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EFFECTS OF PHOSPHORUS APPLICATION AND CUTTING MANAGEMENT ON SEED YIELD AND YIELD COMPONENTS OF WHITE CLOVER (*Trifolium repens* L.)

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ABSTRACT: The objectives of this study were to determine the effects of different phosphorus (P) rates and cutting management on seed yield and the other traits. Experiments were carried out in Çarşamba and Kavak districts (Samsun-Turkey) during 2000 and 2001. In the study, 0, 40, 80 or 120 kg P_2O_5 ha^{-1} rates were applied to Klondike and Nanouk cultivars. Seed was harvested when the 50-67% of inflorescences became brown. In 2001, while the first growth was left for seed in Çarşamba, the second growth was left for seed in some plots. There was not any difference among cultivars and also among P rates for seed yields in both Çarşamba and Kavak locations. Seed yield was higher in the first growth especially in Kavak (101.2 kg ha^{-1}). Despite of the fact that P rates did not affect the seed yield; 80 kg P_2O_5 ha^{-1} rate of P was suitable for seed yield because it increased seed germination vigour.
Key words: White clover, cultivar, phosphorus, cutting management, seed yield

FOSFOR UYGULAMASI VE BİÇİM SIRASININ AK ÜÇGÜLÜN (*Trifolium repens L.*) TOHUM VERİMİ VE VERİM BİLEŞENLERİ ÜZERİNE ETKİSİ

ÖZET: Farklı fosfor dozları ve biçim sırasının ak üçgülün tohum verimi ile diğer bazı özellikler üzerine etkisinin incelendiği bu araştırma, 2000 ve 2001 yıllarında Çarşamba ve Kavak'ta yürütülmüştür. Denemede, Klondike ve Nanouk çeşitlerine 0, 40, 80 ve 120 kg P_2O_5/ha olacak şekilde gübre dozları uygulanmıştır. Tohum hasadı kömeçlerin %50-67'sinin kahverengi olduğu dönemde yapılmıştır. 2001 yılında Çarşamba'da birinci gelişme doğrudan tohuma bırakılmış, bazı parsellerde ise ikinci gelişme tohuma bırakılmıştır. Hem Çarşamba hem de Kavak'ta tohum verimi bakımından çeşitler ve P dozları arasında farklılık görülmemiştir. İlk gelişimi tohuma bırakılan bitkilerin tohum verimi özellikle Kavak'ta (101.2 kg ha^{-1}) daha fazla olmuştur. Fosfor dozları tohum verimini etkilememesine rağmen, tohumun çimlenme gücünü artırdığından 80 kg P_2O_5 ha^{-1} fosfor tohum verimi için uygun bulunmuştur.
Anahtar Sözcükler: Ak üçgül, çeşit, fosfor, biçim sırası, tohum verimi

1. INTRODUCTION

Forage plants are of great importance in sustainable agricultural systems (Şeker, 1998). The proportion of forage production area in total cultivated area is aimed to increase from 5% to 20% in Turkey. The proportion of forage production area in total cultivated area in the Black Sea Region is nearly 6% (Anon., 2002).

Seed production of forage plants is insufficient in Turkey. Recently, the requirement for seeds of different forage plants and cultivars has significantly increased for improving pasture and establishing grassland besides forage cropping. Nowadays, Turkish agriculture has experienced depression in agricultural sector. In this case, forage crop seed industry enables new opportunities to small farmers.

