

Alfalfa Fertility under Conditions of Cross-Fertilization*

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Abstract: This paper presents data on the vitality of male gametophyte (pollen) and the number of unfertilized ovules per ovary in 17 genetically divergent genotypes under conditions of cross-fertilization in a polycross test. Pollen vitality in the studied alfalfa genotypes ranged from 59.3% to 85.3%, or 76.6% on average. The genotypes Ri-11, Di-4 and Rs-2 were distinguished for high pollen vitality - 85.3%, 81.6% and 81.2%, respectively. The other genotypes had significantly lower values of this parameter. The genotypes had 9.3 to 9.8 unfertilized ovules per ovary, without significant differences among them. The obtained results on the vitality of male gametophyte and the number of unfertilized ovules per ovary show that the studied alfalfa genotypes had high fertilization potential. The correlation between pollen vitality and number of ovules per ovary was not significant ($r=-0.445$).

Key words: alfalfa, genotypes, fertility, cross fertilization.

Introduction

For high alfalfa production, varieties with improved genetic potential for forage yield, dry matter quality and seed yield are needed. Breeding and development of alfalfa varieties play an important role in alfalfa production in this as well as in other countries around the world. Study of the vitality of male gametophyte and the number of unfertilized ovules per ovary helps in gaining fuller insight into genetic variability as a prerequisite for the selection of parent components and the application of effective breeding methods in the process of development of superior alfalfa varieties.

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Effect of genetic factors and ecological conditions on alfalfa seed production have been extensively studied (Golodkovskii *et al.*, 1971; Guy *et al.*, 1973; Demarly, 1977; Đukić *et al.*, 1987, 1992, Đukić and Bolanos, 1998; Bolanos *et al.*, 1999; Radović *et al.*, 2003). Among the genetic factors that affect alfalfa seed yield, particularly important are the inflorescence morphology, pollen vitality, number of ovules per ovary and the interaction of these factors with ecological conditions (Guy *et al.*, 1973; Đukić, 1978; Hacquet, 1990; Lukić, 2000). The importance of the interaction genotype x environment for alfalfa seed yield and yield components has been emphasized by numerous researchers (Bolanos-Aquilar *et al.*, 2001; Hill and Baylor, 1983; Rod, 1979). Furthermore, Debrand (1976) pointed out the importance of this interaction for increased fertility in alfalfa genotypes.

In consideration of the importance of new alfalfa varieties with improved potential for forage and seed yields, the objective of this study was to examine the genetic variability for the vitality of male gametophyte (pollen) and the number of unfertilized ovules per ovary under the conditions of cross fertilization. Better knowledge of these characteristics may help in the selection of promising genotypes for breeding of yield-superior alfalfa varieties.

Material and Method

Seventeen genetically divergent genotypes from the alfalfa breeding program at Institute of Field and Vegetable Crops in Novi Sad have been analyzed for the vitality of male gametophyte and the number of unfertilized ovules per ovary.

The study included domestic varieties NS-Slavija (1990), NS-Bačka ZMS I (derived genotype Bačka-136) and NS-Banat ZMS II (derived genotype Banat-139), a French variety Europe (1961, INRA, Lusignan) and a Bulgarian variety Prista 3 (Institute of Agriculture and Seed Science "Obraztsov chiflik", Rouse). The other genotypes were obtained from France, Bulgaria, Denmark and U.S.A.

Experiments were conducted at the experiment field of Forage Crops Department of the Institute. Analyses were conducted in the cytology laboratory of Oilcrops Department of the Institute. The facilities of both departments are located at Rimski Šančevi.

The experiments were established in a randomized blocks design with six replications, on the slightly calcareous chernozem soil, in spring 1999 (A_0). Fertility of alfalfa genotypes from the second growth (A_1 , C_2) was analyzed in the year 2000, from the third growth (A_2 , C_3) in 2001 and from the second growth (A_3 , C_2) in 2002.

