Heterotic effects and genetic distance for the prediction of agronomic performance in crossing divergent alfalfa populations

Dragan Milić

Ksenija Taški-Ajduković, Nevena Nagl Snežana Katanski,
Đura Karagić, Sanja Vasiljević and Slobodan Katić

dragan.milic@ifvcns.ns.ac.rs

Institute of Field and Vegetable Crops, Novi Sad, Serbia

2016 Joint Conference NAAIC, Trifolium, & Grass Breeders
July 12-14, 2016 · Madison, Wisconsin

Overview

PART I

- ***Introduction;***
- ***Objectives;***
- ***Material and methods;***
- ***Results***

PART II

- ***Breeding implications***
- ***First results***
- ***Hybrid seed production***

Introduction

- Alfalfa is an autotetraploid, allogamous species, where each cultivar is a heterogeneous population with heterozygous genotypes;
- The common breeding program approach in alfalfa includes recurrent phenotypic selection;
- Historically, the main goals of alfalfa breeding have not changed since the time of the first breeding programs;
- Alfalfa breeding for yield *per se* has been producing modest results;
- Complex genetic structure prevented the use of the heterotic effect in alfalfa.

Introduction

- The idea of the partial utilization of heterosis in alfalfa has been developing in parallel in the U.S. and Europe.

- A proposal has been made to the start with the development of semi-hybrids.

- This strategy for developing semi-hybrids based on making crosses among populations avoide the need for developing inbred lines and enables the partial utilization of the heterosis effect.

- In Europe, Rotili (Italy) was the first to realize and suggest that heterotic effects could be partially utilized in alfalfa through the development of free hybrids from lines resulting from two to three generations of selfing.

Introduction

- **Researches conducted in the late 20th and early 21st century demonstarte possible utilization of additive and nonadditive gene action in alfalfa breeding;**

- **Previous research clearly shows existence of heterosis in alfalfa (Riday and Brummer, 2002; Bhandari et al., 2007; Madril et al,. 2008; Al Lawati et al., 2010; Milić et al., 2011);**

- **Heterosis is related to inbreeding depression and outbreeding depression, since both, inbreeding between close relatives and outbreeding between distant populations can cause reduced fitness of progeny.**

Introduction

- Outbreeding depression occurs more often than reported;
- Crossing very distant genotypes can cause outbreeding depression in progeny;
- There is an optimal outcrossing distance for expression of heterosis effects.

Objectives

- **This research considers the concept of molecular and genetic basis of heterosis as well as possibilities of using genetic distance in prediction of heterotic effects in autotetraploid alfalfa (M. *sativa* ssp. *sativa* L.).**

- **The aim of this research was to explore the occurrence of heterosis by crossing divergent alfalfa populations and association of heterosis magnitude among the analyzed traits.**

- **Genetic distance and relationship between genetic distance and heterosis.**

- **Improvement of semi-hybrid breeding strategies in alfalfa:**

  - breeding alfalfas within the population;
  - identification of heterotic germplasm;
  - the production of hybrid seed.

Wisconsin (US) (169,639 km²) is **1.92** times bigger than Serbia (88,361 km²).

MATERIAL AND METHODS

STARTING POINT:
2003

Diallel crosses during 2003 and 2004 between 5 alfalfa populations (*Medicago sativa* L. ssp. *sativa*) of different geographic origin

NS Banat ZMS II (Serbia)
Ghareh Yon Geh (Iran)
Zuzana (Czech Republic)
Pecy (France)
RSI 20 (Spain)

MATERIAL AND METHODS

A spaced plant field was established in 2006 five alfalfa populations (parents) and 10 diallel hybrids and their reciprocals (F_1) sown in 3 replications. 20 plants per replication.

Plants were harvested five times in each of 2 years of research (2007 and 2008)

Agronomic traits: dry matter yield g plant^{-1}

plant height, internode number and length, regrowth rate, L/S ratio, stem diameter.