The most common cultivated *Trifolium* species in Turkey are red clover (*Trifolium pratense* L.), white clover (*Trifolium repens* L.) and persian clover (*Trifolium resupinatum* L.) (Acar and Eraç, 1999; Acar and Ayan, 2000). White clover, which is highly resistant to grazing and crushing, has an excellent nutritive value. For this reason, white clover is commonly used with grass species in ranges, football fields, game areas, parks and gardens. It is raised in orchards due to its tolerance to shadow. There is a lack of information on raising of white clover (especially raising for seed production) despite the fact that it occupies a large area in natural flora in Turkey. Seed production for white clover is performed using two different methods; i) in areas used as pasture, ii) in areas established for seed production. In a study conducted in Romania during 1984 and 1985, the Crau –Ladino and Magurek-1 varieties of *T. repens* produced 163 and 407 kg ha^{-1} seed, respectively (Breazu and Krauss, 1988). In experiments conducted between 1981 and 1989, 160 kg ha^{-1} seed was produced from *T. hybridium* as an average for six years. Seed yields were 130 and 110 kg ha^{-1} for first and second cutting, respectively. Seed productions of *T. repens* (as an average of five years) for two cuttings were 170 and 250 kg ha^{-1}, respectively. For seed production, first cutting for *T. hybridum* and second cutting for *T. repens* were recommended (Korobov, 1990). Grazing or cutting prior to flowering is recommended for activation of buds from which inflorescences in the stolons emerged (Açıkgöz, 2001). In a study conducted in Erzurum was obtained between 180-450 kg ha^{-1} seed yield from white clover (Serin and Tan, 1996). Nanouk, cultivar with small leaves, has short and intensive growing tendency (DLF Ltd., 2000). A high inflorescence count in a unit area and an adequate pollination are required to attain a high seed production level. Number of flowers count in inflorescences of white clover was reported to range

from 50 to 350 (Marshall and Hides, 1988; Açıkgöz, 1991). Also, 1000 seed weight of white clover was reported to range from 0.5 to 0.7 g (Andreda et al., 1990; Pederson and Brink, 2000).

One of the most important organic phosphate compounds in plants is phytin. Phytin is found primarily in seeds in the salt forms of Ca and Mg and it is formed during seed formation. For this reason, there is an increase in P flowing towards the emerging and developing seeds immediately after pollination (Link and Swanson, 1960). Seed and fruit formation impairs in plants, which can not take enough available P and fruit formation delays (Aydemir and İnce, 1988). In a study conducted with A-2 variety of Lucerne with 0, 40 and 80 kg ha^{-1} P_2O_5 rates did not affect average seed yield (Patel et al., 1991).

In this study, it was aimed to determine the effects of P fertilization and cutting sequence on seed yield of white clover.

2. MATERIALS AND METHODS

This study was conducted in two locations, Çarşamba (36^o 43' E, 41^o 12' N, 5 m altitude) where rainfall, moisture and soil water level is high, and is located in coastal area and Kavak (36^o 02' E, 41^o 03' N, 575 m altitude) where rainfall and moisture is lower, and is located in the inner part of the Black Sea Region of Turkey, during 2000 and 2001 years.

Soil samples were taken prior to study and during the fall in first year from the locations before the second P applications (Table 1).

While soil in Çarşamba was clay loam, slightly alkaline, calcerous, saltless, deficient in organic matter (OM), rich in K and intermediate in P, soil in Kavak was clay loam, slightly alkaline, calcerous, saltless, intermediate in OM, rich in K and intermediate in P in first year and rich in P in second year. Available phosphorus contents of the soil in Carsamba location were dramatically decreased due to the fixed phosphorus by high lime, low organic matter and some chemical reactions in the second year.

Total rainfall in Çarşamba is 625.4 mm and 545.3 mm in Kavak in 2000. In 2001, total rainfall was 311.5 in Çarşamba and 265.4 mm in Kavak until June (harvest was performed in June). In June 2000, there was no rainfall in Çarşamba and Kavak. There was a less rainfall in 2000 and 2001 for both locations compared to the long-term averages. In both locations air temperature in seed maturing period (June and July) was higher than long term mean air temperature.

Klondike and Nanouk cultivars of white clover (*Trifolium repens L.*) were used in this study. 25 kg N ha^{-1} fertilization (CAN with 26% N) was performed as an initial fertilizer. P fertilization was accepted as factor and 0, 40, 80 or 120 kg P_2O_5 ha^{-1} rates (TSP containing 42-44% P_2O_5) were used. All P rates applied two times in both locations. The first P application was performed during sowing, second P application was performed at the end of the autumn in the establishment year (Stefan and Motca, 1990). N fertilizer was broadcast-applied, P fertilizer was drillied in the first application and broadcast-applied in the second application.

The experiment was planned according to the split plot design with four replicates. Cultivars were main plots, P rates were subplots. Row place and row length were 30 cm (Perepravo and Zolotarev, 1990) and 5 m, respectively, and there were seven rows in each subplot.

