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COMBINING ABILITIES AND HETEROSIS FOR DRY MATTER YIELD IN ALFALFA DIALLEL CROSSES

Marijana Tucak[1]*, Svetislav Popović[1], Tihomir Čupić[1], Valentina Španić[1], Branimir Šimić[1], and Vladimir Meglič[2]

[1]Agricultural Institute Osijek, Južno predgrađe 17, 31000 Osijek, Croatia.

[2]Agricultural Institute of Slovenia, SI-Hacquetova 17, 1000 Ljubljana, Slovenia.

*Corresponding author. E-mail: marijana.tucak@poljinos.hr

ABSTRACT

The combining ability and heterosis for dry matter yield of seven previously selected high yielding alfalfa cultivars were estimated using a half-diallel crosses. These cultivars originated from Croatia (OS-88, Slavonka), Denmark (Resis), Bulgaria (Prista), USA (Columbia 2000) Australia (Super 10) and Argentina (Lujan). Dry matter yield of the 21 F_1 progenies and their parents were evaluated during two growing seasons (2008 and 2009) at the experimental field of the Agricultural Institute Osijek in Croatia. The analysis of variance indicated significant differences between parents and F_1 progenies for dry matter yield. An analysis of the components of combining ability showed that general and specific combining ability effects were highly significant. Significant positive general combining ability effects were recorded in parents OS-88 and Columbia 2000. The five F_1 progenies were obtained between the six highest-yielding crosses throughout the experiment and they demonstrated significant positive specific combining ability effects. Mid-parent heterosis ranged from -18.42 to 61.47% and high-parent heterosis ranged from -22.94 to 58.70%. The highest yields, significant specific combining ability effects and highest mid and high parent heterosis were found in the progenies OS-88 x Columbia 2000, OS-88 x Super 10, Slavonka x Columbia 2000 and Slavonka x Super 10. Therefore cultivars OS-88 and Columbia 2000 demonstrated favourable genetic potential that could contribute to the improvement of alfalfa yield in our breeding programme. The results of this study indicated that the development of progenies superior in yield is possible in crosses of high yielding *Medicago sativa* cultivars of different geographic origin.

Key words: alfalfa, dry matter yield, diallel crosses, combining abilities, heterosis, breeding.

INTRODUCTION

The alfalfa breeding programs have mainly focused on increasing forage yield and on improvement of the persistence, diseases or insect resistance and biomass quality. Although previous work of most the programs achieved excellent progress in improving resistance and other economically important traits, less progress has been made in genetic improvement of forage yield per se. The genetic increases in alfalfa yield have been small compared with those realized in other crops. According to estimates from the middle of the last century the yield of alfalfa increased only from 0.15 to 0.30% or less per year, while the maize yield in the same period increased in average 2% per year (Skinner et al., 2000).

There are many reasons contributing the slow progress in the improvement of alfalfa yield such as: complexity genetics of autotetraploids, perennial nature, inability to exploit heterosis in commercial cultivars, use of inefficient selection methods that make usage of additive genetic variance (Casler and Brummer, 2008; Tucak et al., 2008, 2009; Kumar, 2011).

Previous researches have demonstrated that improved alfalfa yield can be possible by utilization of additive and nonadditive gene effects, including higher-order allelic and complementary gene interactions associated with autotetraploid inheritance. Large portion of the genetic variation for forage yield has been explained by nonadditive components, and it was found that approximately two-thirds of the variance for yield in alfalfa was

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nonadditive (Rowe and Hill, 1981; Dudley et al., 1969).

The importance of general (GCA) and specific combining ability (SCA) in determining forage yield has been documented. Several studies reported a significant amount of GCA (additive gene action) contributing to genetic variation of forage yield, while SCA (nonadditive gene action) was not significant which is explained by the fact that in some experiments half-sibs were used as parents instead of clones. Also, detection of SCA effect in alfalfa, which is an autotetraploid, is very difficult due to existence of both trigenic and tetragenic gene action. According to Riday and Brummer (2002), Segovia-Lerma et al. (2004) and Bhandari et al. (2007), SCA effects can be detected by crossing genetically divergent and distant genotypes/populations of alfalfa to produce hybrids/progenies. When diallel analysis is used in crossing divergent populations, the degree of expression additive and nonaditive genetic variance, possible heterotic groups in the parental components and degree of heterosis expression can be determined, and this may contribute in increasing forage yield per se in alfalfa breeding programs (Brummer, 1999; Riday et al., 2003; Milić et al., 2011).