From each parental population, 10 individual samples were taken for DNA isolation and further RAPD analysis. Total genomic DNA was isolated from leaves according to the protocol of Somma.

DATA ANALYSES

Diallel analyses:

Zhang. Y.. M.S. Kang. and K.R. Lamkey. 2005. DILALLEL-SAS05: A Comprehensive Program for Griffing's and Gardner-Eberhart Analyses. Agronomy Journal 97: 1097-1106.

HPH= 100 * [(F1 – HP) /HP]

Contrasts Steel an Torrie (1980)

LSD test three way analysis.

Jaccard's coefficient of similarity was calculated and dendograms for varieties and individual samples were constructed by using Unweighted Pair Group Method of Arithmetic Mean (UPGMA).

MATERIALS AND METHODS

Current/future work:

During 2013-2015 at the Experiment Field of the IFVCNS, Novi Sad, Serbia hybrid seed was produced on two ways using ESP and FRA heterotic groups:

F_2 and F_3 hybrid seed, and seed of population hybrids (PH).

2014, 2015,2016 - ?Trials in swards, plot size 5 m^2.

Table 1. Mean values for list of alfalfa parents and hybrids.

Parents and hybrids	DM yield (g/plant)	Plant Height (cm)	Number of stems/plant	Internode number	Internode length (cm)	Stem diameter (mm)	Regrowth Rate (cm)	L/S ratio (%)
NS Banat ZMS II	108.6	82.0	48.3	13.2	6.2	4.0	40.4	49.2
Ghareh Yon Geh	112.2	79.0	55.0	13.0	6.0	3.6	38.8	49.9
Zuzana	96.6	72.9	50.7	12.5	5.9	4.0	33.3	51.2
Pecy	93.5	73.1	48.9	13.0	5.8	3.9	32.9	52.2
RSI 20	112.1	75.3	55.3	13.3	5.6	3.7	43.6	54.0
Parent average	104.6	76.5	51.6	13.0	5.9	3.8	36.7	50.0
♀ Banat x ♂GYG	107.5	77.6--	59.3	13.2	5.9--	3.8--	36.0--	46.8--
♀ Banat x ♂Zuzana	111.9	73.8--	54.3	12.7-	5.8--	4.0	33.2--	48.2--
♀ Banat x ♂Pecy	100.2	72.8--	49.9	13.0	5.6--	4.0	34.2--	51.6
♀ Banat x ♂RSI 20	119.9	79.4	59.9	13.5	5.9--	4.0	38.3--	50.2--
♀ GYG x ♂ Banat	118.4	74.6--	62.5*	13.1	5.7--	3.6--	37.3--	50.2
♀ GYG x ♂Zuzana	115.8	74.1--	58.0	12.6-	5.9	4.1	32.8--	50.7
♀ GYG x ♂Pecy	110.8	74.3--	56.5	13.3	5.6--	3.9	34.5--	50.8-
♀ GYG x ♂RSI 20	**122.1***	**78.5**	**53.9**	**13.3**	**5.9**	**4.2****	**37.5--**	**50.8--**
♀Zuzana x ♂ Banat	114.0	76.3-	62.7**	13.2	5.8--	4.0	35.1--	50.2
♀Zuzana x ♂GYG	119.4	77.0	58.7	13.1	5.9	3.8--	38.2	49.6-
♀Zuzana x ♂Pecy	98.7	69.7-	53.5	12.7	5.5--	3.8--	31.2	52.3
♀Zuzana x ♂RSI 20	117.0	75.8	60.2	12.8-	5.9	3.8--	37.8--	50.3--
♀Pecy x ♂ Banat	111.4	73.6--	54.6*	13.1	5.6--	3.9	34.5--	51.9
♀Pecy x ♂GYG	119.3	79.7	57.0	13.7**	5.8-	3.9	39.0	50.0--
♀Pecy x ♂Zuzana	105.9	70.9	54.9	12.9	5.5--	3.8--	32.8	54.1**
♀Pecy x ♂RSI 20	**128.9****	**79.0****	**57.8**	**13.6**	**5.8**	**4.1****	**39.3--**	**52.4-**
♀RSI 20 x ♂ Banat	118.9	79.5-	59.0	13.5	5.9--	4.0	39.2--	48.3--
♀RSI 20 x ♂GYG	122.7*	77.8	61.6	13.2	5.9	3.7	40.5-	51.0--
♀RSI 20 x ♂Zuzana	118.8	77.0	59.9	13.1	5.9	3.8--	39.5--	49.6--
♀RSI 20 x ♂Pecy	**124.0***	**77.2**	**55.5**	**13.1**	**5.9**	**4.0**	**40.9**	**51.1--**
Hybrid average	115.3	75.9	57.5	13.1	5.8	3.9	36.6	50
LSD 0.05	10.0	2.7	5.9	0.4	0.2	0.1	2.9	1.4
0.01	13.2	3.5	7.7	0.6	0.3	0.2	3.8	1.9

