within *Medicago sativa* ssp. *sativa* complex (Brummer 2008). Primary question of the past ten years in alfalfa research has been the examination of possible heterotic groups, particularly between the semidormant and nondormant germplasm groups (Sakiroglu et al. 2007, Bhandari et al. 2007, Al Lawati et al. 2010), as Brummer (1999) previously proposed.

Crossing a large number of germplasms on the basis of special combining ability, makes possible determination of the effects of intra- and inter-allelic interactions in alfalfa using diallel mating design (Riday & Brummer 2002, Segovia-Lerma et al. 2004, Bhandari et al. 2007, Madril et al. 2008, Al Lawati et al. 2010, Milic et al. 2011b, Tucak et al. 2012). The intensity of these interactions (heterosis) is helpful in identifying components suitable for the development of population hybrids. Heterosis could be determined by crossing germplasms using a diallel mating design, while the production of F_1 seed and its utilization could be used for the assessment of hybrid vigor (Milic et al. 2011b). One disadvantage of population hybrids may be that populations used as heterotic groups can carry a heavier genetic load, so complementariness among populations may not be sufficient to cover undesirable alleles (Riday et al. 2002). Heterosis cannot be expected to occur in highly recombinant germplasm either, since crossing populations that are very distant leads to a loss of desirable epistatic effects (complementary gene interactions) that exist within parental populations (Li & Brummer 2009).

Using diallel mating design nowadays is more efficient due to presence of various statistical software programs, but still presents challenge because of enormous manual work. In this phase it is of great importance to cross divergent genotypes and populations created in the previous phase, or use materials from other breeding sites. One of possible solutions in this stage could be to recurrently produce population hybrids that express heterosis and to develop “heterotic pools” of germplasm directly, by subdividing adapted, elite germplasm into two groups, which could then be improved concurrently within a reciprocal recurrent selection program (Brummer 2008).

The populations that exhibited heterosis in a set of crossings are recommended for use as parent components for development of high-yielding population hybrids (Brummer 2008, Milić et al. 2011b).

C) **Seed production of semi or free hybrid of alfalfa**

At this point semi hybrid breeding strategy can be divided in two separate directions, but significantly related processes of producing seed of alfalfa free or semi hybrids:

1) *Seedling of F_1 and multiplication of advanced generation ($F_2 – F_4$) in space isolation for free hybrid seed production*

Our assumption is that plants of high-yielding, genetically divergent, and homogeneus populations would produce hybrids with improved agronomically important traits, thus contributing to an increase in alfalfa biomass yields.

Fig. 1. Multiplication of $F_2 – F_4$ hybrid seeds from F_1 heterotic generation

This approach presents assay in order to fix nonadditive gene action (complementary gene interactions that is represented by significant SCA effects), and detect level of heterosis expression followed by the yield increases. This procedure has to be in space isolation in order to prevent gene flow and contamination with pollen from other undesirable alfalfa plants (Fig. 1). Our assumption is that hybrid plants will preserve and maintain significant level of heterosis, during years of multiplication, have fixed heterozygosity due to the existence of heterotic effects and that they will exhibit heterosis in the following years in preliminary trials comparing to parental populations, but more importantly with commercial check cultivars.

2) *Producing seed of population hybrids*

This option presents seedling of parental heterotic populations in rows isolation fields as it is shown in Figure 2. In the case of a hybrid between two populations, assuming equal hybridization among all plants, the resultant seed will consist of one-half inter-population hybrids and one-half hybrids among plants within populations (one quarter for each parental population). This population hybrid, however, can still capture partial heterosis (Brummer 1999).

Fig. 2. Heterotic parental populations in space isolation - seed production of population hybrids

A "semi-hybrid" breeding approach will work best if complementary populations, i.e. populations that carry favorable alleles for yield (or any other trait of interest) at a complementary set of loci that are affecting the trait – either exist in nature or have been (or can be) created by breeders (Brummer 2008).

In producing the seed of population hybrids, traits that are not directly related to hybrid vigor can be important as well. For instance, the maturation periods of the heterotic groups should be of equal length so that their ripening is simultaneous in order to secure as much cross pollination as possible (Milić et al. 2011b). In order to get as much crossing as possible in alfalfa, the reproductive biology of this crop should be studied in greater detail for the purposes of hybrid seed production, no matter how unattainable this goal might seem at the present level of knowledge.