The objectives of this study were: (i) to estimate combining abilities and heterosis for dry matter yield using a half-diallel crosses system between alfalfa cultivars of different geographical origin, (ii) to evaluate dry matter yield of the parents and their F_1 progenies for determining heterotic responses, and (iii) to demonstrate the genetic potential of cultivars which will be used to improve alfalfa yield in our breeding programme.

MATERIAL AND METHODS

Selection of parental cultivars

Seven alfalfa cultivars of different geographic origin were used as parents in this experiment: OS-88 and Slavonka created at the Agricultural Institute Osijek from Croatia, Columbia 2000 from USA, Resis from Denmark, Super 10 from Australia, Prista from Bulgaria and Lujan from Argentina. In previous research (from 2004 till 2006) in field trials (nurseries, RCBD design in three replications, 108 spaced plants/germplasm) at the Agricultural Institute Osijek, these seven cultivars were selected for the current study, because of their highest yielding performance among 72 alfalfa germplasm.

Crossing, experimental design and field evaluation

The seven selected parental cultivars were crossed in the field during July 2007 in a half-dialel mating design. For each pairwise cross, five individual plants were randomly chosen from each of the two cultivars. Plants were hand-crossed without emasculation (approximately 80 flowers/cross pair, i.e. 400 flowers/combination). After elimination of all open flowers on the inflorescence of female plant, the remaining closed flowers were opened, pollinated by sterile glass containing pollen of male plant and finally protected by small transparent paper bags. Seeds from each female plant in all cross combinations were harvested separately by hand at the end of August 2007. Five seed samples of each combination were bulked in equal frequency. The seven selected parental cultivars and their 21 F_1 progenies were sown at March 2008 in a peat pellets and grown in a greenhouse for the next one months. Well-developed young plants (144 plants/progenies and parents in total) were transplanted in the field of the Agricultural Institute Osijek. Field experiment was designed as a randomised complete block with four replications. Each plot consisted of three 5.5 m long rows spaced approximately 40 cm apart. Twelve plants spaced approximately 50 cm apart were planted in each row for a total of 36 plants for each progeny/parent per plot. The plants were cut three times during 2008 (July-September) and six times during 2009 (May-October). In both growing seasons, forage yield data were collected on all individual plants of each progeny/parent in all cuts. The cutting was done manually by sickle at a cutting height of approximately 7 cm above the ground. Immediately after cutting, the green mass yield of each plant was weighed in the field using an electronic balance (Ohaus Scout II).

To determine the dry matter yield fresh forage samples (approximately 500 g) were taken randomly from each plot of all progenies/parents at each cutting. After weighing, the samples were dried at 105°C for 24 h and weighed again to determine the percentage of dry matter. The dry matter yield (g plant^{-1}) was calculated as dry matter content × green mass yield/100, and expressed as a two-year average of the total yield per year.

Data analysis

The analyses of variance (ANOVA) for general combining ability (GCA) and specific combining ability (SCA) for dry matter yield were carried out according to Griffing's (1956) Method 2 (parents and F_1 progenies) Model 1 (fixed effects) with the diallel analysis package of MSTAT-C software (MSTAT-C, 1990). Comparisons of the yield performance of the parents and their progenies were made on the basis of a least significant differences (LSD) mean separation test.

Mid-parent heterosis (MPH), which describes the performance of crosses (F1) relative to average performance of their parents (P_1 and P_2), and high-parent heterosis (HPH), which describes performance of crosses (F1) relative to the highest-yielding parent (HP), were estimated according to Hallauer and Miranda (1988) and computed as follows:

$$MPH = 100 \times [F1 - \{(P1 + P2)/2\}/ (P1 + P2)/2] \text{ and } HPH = 100 \times (F1 – HP)/HP$$

The significance of heterosis effects was estimated using a Student t-test (Al Lawati et al., 2010).