Table 2. Percent high-parent heterosis (HPH %) effects for followed traits

Hybrids	DM yield	Plant height	Number of stems	Int. number	Int. length	Stem diameter	Regrowth rate	L/S ratio
♀ Banat x ♂GYG	-4.4	-5.7**	7.3	0.0	-5.1*	-5.3*	-12.2**	-6.6**
♀ Banat x ♂Zuzana	2.9	-11.1**	6.6	-3.9*	-6.9**	0.0	-21.7**	-6.2**
♀ Banat x ♂Pecy	-8.4	-12.6**	2.0	-1.5	-10.7**	0.0	-18.1**	-1.2
♀ Banat x ♂RSI 20	6.5	-3.3	7.7	1.5	-5.1**	0.0	-13.8**	-7.6**
♀ GYG x ♂ Banat	5.2	-9.9**	12.0*	-0.8	-8.8**	-11.1**	-8.3**	0.6
♀ GYG x ♂Zuzana	3.1	-6.6**	5.2	-3.2*	-1.7	2.4	-18.3**	-1.0
♀ GYG x ♂Pecy	-1.3	-6.3**	2.7	2.3	-7.1**	0.0	-12.5**	-2.8*
♀ GYG x ♂RSI 20	**8.2***	-0.6	-2.6	0.0	-1.7	**11.9****	-16.3**	-6.3**
♀Zuzana x ♂ Banat	4.7	-7.5**	19.1**	0.0	-6.9**	0.0	-15.1**	-2.0
♀Zuzana x ♂GYG	6.0	-2.6	6.3	0.8	-1.7	-5.3*	-1.6	-3.2*
♀Zuzana x ♂Pecy	2.1	-4.9**	5.2	2.4	-6.8**	-5.3*	-6.7	0.2
♀Zuzana x ♂RSI 20	4.2	0.7	8.1	-3.9*	0.0	-5.3*	-15.3**	-7.4**
♀Pecy x ♂ Banat	2.5	-11.4**	10.4*	-0.8	-10.7**	-2.6	-17.1**	-0.6
♀Pecy x ♂GYG	6.0	0.9	3.5	5.1**	-3.4*	0.0	0.5	-4.4**
♀Pecy x ♂Zuzana	8.8	-3.1	7.7	-0.8	-7.3**	-5.3*	-1.5	3.5**
♀Pecy x ♂RSI 20	**13.0****	**4.7****	4.3	2.2	0.0	**4.9***	-10.9**	-3.1*
♀RSI 20 x ♂ Banat	5.7	-3.1*	6.3	1.5	-5.1**	0.0	-11.2**	-11.8**
♀RSI 20 x ♂GYG	8.6	-1.5	10.2*	-0.8	-1.7	0.0	-7.7**	-5.9**
♀RSI 20 x ♂Zuzana	5.6	2.2	7.7	-1.5	0.0	-5.3*	-10.4**	-8.9**
♀RSI 20 x ♂Pecy	**9.6***	2.5	0.4	-1.5	1.7	2.5	-6.6	-5.7**