At the flower stage, racemes were randomly sampled for the analyses of pollen vitality and the number of unfertilized ovules per ovary. Pollen vitality (%) was analyzed in laboratory by the staining method (Alexander, 1969). Nine florets per genotype (3 florets x 3 replications) were sampled from different racemes for the analysis of pollen vitality. Anthers from randomly sampled florets (in each replication) were mounted on slides and pollen staining was performed. Vital pollen grains stained red, sterile stained green. Pollen staining was performed on a total of 1500-2000 pollen grains. Pollen vitality (%) was calculated on the basis of the proportion of vital in the total number of pollen grains.

Number of ovules per ovary was determined according to the methodology of Dattée (1972). Ovules were counted under a stereo microscope, in ovaries separated from 10 randomly selected florets per genotype.

Results of pollen vitality analysis were processed after transformation into $\arcsin \sqrt{\text{percentage}}$. Significance of mean values for both characteristics was determined by the analysis of variance (ANOVA) and *t*-test. Standard deviation (S), variation coefficients (CV%) and correlations coefficients (r) were also calculated (Hadživuković, 1989).

Results and Discussion

Together with other alfalfa seed yield components, pollen vitality has a significant effect on the number of pods formed and the number of seeds per pod.

In the three-year study, pollen vitality was fairly uniform, from 74.6% to 78.8% or 76.6% on average. Highest pollen vitality was exhibited by the genotypes Ri-11, Di-4 and Rs-2 (85.3%, 81.6% and 81.2%, respectively). Significantly lower vitality was registered in S-64 and Bačka-136, 64.1% on average (Table 1).

In the first year of study (A_1), highest values of pollen vitality were registered in 177-B and Ri-11, 91.5% and 90.3%, respectively. In relation to the average vitality for that year (78.8%), increased values were found in Europe, Prista 3, V-137 and V-117, 89.1%, 88.8%, 85.5% and 83.5%, respectively. The lowest value was exhibited by the genotype Ao-3, 67.9%. The differences were significant.

In the second year of study (A_2), the average pollen vitality was 74.6%, the values ranging from 85.9% and 83.8% (in Ri-11 and Banat-139, respectively) to 57.9% (in Bačka-136). The differences were highly significant. In the third year of study (A_3), the average pollen vitality was 76.3%. Highest values were registered in Di-4 and Europe (89.3% and 89%, respectively), lowest in S-64 and V-137 (38.9% and 57.4%, respectively). The differences were highly significant (Table 1).

The average variability of pollen vitality was fairly high (CV=8.05%), as indicated by the value of standard deviation (S=6.16).

Similar results for pollen vitality in alfalfa genotypes have been obtained by Đukić and Kraljević-Balalić (1992), Aleksić and Đukić (2004). These papers indicated that the variability of pollen fertility depended on genotype, year and the interaction genotype x year.

Lukić (1985) reported that pollen vitality ranged from 60.9% to 95.3% in parent plants and from 55.1% to 85.9% in F_1 hybrids *Medicago sativa* x *M. falcata*.

Besides pollen vitality, number of ovules per ovary is another important yield component in alfalfa. Based on a two-year study, the average number of ovules per ovary was 9.6. Of the total number of analyzed florets, 51.2% has 9-10 ovules and 91.2% had 8-12 ovules/ovary. The variability of the genotypes for this parameter was low, CV=4.63%, S=0.44 (Table 1).

In the first year of study (A_2), the average number of ovules per ovary was 9.8, the actual values ranging from 10.6 and 10.5 (in Le-6 and Banat-139, respectively) to 9.0 (in Ja-7). The differences were not significant. In the second year of study (A_3), the average number of ovules per ovary was 9.3 ovule, from 8.2 (in Europe) to 10.1 (in both Banat-139 and Bačka-136).

The above results as well as the analysis of variance indicated that there were no significant differences in number of ovules per ovary among the tested alfalfa genotypes (Table 1).