RESULTS AND DISCUSSION

The analysis of variance of the diallel crosses revealed significant differences between parents and F_1 progenies for dry matter yield.

The highly significant GCA and SCA effect obtained in this study indicated that both additive and nonaditive genetic effects were involved in the control of dry matter yield in alfalfa (Table 1). This implies that dry matter yield improvement can be achieved by accumulating favourable alleles and exploiting heterosis effects.

Table 1. Analysis of variance in alfalfa diallel crosses for dry matter yield, conducted according to Griffing's Method 2 (1956)

Sources of variation	Degrees of freedom	Sum of squares	Mean square	F-value
Blocks	2	2984.00	1491.99	1.19
Genotypes	27	418403.45	15496.42	12.32**
GCA	6	232817.14	38802.85	30.84**
SCA	21	185586.30	8837.44	7.02**
Error	54	67946.90	1258.27	

**Significant at P < 0.01 level, GCA = General combining ability, SCA = Specific combining ability.

In the present study, the mean square of GCA was nearly fourfold higher compared to that for SCA, suggesting that additive effect was of the major importance. The predominance of GCA in determining forage yield of alfalfa has been documented in previous studies of several authors (Song and Walton, 1975; Hill, 1983; Groose and Bingham, 1988; Roy et al., 2002; Segovia-Lerma et al., 2004; Bhandari et al., 2007; Milić et al., 2011). Yield performance of the parents and their F_1 progenies are presented in Table 2. Parent cultivars differed significantly in average dry matter yield, which ranged from 205.16 g plant^{-1} (cultivar Prista) to 289.46 g plant^{-1} (cultivar Columbia 2000). Significantly higher yields, in comparison to other cultivars, had Columbia 2000 and Lujan (274.26 g plant^{-1}). These two cultivars achieved yields, of 14.46% and 8.54% higher, respectively, in comparison to average dry matter yield of all parents (252.66 g plant^{-1}). The average yield of all progenies was 284.25 g plant^{-1}. Nine progenies (OS-88 x Columbia

2000, Slavonka x Columbia 2000, OS-88 x Super 10, Slavonka x Super 10, Columbia 2000 x Super 10, and all cross combinations with cultivar Lujan, excluding those with cultivars Prista and Resis) of the total 21, had yields significantly higher than the average of the parents. This progenies obtained higher yields, in a range from 23.10% (311.04 g plant^{-1} in crosses Super 10 x Lujan) to 72.30% (435.34 g plant^{-1} in crosses OS-88 x Columbia 2000), when compared with the average yield of the parents. The progeny which had parents with high yielding performance from the same geographical origin (Croatian cultivars OS-88 and Slavonka) had lower yield than progenies that were obtained from crosses between genetically divergent cultivars. These results are consistent with previous studies of Segovia-Lerma et al. (2004), Katić et al. (2010) and Tucak et al. (2011), where significant positive associations were found between genetic diversity of parental cultivars/populations/germplasm and yield of their single-cross progenies/hybrids.

Table 2. Average dry matter yield (g plant^{-1}) of parents (diagonal, underlined) and their diallel F_1 progenies (above diagonal), over two years (2008, 2009)

Parents/Crosses	1	2	3	4	5	6	7
(1) OS-88	<u>267.99</u>	254.24	435.34**	233.45	425.32**	212.22	358.57**
(2) Slavonka		<u>244.33</u>	389.73**	213.17	376.54**	192.78	353.41**
(3) Columbia 2000			<u>289.46</u>**	231.96	326.08**	223.05	332.54**
(4) Resis				<u>228.62</u>	222.49	176.94	237.33
(5) Super 10					<u>258.80</u>	224.65	311.04**
(6) Prista						<u>205.16</u>	238.42
(7) Lujan							<u>274.26</u>*
Average parent: 252.66 g plant^{-1}, LSD 0.05 = 18.68, LSD 0.01 = 26.19 Average F_1 progeny: 284.25 g plant^{-1}							

*, ** = Yields significantly higher than the average of the parents at P < 0.05 and P < 0.01 level, respectively.