Sowing was made in the spring of 2000 and applied in order to reach 2 kg seed ha^{-1} rate in 1.5 cm depth by hand. A hoe was used in seedling period. Seed harvest was performed by picking inflorescences when the 50-67% of pods converted to yellow-brown (Manga et al., 1995). In Çarşamba, half of the subplots were cut at 5 cm stubble height at flowering time in the second year (19 May 2001) and then the second growth was left for seed (T_1). The first growth of the plants in the other half of the subplots was left for seed (T_0). Seed was harvested for T_0 on 10_{th} July 2001, and for T_1 on 26_{th} July 2001. Cutting sequence could not be applied in Kavak because of the undesirable grazing of the land by sheep. Cutting sequence was evaluated using *t* test.

Prior to winter (in November 2000), plants were cut at 5 cm height to remove the stubble residues and P was applied as the second year treatment (Stefan and Motca, 1990). Plants were irrigated using flood irrigation up to field capacity after the cuttings.

Table 1. Soil properties of experimental fields in Çarşamba and Kavak

Properties	Çarşamba					Kavak				
	1st year	2nd year				1st year	2nd year			
		P_0	**P_{40}**	**P_{80}**	**P_{120}**		**P_0**	**P_{40}**	**P_{80}**	**P_{120}**
pH	7.90	8.00	7.95	7.80	7.85	8.00	7.75	7.80	7.95	7.85
Calcerous, %	9.12	9.25	8.44	9.25	8.93	8.44	9.71	8.89	10.37	9.87
Total salt ,%	0.04	0.03	0.03	0.03	0.03	0.03	0.04	0.04	0.04	0.03
P_2O_5 kg ha^{-1}	68.19	25.49	33.97	37.10	37.86	64.19	73.16	90.21	92.08	86.51
K_2O kg ha^{-1}	621.7	416.0	445.7	410.6	445.7	457.5	492.7	551.3	563.0	586.5
Organic matter, %	1.89	1.88	2.32	2.21	1.99	2.88	3.04	4.65	4.76	3.71
Saturation, %	60	62	62	62	62	55	62	66	66	66

During the seed harvest, inflorescence density was determined from 1 m^2 for the centre of subplot. 10 inflorescences were taken from each subplot randomly and then flowers in each inflorescence were counted. The diameter and length of inflorescences were measured using callipers. 8 x 100 seeds were taken randomly from the seeds obtained from each subplot (with eight replicates) and 1000-seed weight was determined by averaging these eight replicates. After six months from the harvest, these seeds were moistened in Petri dishes with filter paper and were exposed to pre-cooling process (five days at 4 ^{0}C). Afterwards, they were sprouted in a climate room at 20 ^{0}C. Germination rate (%) and germination vigour were determined counting the seeds at the seventh day (for germination rate) and at the fifteenth day (for germination vigour) (Şehirali, 1997).

Results obtained from the experiment were analyzed according to the Two Factor Randomized Complete Block Design with Split Plot Combined Over Years using MSTAT-C pocket programme. DUNCAN test was used to compare group means.

3. RESULTS AND DISCUSSION

3.1. Seed Production Values for the First Plant Growth

There were not statistically differences among P doses in term of seed yield, inflorescence density, inflorescence diameter and length, inflorescence peduncle length, number of flower/inflorescence and 1000 seed weight in the experiments.

Mean seed yield and 1000 seed weight of the two cultivars were statistically similar in Çarşamba and Kavak. Seed yield for Klondike was around 6% greater than that Nanouk in Çarşamba, and 2% less than that for Nanouk in Kavak. Seed yield in Kavak (101.2 kg ha^{-1}) was higher than that in Çarşamba (70.2 kg ha^{-1}). Seed yields of this study conducted in different environment conditions were lower than those reported by Serin and Tan (1996), Stefan and Motca (1990) and Açıkgöz (2001). The temperature difference between day and night in Kavak was higher than that in Çarşamba. This means that a lower proportion of metabolites produced during day hours is consumed during night and consequently a higher amount of metabolites deposit in seeds (Eser, 1986; Kevseroğlu, 1999). Furthermore, loss of pollen was lower and pollinator activity was higher in Kavak due to low level of relative humidity and rainfall. For this reason, higher seed yield was obtained in Kavak than in Çarşamba. P rate(s) did not affect the seed yield. Especially in Kavak, higher P content in the second year might remove the effect of P rates (Table 1). This supports the findings of Patel et al. (1991).