Better understanding of the molecular genetic mechanisms causing heterosis could assist breeders in reliably creating high-yielding hybrids (Li et al. 2009). The studies of the relationship between the molecular variability of alfalfa populations and heterosis in their hybrids could contribute to a more precise selection of parental populations to be used for crossing in semi-hybrid alfalfa breeding procedure, with the aim to reduce number of necessary crossings and therefore make future alfalfa breeding programs more efficient. (Katić et al. 2011, Nagl et al. 2011). Until now marker assisted selection (MAS) has not broadly been implemented in perennial forage breeding programs (Riday 2011), with the main reasons being: a) small breeding programs with limited resources; b) lack of genomic tools and infrastructure and c) ploidy level of main forage species such as alfalfa. Applying molecular markers in alfalfa breeding should be as Brummer (2004) proposed, in order to facilitate creation and maintenance of genetic variation for main quantitative traits in diverse elite breeding populations, and that direction could help also in semi-hybrid breeding strategy in alfalfa.

Final step of this research will be comparison of free hybrids (F_2-F_4), and population hybrids versus heterotic parental populations and standard commercial cultivars. This type of preliminary trials should be in meadow conditions with large basic plots (10 m^2 at least), because heterosis expression in swards was lower than in the spaced planted nurseries (Riday & Brummer 2004), due to increased plant to plant competition related to plant morphology. Representing the task for future more precise analysis, the other issue is after how many generations of seed multiplication heterosis will still be present in the way that we proposed in this paper. Our assumptions are that seed multiplication must be stopped in isolated fields after 3-4 years. All these pointed issues, proposed methods should be studied in the following years more precisely in public and private breeding centers of alfalfa and in their partnership because addressed topic is one of the real and most interesting challenges for alfalfa breeding community in the 21st century.

Conclusions

The main future objective of alfalfa breeders should be to discover how to translate heterosis from single plants in hybrids planted in dense stand to generate "yield for free" (capture heterosis) in alfalfa semi hybrids.

Approach given in this paper presents the first attempt to explore and improve breeding of alfalfa in Serbia, in the way that is more familiar with other diploid field crops (maize, sunflower), but still with differences in the methodology applied.

Alfalfa reproductive biology should be studied in greater detail for the purposes of hybrid seed production, no matter how unattainable this goal might seem at the present level of knowledge.

The studies of the relationship between the molecular variability of alfalfa populations and heterosis in their hybrids could contribute to a more precise selection of parental populations to be used for crossing in semi-hybrid alfalfa breeding procedure.

There is a possibility that complex traits such as yield of alfalfa can be improved more successfully than has been done in the past. More options are arising on the horizon and their utilization and level of effectiveness, will keep alfalfa breeders community occupied and busy in the following years.

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Heterosis u oplemenjivanju lucerke

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Sažetak: Napredak u oplemenjivanju lucerke je spor najviše zbog njene složene genetičke strukture (autotetraploidna vrsta), kao i tetrasomičnog načina nasleđivanja. Aktuelni programi oplemenjivanja lucerke se oslanjaju na rekurentnu fenotipsku selekciju sa ili bez primene testova potomstava. Genetička kontrola važnih agronomskih osobina uslovljena je kako aditivnim delovanjem gena akumulacijom frekvencije poželjnih alela predstavljenim u značajnim efektima opštih kombinacionih sposobnosti (OKS) tako i neaditivnim delovanjem gena komplementarnim genskim interakcijama predstavljenim kroz značajne efekte posebnih kombinacionih sposobnosti (PKS). Ovakav način ispoljavanja genskih akcija kod lucerke uslovljava i način oplemenjivanja i promenu u metodama, pa se javlja ideja o semihibridnom oplemenjivanju lucerke. Ovaj koncept podrazumeva oplemenjivanje lucerke u okviru populacije, identifikaciju heterotične germplazme i proizvodnju semena. Istraživanja odnosa između molekularne varijabilnosti kod populacija lucerke i heterozisa kod hibrida nastalih ukrštanjem ovih populacija može doprineti preciznijoj i efikasnijoj selekciji roditeljskih populacija koje će biti korišćene u semihibridnom oplemenjivanju lucerke u cilju smanjivanja broja potrebnih ukrštanja čime se program oplemenjivanja čini značajno efikasnijim. Budući zadatak oplemenjivača lucerke je da se otkrije način iskorišćavanja heterozisa dobijenog na pojedinačnim biljkama i prenese na uslove useva u gustom sklopu i time omogući iskorišćavanje efekata heterozisa u vidu većih prinosa biomase u semihibridima lucerke.

Ključne reči: heterozis, hibridi, lucerka, oplemenjivanje biljaka, prinos useva, semihibrid