GCA ability estimates ranged from -59.16 for cultivar Prista to 34.08 for cultivar Columbia 2000 (Table 3). Significant positive GCA effect was recorded in parents OS-88 and Columbia 2000, indicating that these two cultivars are good combiners and possess potential to improve dry matter yield in our environmental conditions. Also, positive GCA effects were demonstrated by cultivars Super 10, Slavonka and Lujan, while cultivars Resis and Prista had significantly negative GCA effect. Differences between alfalfa genotypes/populations/cultivars for GCA effects have been previously reported by Segovia-Lerma et al. (2004), Sakiroglu and Brummer (2007), Bhandari et al. (2007) and Milić et al. (2011).

Table 3. Estimates of diallel effects for general combining ability (GCA) and specific combining ability (SCA) for alfalfa dry matter yield

Effect	Estimate	Effect	Estimate	Effect	Estimate	Effect	Estimate
GCA_1	27.14*	SCA_{12}	-55.66**	SCA_{24}	-20.90	SCA_{37}	3.32
GCA_2	6.41	SCA_{13}	97.75**	SCA_{25}	72.34**	SCA_{45}	-26.59
GCA_3	34.08**	SCA_{14}	-21.35	SCA_{26}	-30.82	SCA_{46}	8.43
GCA_4	-48.69**	SCA_{15}	100.39**	SCA_{27}	51.86*	SCA_{47}	-9.10
GCA_5	21.43	SCA_{16}	-32.12	SCA_{34}	-29.78	SCA_{56}	13.97
GCA_6	-59.16**	SCA_{17}	36.29	SCA_{35}	-5.80	SCA_{57}	-5.52
GCA_7	18.77	SCA_{23}	72.87**	SCA_{36}	-28.23	SCA_{67}	2.44
*g_i 0.05 = 22.00 **gi 0.01 = 29.33		*sij 0.05 = 41.16 **sij 0.01 = 54.85					

Diallel effect (GCA) of parental cultivars designated as 1 = OS-88; 2 = Slavonka; 3 = Columbia 2000; 4 = Resis; 5 = Super 10; 6 = Prista; 7 = Lujan. SCA designations such as 12 reflect the SCA of the progeny between OS-88 (1) and Slavonka (2), 13 reflect the SCA of the progeny between OS-88 (1) and Columbia 2000 (3) etc.
gi = least significant differences between GCA effects of parents.
sij = least significant differences between SCA effects of the F_1 progenies.

The estimates of GCA effect in our study were different in magnitude compared with the results of these authors, which is probably associated with the type of population evaluated (forage yield range of parental population). Five of 21 F_1 progenies (OS-88 x Columbia 2000, OS-88 x Super 10, Slavonka x Columbia 2000, Slavonka x Super 10 and Slavonka x Lujan), demonstrated significant positive SCA effects, which indicates that desirable recombinations of genes were present in these crosses (Table 3). These F_1 progenies were between the six highest-yielding crosses in the whole experiment. The progenies OS-88 x Columbia were produced by crossing two best general combiners (significant positive GCA effects), while all other progenies were obtained by crosses low x high and/or high x low, low x low general combiners. Dry matter yield of these progenies was superior to the average yield of their parental cultivars. In most cases, negative SCA effects were found in progenies obtained from crossing combinations with the parent that had lowest yielding. Negative SCA effects were found in the crosses between OS-88 x Slavonka, Columbia x Super 10 and Super 10 x Lujan.

Significant positive values for MPH (mid-parent heterosis) and HPH (high-parent heterosis) were found in four progenies (OS-88 x Columbia 2000, OS-88 x Super 10, Slavonka x Columbia 2000, Slavonka x Super 10). These results indicate that overdominance and/or complementary partial to complete dominance and epistasis were contributing toward their performance.

The progenies OS-88 x Super 10 had highest MPH (61.47%) and HPH (58.70%) values (Table 4). All progenies which had cultivars Prista or Resis as one of the parent, as well as the progeny derived from crossing these two cultivars, demonstrated negative MPH and HPH (lowest values: -18.42% for MPH in the Resis x Prista and -22.94% for HPH in the Prista x Columbia 2000).