Inflorescence density was higher in Kavak than in Çarşamba. The higher incidence of clear and sunny days in Kavak compared to Çarşamba during the seed emerging period (less rainfall in June) caused the increased flowering ratio in Kavak. One of the most important factors affecting the seed yield is inflorescence density. Inflorescence counts obtained in Kavak were similar to those reported by Marshall and Hides (1990). Nanouk cultivar produced more inflorescence compared to Klondike cultivar because of its small leaves and more intensive growth trend.

1000-seed weight and inflorescence density of Nanouk cultivar in both Çarşamba and Kavak were higher than Klondike cultivar (Table 2). Average 1000-seed weight values were 0.565 and 0.561 for Çarşamba and Kavak, respectively. These findings were higher than those of Pederson and Brink (2000) and lower than those of Andreda et al. (1990). 1000-seed weight may vary due to variations in ecological conditions (Zapletalova, 1990).

Klondike cultivar produced more flower compared to Nanouk cultivar (Table 2). These findings are in consistence with those of Marshall and Hides (1990) and Açıkgöz (1991). When inflorescence density decreased, inflorescence diameter, length and flower count in per inflorescence increased as reported by Marshall and Hides (1990).

Table 2. The two-year average values of some agronomic traits of white clover varieties obtained from T_0 in Çarşamba and Kavak locations

	Çarşamba		Kavak	
	Klondıke	Nanouk	Klondıke	Nanouk
Seed yield (kg ha^{-1})	72.0	68.3	100.2	102.3
Inflorescence density	135.5b*	208.4a	316.7	333.3
Inflorescence diameter (mm)	15.32a**	13.18b	11.22A*	9.84B
Inflorescence length (mm)	14.11a*	11.80b	11.11A**	9.72B
Inflorescence peduncle length (cm)	25.56a*	20.74c	23.75ab	22.58bc
Number of flower in each inflorescence	57.31a**	45.27b	51.48A**	37.47B
1000-seed weight (g)	0.556	0.576	0.554	0.572

Means within the same group shown by the same letters are not different * P≤ 0.05, ** P≤ 0.01.

Average germination vigour for entire experiment was 17.6%. The highest germination vigour was found for P_{120} rate in Kavak. There were no differences between P_{40} and P_{80} rates in Çarşamba (Table 3). Germination vigour increased 27% with 40 kg P_2O_5 ha^{-1} in Çarşamba and 20% with 120 kg P_2O_5 ha^{-1} in Kavak (Table 3). Germination vigour of white clover was reported to range from 69 to 76% (Nykänen-Kurki, 2007). Lower germination vigour values observed in this study compared to the other reports can be attributed to the hard shells of the seeds used in this study. As a matter of fact, germination vigour could be increased up to 100% when the shells of seeds were scratched. Hard seed shell is highly influenced from climate factors. Hot and dry climate conditions during seed maturing period increase hard seed development (Şehirali, 1997). In this period, there was not any precipitation in both Çarşamba and Kavak locations. Air temperature in this period was also hotter than average air temperature of long term. Methods of breaking seed coat dormancy include scarification, hot water, dry heat, fire, charate, acid and other chemicals, mulch, water, cold and warm stratification, and light (Deno, 2004; Hickman, 2005). Manga et al. (1995) reported that red clover grown under Samsun ecological conditions had hard seed shell, and germination vigour can be enhanced by scarification.