**, * significant at the 0.01 and 0.05 level of probability;

Table 3. Estimates of genetic distances (GD) by Nei's coefficients based on marker data for alfalfa parental populations

Parents	Banat	GYG	Zuzana	Pecy	RSI 20
Banat	0.00	0.09	0.09	0.09	0.17
GYG		0.00	0.07	0.09	0.12
Zuzana			0.00	0.06	0.13
Pecy				0.00	0.11
RSI 20					0.00

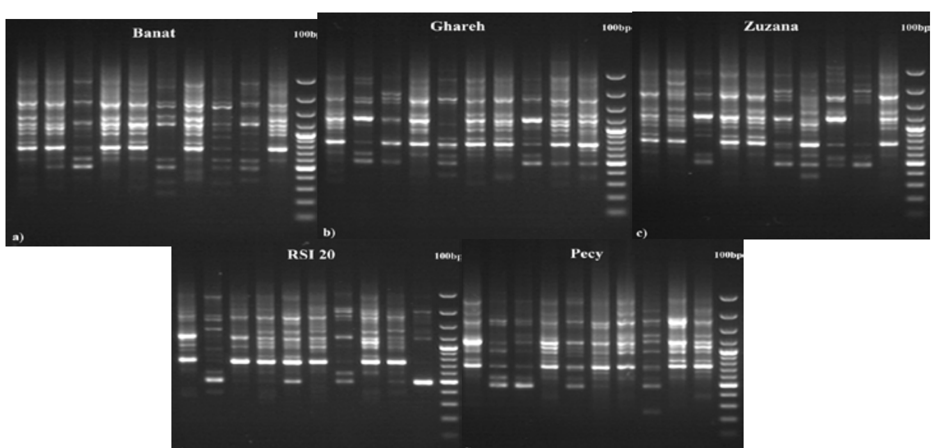
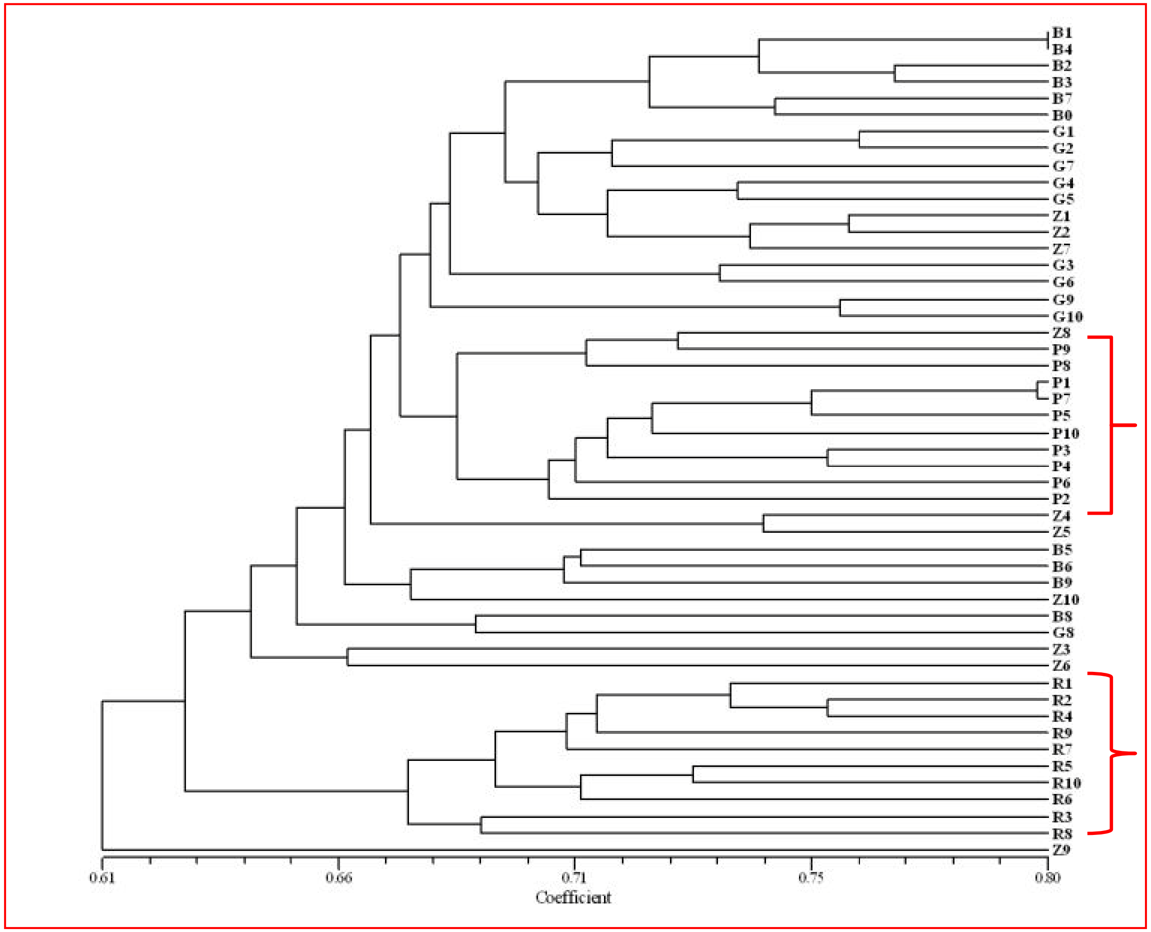