Negative heterosis obtained in these combinations might be the result of high non complementarity between parental cultivars and/or a stronger negative effect of genes present in both parental components. Also, low MPH and HPH values of progenies which had parents Prista or Resis reflected the fact that they were the lowest yielding cultivars in this study.

Table 4. Mid-parent heterosis (MPH, above diagonal) and high-parent heterosis (HPH, below diagonal) for dry matter yield in diallel crosses between seven alfalfa cultivars

Parents	1	2	3	4	5	6	7
(1) OS-88	-	-0.75	56.19**	-5.98	61.47**	-10.29	32.25
(2) Slavonka	-5.13	-	46.02*	-9.85	49.67**	-14.22	36.29
(3) Columbia	50.40**	34.64*	-	-10.45	18.95	-9.81	17.98
(4) Resis	-12.88	-12.75	-19.86	-	-8.70	-18.42	-5.60
(5) Super 10	58.70**	45.49**	12.65	-14.02	-	-3.15	16.69
(6) Prista	-20.81	-21.10	-22.94	-22.60	-13.19	-	-0.53
(7) Lujan	30.74	28.85	14.88	-13.46	13.40	-13.06	-

*, ** = Significant at P < 0.05 and P < 0.01 level, respectively

The highest yields, significant SCA effects and highest MPH and HPH values were found in the progenies OS-88 x Columbia 2000, OS-88 x Super 10, Slavonka x Columbia 2000 and Slavonka x Super 10. Our results showed that the selection based on MPH and HPH could be successful or/and useful in the choice of parental components for the improvement of alfalfa yield in our breeding programme.

A similar result was reported by Milić et al. (2010), while Bhandari et al. (2007) indicated that parent selection based strictly on MPH or HPH may sometimes be misleading, and in plant breeding programs the choice of hybrids/progenies should be based on their performance relative to commercial check cultivars, rather than their absolute or relative heterosis response.

CONCLUSIONS

The results of this study indicated that contributions of both GCA and SCA effects were important in explaining variation for dry matter yield between the alfalfa progenies. Development of progenies superior in yield may be possible by crossing high yielding *Medicago sativa* cultivars of different geographic origin. Five of nine highest yielding crosses had at least one parent which possessed significant positive GCA effect. These progenies were between the six highest-yielding crosses in the whole experiment. The magnitude of GCA effects were four times larger than that for SCA effects, and five highest yielding crosses demonstrated significant positive SCA effects, indicating relative importance of this effects in determining yield of the progeny. The obtained results showed clearly that alfalfa yield can be improved by exploiting additive and non additive gene effects. The highest yields, significant SCA effects and highest MPH and HPH values were found in the progenies OS-88 x Columbia 2000, OS-88 x Super 10, Slavonka x Columbia 2000 and Slavonka x Super 10. Our results suggested that the selection based on MPH and HPH could be successful or/and useful in the choice of parental components for the improvement of alfalfa yield in our breeding programme.

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REFERENCES

Al Lawati, A.H., Pierce, C.A., Murray, L.W., Ray, I.M., 2010. *Combining ability and heterosis for forage yield among elite alfalfa core collection accessions with different fall dormancy responses.* Crop Science, 50: 150-158.

Bhandari, H.S., Pierce, C.A., Murray, L.W., Ray, I.M., 2007. *Combining abilities and heterosis for forage yield among high-yielding accessions of the alfalfa core collection.* Crop Science, 47: 665-673.

Brummer, E.C., 1999. *Capturing heterosis in forage crop cultivar development.* Crop Science, 39: 943-954.

Casler, M.D., Brummer, E.C., 2008. *Theoretical expected genetic gains for among-and-within-family selection methods in perennial forage crops.* Crop Science, 48: 890-902.

Dudley, J.W., Busbice, T.H., Levings, III C.S., 1969. *Estimates of genetic variance in Cherokee alfalfa (Medicago sativa L.).* Crop Science, 9: 228-231.