3.2. Seed Production Values for the Second Growth

Seed yield obtained from the first plant growth was 325% higher than seed yield obtained from the second growth after cutting in full flowering time (t=8.739**, n=32) (Table 4). Rainfall in June 2001 was too low compared to long-term measurements. Drought occurred after cutting and mouse damage caused the plants to complete vegetative and generative developments in a short time. Consequently, inflorescence density, lower inflorescence diameter and length decreased seed yield. One of the most important factors affecting seed yield is inflorescence density (Domingues et al., 1993). Despite Manga et al. (1995) had the highest seed yield in red clover plants in the second developments left for seed, the highest seed yield in this study was obtained from plants in the first developments left for seed. According to the *t* test, there were significant differences between cutting treatments (T_0 and T_1) with respect to inflorescence density, inflorescence diameter, inflorescence length, inflorescence peduncle length, germination rate and germination vigour (t=8.068**, t=19.760**, t=4.260**, t=8.780**, t=4.14**, t=3.93** n=32, respectively) but no differences in terms of number of flower in inflorescence and 1000 seed weight between cutting sequences. There were differences between cultivars for inflorescence and inflorescence peduncle length in T_1 treatment. Date for the both properties was longer in Klondike cultivar.

Germination rate and germination vigour were found higher in T_0 compared to the T_1 treatment. Drought and high temperatures occurred during seed ripening period probably increased the incidence of hard seed coat. An increase in the incidence of hard seed coat appears to decrease germination rate as well as germination vigour (Şehirali, 1997).

The results obtained from this study and some proposals were mentioned below:

1. Seed yield of Klondike cultivar, albeit was not statistically significant, Klondike cultivar can be recommended for use by farmers due to its higher seed yield.

2. Seed yield, inflorescence density, inflorescence diameter and length were higher in Kavak district compared to Çarşamba. There were suitable ecological conditions for seed production in Kavak compared to Çarşamba.

3. P rates affected germination rate and germination vigour in both locations. Even though P rates did not affect seed yield and related traits affecting seed yield. Germination rate and germination vigour, which are important quality criteria in seed production, were affected by P rates.

4. Seed yield, inflorescence density, inflorescence length, inflorescence peduncle length, inflorescence diameter, 1000 seed weight, germination rate and germination vigour values were found to be higher for plants in the first growth left for seed production compared to the second growth.

Furthermore, the possibilities of enhancing germination rate and germination vigour by using suitable methods to decrease the incidence of hard seed coat, which is the most important factor affecting germination rate and germination vigour, must be investigated.

Table 3. The two-year average values of germination vigour values (%) of white clover seeds obtained from T_0 in Çarşamba and Kavak locations

Location	Variety	P_0	P_{40}	P_{80}	P_{120}
Kavak	Klondıke	16.3	13.5	17.5	17.8
	Nanouk	16.2	16.8	19.8	21.0
Average*		16.25 ab	15.15 b	18.7 ab	19.4 a
Çarşamba	Klondıke	16.0	18.3	17.8	17.0
	Nanouk	15.5	21.5	20.8	17.2
Average*		15.75 B	19.9 A	19.4 A	17.1 AB
General Average		15.97	17.51	18.96	18.22

*Means within the same group shown by the same letters are not different P≤ 0.05

Table 4. Some properties of white clover obtained with T_0 and T_1 treatments in Carşamba in 2001.

	Cultivar	T_0	T_1
Seed yield (kg ha $^{-1}$)	Klondike	116.3	31.2
	Nanouk	111.9	22.5
	Average*	114.1a	26.8b
Inflorescence density	Klondike	212.4b	93.8
	Nanouk	322.8a	97.4
	Average*	267.6a	95.6b
Inflorescence diameter (mm)	Klondike	18.98	14.53a
	Nanouk	16.06	12.94b
	Average*	17.52a	13.73b
Inflorescence length (mm)	Klondike	16.35	12.42
	Nanouk	12.70	11.35
	Average*	14.52a	11.88 b
Number of flower in inflorescence	Klondike	49.32	49.99
	Nanouk	36.66	36.10
	Average*	42.99	43.04
İnflorescence peduncle length (mm)	Klondike	23.65	19.93 a
	Nanouk	20.53	16.17 b
	Average*	22.09a	18.05b
1000 seed weight (g)	Klondike	0.583	0.574
	Nanouk	0.568	0.566
	Average*	0.575	0.570
Germination rate (%)	Klondike	15.97	10.50
	Nanouk	19.97	14.91
	Average*	17.47a	12.70b
Germination vigor (%)	Klondike	17.05	11.41
	Nanouk	19.47	16.00
	Average*	18.26a	13.70b

*Means within the same group shown by the same letters are not different P≤ 0.01.

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