Table 1. Pollen vitality (%) and number of ovules per ovary in alfalfa genotypes

Genotype	Pollen vitality (%)				No. of ovules/ovary		
	A_1, C_2	A_2, C_3	A_3, C_2	$\bar{X}$	A_2, C_3	A_3, C_2	$\bar{X}$
NS-Slavija	71.6	75.6	66.9	71.4	9.2	9.9	9.5
Rs-2	75.5	81.9	86.3	81.2	10.3	9.3	9.8
Ao-3	67.9	76.1	84.0	76.0	10.1	9.3	9.7
Di-4	73.9	81.5	89.3	81.6	9.2	8.5	8.8
Europe	89.1	63.2	89.0	80.4	10.0	8.2	9.1
Le-6	69.0	69.3	78.9	72.4	10.6	9.5	10.1
Ja-7	71.7	74.3	82.5	76.2	9.0	8.6	8.8
Ci-8	79.0	81.7	80.0	80.2	9.4	9.1	9.2
V-117	83.5	79.5	78.3	80.4	10.0	9.1	9.5
Ri-11	90.3	85.9	79.7	85.3	10.1	9.1	9.6
177 (B)	91.5	62.5	71.5	75.2	9.5	9.5	9.5
Prista 3	88.8	72.3	75.5	78.9	10.0	8.8	9.4
Banat-139	74.0	83.8	81.0	79.6	10.5	10.1	10.3
S-64	76.6	62.3	38.9	59.3	10.1	10.0	10.1
V-137	85.5	79.3	57.4	74.1	9.7	9.9	9.8
Bačka-136	72.0	57.9	76.8	68.9	9.7	10.1	9.9
Sap-134	79.4	81.2	81.9	80.8	9.2	9.1	9.1
$\bar{X}$	78.8	74.6	76.3	76.6	9.8	9.3	9.5
LSD _{0,05}	2.62	3.41	3.33	-	5.01	4.92	-
0,01	3.43	4.47	4.36	-	6.56	6.44	-
S	7.88	8.65	12.46	6.16	0.48	0.57	0.44
CV _(%)	10.00	11.59	16.32	8.05	4.95	6.20	4.63

Numerous investigations have indicated that the average number of ovules per ovary ranges between 9 and 12. Aleksić and Đukić (2004) reported the average number of 9.6 ovules for five alfalfa genotypes. Rod (1979) stated that variability in the number of ovules in fertile alfalfa florets depended on alfalfa type and clone, i.e., it is genetically controlled; however, it may be modified by ecological conditions.

Based on the results of Đukić (1978), Bolanos *et al.* (1999) and the results of this study, pollen vitality and number of ovules per ovary should not be

limiting factors in alfalfa seed production. This study also showed that there was no significant correlation between pollen vitality and number of ovules per ovary ($r=-0.445$).

Conclusion

Under the conditions of cross fertilization, the studied alfalfa genotypes had the average pollen vitality of 76.6%, i.e., from 59.3% to 85.3%. The differences were significant (CV=6.62%).

The alfalfa genotypes had 9.6 ovules per ovary on average. The differences were not significant.

Genetic characteristics of alfalfa, environmental factors and the interaction genotype x environment had significant effects on pollen vitality, but this was not the case with number of ovules per ovary.

Based on the obtained results, the genotypes Rs-2, Europe, Ja-7, Ci-8, V-117 and Bačka-136 are recommended for alfalfa breeding for increased production potential for seed yield.

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FERTILNOST LUCERKE U USLOVIMA SLOBODNE OPLODNJE

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Rezime

U radu je dat prikaz vitalnosti muškog gametofita (polena) i broj neoplođenih ovula po ovarijumu 17 genetički divergentnih genotipova u uslovima slobodne oplodnje (*polycross*). Na osnovu rezultata istraživanja, genotipovi lucerke su imali vitalnost polena od 59,3% do 85,3%, ili prosečno 76,6%. Sa visokom vitalnošću polena ističu se genotipovi Ri-11 (85,3%), Di-4 (81,6%) i Rs-2 (81,2%), dok su ostali genotipovi imali signifikantno nižu vitalnost. Genotipovi lucerke su imali 9,3-9,8 neoplođenih ovula/ovarijumu, a razlike nisu bile značajne. Rezultati vitalnosti muškog gametofita i broja ovula/ovarijumu u uslovima slobodne oplodnje ukazuju da ispitivani genotipovi lucerke imaju dobar potencijal za oplodnju. Takođe, korelacija između vitalnosti polena i broja ovula po ovarijumu nije bila značajna ($r=-0,445$).