Griffing, B., 1956. *Concept of general and specific combining ability in relation to diallel crossing systems.* Australian Journal of Biological Sciences, 9: 463-493.

Groose, R.W., Bingham, E.T., 1988. *Combining ability differences between isogenic diploid and tetraploid alfalfa.* Crop Science, 28: 7-10.

Hallauer, A.R., Miranda, J.B., 1988. *Quantitative genetics in maize breeding*. Iowa State University, Ames: 337-373.

Hill, R.R., 1983. *Heterosis in population crosses of alfalfa.* Crop Science, 23: 48-50.

Katić, S., Milić, D., Mihailović, V., Vasiljević, S., Karagić, D., 2010. *Effects of heterosis for yield and yield components obtained by crossing divergent alfalfa populations.* Field and Vegetable Crops Research, 47: 203-208.

Kumar, S., 2011. *Biotechnological advancements in alfalfa improvement.* Journal of Applied Genetics, 52: 111-124.

Milić, D., Katić, S., Mikić, A., Karagić, D., 2010. *Heterotic response from a diallel analysis between cultivars of different geographic origin.* In: Huyghe C, ed. Sustainable Use of genetic Diversity in Forage and Turf Breeding. Springer, New York: 551-556.

Milić, D., Katić, S., Karagić, D., Gvozdanović-Varga, J., Petrović, S., Boćanski, J., 2011. *Genetic control of agronomic traits in alfalfa (M. sativa ssp. sativa L.).* Euphytica, 182: 25-33.

MSTATC, 1990. *MSTATC user guide: A microcomputer program for the design, management, and analysis of agronomic research experiments*. Michigan State University, East Lansing, MI.

Riday, H., Brummer, E.C., 2002. *Forage yield heterosis in alfalfa.* Crop Science, 42: 716-723.

Riday, H., Brummer, E.C., Campbell, T.A., Luth, D., Cazcarro, P.M., 2003. *Comparisons of genetic and morphological distance with heterosis between Medicago sativa subsp. sativa and subsp. falcata.* Euphytica, 131: 37-45.

Rowe, D.E., Hill, R.R.Jr., 1981. *Inter-population improvement procedures for alfalfa.* Crop Science, 21: 392-397.

Roy, N.C., Jettopujov, V.N., Solanik, N.M., 2002. *Combining ability for some agronomic characters in alfalfa (Medicago sativa L.).* Pakistan Journal of Agricultural Research, 17: 346-350.

Sakiroglu, M., Brummer, E.C., 2007. *Little heterosis between alfalfa populations derived from the midwestern and southwestern United States.* Crop Science, 47: 2364-2371.

Skinner, D.Z., Loughin, T., Obert, D.E., 2000. *Segregation and conditional probability association of molecular markers with traits in autotetraploid alfalfa.* Molecular Breeding, 6: 295-306.

Segovia-Lerma, A., Murray, L.W., Townsend, M.S., Ray, I.M., 2004. *Population-based diallel analyses among nine historically recognized alfalfa germplasms.* Theoretical and Applied Genetics, 109: 1568-1575.

Song, S.P., Walton, P.D., 1975. *Combining ability, genotype × environment interactions and genotypic correlations of agronomic characters in Medicago sativa L.* Euphytica, 24: 471-481.

Tucak, M., Popović, S., Čupić, T., Grljušić, S., Bolarić, S., Kozumplik, V., 2008. *Genetic diversity of alfalfa (Medicago spp.) estimated by molecular markers and morphological characters.* Periodicum Biologorum, 110: 243-249.

Tucak, M., Popović, S., Čupić, T., Šimić, G., Gantner, R., Meglič, V., 2009. *Evaluation of alfalfa germplasm collection by multivariate analysis based on phenotypic traits.* Roman. Agric. Res., 26: 47-52.

Tucak, M., Popović, S., Grljušić, S., Čupić, T., Bolarić, S., 2011. *Implementation of molecular markers diversity in parental selection of alfalfa (Medicago sativa L.).* Biotechnology & Biotechnological Equipment, 25: 2310-2314.