Figure 1. Dendogram of 50 individual samples based on unweighted pair group arithmetic mean method cluster analysis of Jaccard's similarity coefficient. (B1 - B10 - Banat, G1 - G10 - Ghareh, Z1 - Z10 - Zuzana, P1 - P10 - Pecy, R1 - R10 - RSI 20). Numbers represent bootstrap confidence limits for 1000 replicates



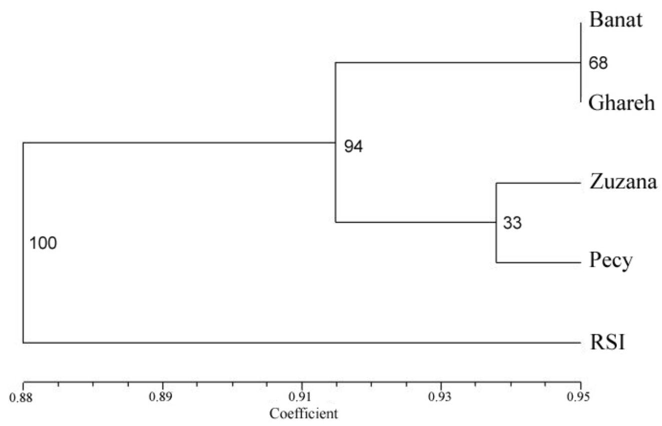


Table 4. Correlations coefficients between mean values of observed traits of alfalfa hybrids (20 means)

HPH	Plant height	Number of stems	Int. number	Int. lenght	Stem diameter	Regrowth rate	Leaf to stem ratio in DMY
Dry matter yield	0.80**	0.51*	0.56*	0.72**	0.29 ns	0.83**	-0.14 ns
Plant height	-	0.47*	0.80**	0.80**	0.26 ns	0.84**	-0.48*
Number of stems/plant		-	0.31 ns	0.53*	-0.40 ns	0.47*	-0.38 ns
Internode number			-	0.32 ns	0.14 ns	0.69**	-0.15 ns
Internode length				-	0.23 ns	0.69**	-0.62**
Stem diameter					-	-0.05 ns	-0.04 ns
Regrowth rate						-	-0.25 ns

**,* significant at the 0.01 and 0.05 level of probability; ns = not significant.

Part II
Breeding implications

- Current task of alfalfa breeders should be to discover how to translate heterosis from single plants in hybrids planted in swards to generate "yield for free" (capture heterosis) in alfalfa semi hybrids.

FOCUS:
Medicago sativa ssp.*sativa* x *Medicago sativa* ssp.*sativa*

Part II
Breeding implications

- Heterosis expression in alfalfa determines the way in which breeding is carried out and brings about changes in the methods used.
- It has given rise to the idea of the semi-hybrid breeding of this crop which involves:

a) breeding alfalfas within the population;

b) identification of heterotic germplasm (same/close dormancy classes)

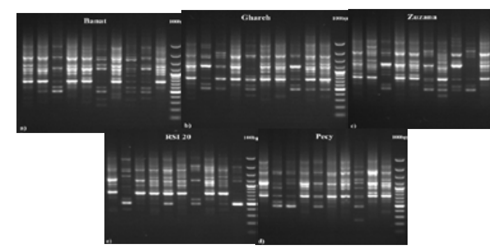
c) production of seed of the free/semi hybrids.

Breeding implications

A) BREEDING ALFALFA WITHIN THE POPULATION (RPS, continuously)

B) IDENTIFICATION OF HETEROTIC GERMPLASM

A x B → F_1



DNA markers:

- Selection of heterotic groups;
- Estimation of genetic distance
- Reduction of redundant crosses

C) PRODUCTION OF THE HYBRID SEED: FOLLOWING STEPS:

Planting of F_1 hybrids:

F_2 seed multiplication - space plants in space isolation (2013-2014)

F_3 seed multiplication in space isolation (2015-2016 1 acre)

Planting of heterotic populations in rows: population hybrids (PH)

A B A B A

PH_1 seed multiplication in space isolation (2013-2014)

PH_2 seed multiplication in space isolation (2015-2016) 1 acre

Table 4. Preliminary results of different hybrid combinations, commercial checks and parental populations in 2015. (6 cuts)

Sorta	DMY t ha^{-1}	Plan height cm	Regrowht rate cm
F_2 ♀Pecy x ♂RSI 20	18.8	62.9	37.2
PH_1 2013	19.9	63.4	37.3
Pecy PH	18.5	61.7	34.4
RSI 20 PH	19.7	64.8	38.0
Pecy (parent)	16.1^{--}	55.0	31.0
RSI 20 (parent)	19.3	64.6	38.4
Banat VS	20.2	62.2	37.0
NS Mediana ZMS II	20.1	64.1	37.2
Average	19.0	62.3	36.3
CV%	5.3	2.2	2.6
LSD 0.05 0.01	2.1 2.8	2.6 3.5	1.3 1.8

Part II
Breeding implications

FINAL STEPS

↓

Comparison: hybrids (F_2-F_3) , pop. hybrids (PH_1 – PH_2), versus check varieties and heterotic populations;
2014-2020

- **Alfalfa is an autotetraploid species, and it is expected that the effects of heterosis will sustain throughout the few generations of multiplying semi-hybrid seed, which remains to be experimentally confirmed.**

- **Attempt to uncover the relations among heterosis effects and genetic distance between and inside alfalfa heterotic groups.**

- **This research can contribute to improvement of alfalfa breeding methods.**

- **Further understanding of molecular and genetic base of heterosis, will help to better utilize this important biological phenomenon, which is the backbone of multi-billion dollar business in agriculture.**

ACKNOWLEDGMENTS:

- Scientific board NAAIC 2016
- Charlie Brummer

Crew at IFVCNS, Forage crops Department:
Dr. SLOBODAN KATIC
Dr. Đura Karagic
Dr. Sanja Vasiljevic
Dr Ksenia Taski-Ajdukovic
Dr Nevena Nagl
Snezana Katanski
Branko Milosevic

THANKS FOR YOUR ATTENTION