NARDI FUNDULEA, ROMANIA ROMANIAN AGRICULTURAL RESEARCH, NO. 42, 2025
www.incda-fundulea.ro; doi.org/10.59665/rar4264 First Online: June, 2025. DII 2067-5720 RAR 2025-183

Effects of Plant Growth Regulator and Row Spacing on Seed Yield and Yield Components of Alfalfa (*Medicago sativa* L.)

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ABSTRACT

This research was conducted to determine the effects of plant growth regulator (*Naphthalene acetic acid*) applied to four different row spacing (*17.5, 35, 52.5* and *70* cm) on seed yield and yield components of alfalfa (*Medicago sativa* L) under temperate climate conditions in Turkey. The experimental design featured a split plot with three replications during the growing season of 2019-2023. In the research, biomass yield, harvest index, racemes per stem, pods per raceme, and seeds per pod were examined as well as seed yield. According to the research results, plant growth regulator increased seed yield and yield components except biomass yield. Seed yield varied among row spacing and the highest seed yield was determined at 52.5 cm row spacing. In present study, it was concluded that 52.5 cm row spacing was suitable for alfalfa seed yield for similar ecologies, and further research should be conducted for plant growth regulator application in forage crops.

Keywords: alfalfa, plant growth regulator, row spacing, seed yield, naphthalene acetic acid.

INTRODUCTION

Due to its wide adaptability and ability to be grown in different climatic and soil conditions, alfalfa (*Medicago sativa* L.) is called the queen of forage plants (Dokiç et al., 2009). Environment, genotype and different agronomic practices affect the seed yield and quality of alfalfa (Dordas, 2006). Although genetic changes in alfalfa seed yield have been explained (Knapp and Teuber, 1994; Rosellini et al., 1998; Bolanos-Aguilar et al., 2000), breeding studies on increasing seed yield in alfalfa are very few (Bolanos-Aguilar et al., 2002). While alfalfa breeders focus on developing varieties resistant to diseases, pests and abiotic stresses (Petcu et al., 2019), as well as traits that increase hay yield and quality (Schitea et al., 2007, Popa et al., 2024) studies on increasing seed yield remain in the background (Martura, 2001, Dragoz et al., 2002; Iannucci et al., 2002). Therefore, different agronomic practices need to be developed in order to obtain high and quality seeds in alfalfa (Zhang et al., 2009).

Row spacing is an important factor in alfalfa seed production (Bekovic et al., 2016), because there is a constant competition among plants due to the ability of plants to regrow after cutting (Askarian et al., 1995). The optimum row spacing recommended in alfalfa seed production shows great changes in the literature (Avcı et al., 2017). While studies on alfalfa seed production reported that seed yield was higher in wide row spacing production than in narrow rows (Lukic, 2000); some researchers emphasized that high seed yield could not be obtained in wide row spacing compared to narrow rows (Avcı et al., 2017; Zhang et al., 2017). At the same time, depending on the years and environmental conditions, seed yield in alfalfa gives different results in row spacings (Zhang et al., 2008).

Plant growth regulators have opened new horizons for researchers to increase seed production of grasses and legumes (Lorenzetti, 1993). Plant growth regulators reduce plant height and lodging rate by changing hormone biosynthesis, thus, they increase seed yield of plants by inhibiting gibberellin synthesis or accelerating ethylene release (Wenhua et al., 2008). In soybean and pea, which are annual plants, the available

Received 16 May 2025; accepted 17 June 2025.

energy is allocated to seed production, thus mobilizing vegetative reserves for filling the seeds. In contrast, in alfalfa, which is a perennial plant, there is a balance between forms and regrowth for grass production, but this balance is usually unclear in seed production. It has been reported that various factors that negatively affect the distribution of assimilates may be the reason for the low seed yield of alfalfa, and plant growth regulators may play an active role in providing this balance (Genter et al., 1997).

In this study, the effects of plant growth regulator on alfalfa seed yield and yield components in different row spacing were investigated.

MATERIAL AND METHODS

Site Description

This research was carried out for five years (2019-2023) at the experimental area of Ondokuz Mayıs University in Samsun-Bafra (41°56'N, 35°86'E; altitude 15 m). In the research area, the soil structure is clayey loam, the soil reaction is neutral (pH: 7.01), moderately calcareous (6.2% $CaCO_3$), poor in organic matter (1.23%), moderate in available phosphorus level (75 kg ha^{-1} P_2O_5 phosphorus), rich in available potassium (1250 kg ha^{-1} K_2O), and harmless in terms of total salt (0.027%). The total precipitation and average temperature data for the experimental area are shown in Table 1 and 2.

Experimental Design and Treatments

The field experiment was carried out by the method of split plots with three replications. The main areas plant growth regulator [Naphthalene acetic acid application of 20 g a.i. ha; sub-plots - (17.5, 35, 52.5 and 70 cm) - row spacing].

Agricultural Practices

The trial was established in mid-March of 2019. 10 kg of seed was used per hectare. The trial was established in production plot of Albatur alfalfa cultivar where Ondokuz Mayıs University research area in Samsun-Bafra. The Albatur cultivar was bred by Ondokuz Mayıs University. Seed sowing was done with a drill at 17.5 row spacing. After emergence, plots were created according to 17.5 35, 52.5 and 70 cm row spacing. The plots were 5x2.1 m. 100 kg of diammonium phosphate was applied per hectare along with planting. The first year of the experiment was the establishment year and no application was made. Plant growth regulator was applied in the second and subsequent years.

Table 1. Total precipitation (mm) and average temperature (°C) in research area

Months	2019	2020	2021	2022	2023	Long period
Total precipitation (mm)						
March	41.9	26.5	91.7	117.2	83.4	67.4
April	45.5	26.1	56.5	34.6	102.2	56.6
May	23.6	23.0	77.4	31.3	60.6	48.8
Jun	42.5	58.4	65.8	47.8	102.4	46.2
July	75.7	1.5	8.2	6.5	71.4	34.8
August	55.4	43.3	49.3	3.0	1.2	37.5
Total	284.6	178.8	348.9	240.4	421.2	291.3
Average temperature (°C)						
March	7.7	9.1	7.4	5.5	8.9	8.0
April	11.6	10.4	11.1	12.4	12.2	11.3
May	18.0	17.1	16.6	15.2	14.6	15.6
Jun	23.5	21.5	20.2	21.3	20.4	20.2
July	22.9	24.2	24.9	23.0	23.8	23.2
August	22.9	22.7	24.0	25.4	25.3	23.6
Mean	17.77	17.50	17.37	17.13	17.53	16.98

During the research period, the first cutting was done in the 10% flowering period (approximately; between April 15 - May 5) in the alfalfa plots. It was reported that the climate conditions were more suitable for the seed yield in the second growth period in alfalfa (Karagiç et al., 2007).

In the second growth periods of the plants, seed yield operations were carried out. Plant growth regulator was applied twice (mid-May and early June) in the branching period and the beginning of budding of the alfalfa plots (Zhang et al., 2009).

Plant growth regulator (Naphthalene acetic acid) dose (20 g a.i. ha) was taken according to the results of Zhang et al. (2009) and Chen et al. (2016). In order to avoid the negative effect of the sun during the application, the growth regulators were applied before 9 am or after 6 pm (Chen et al., 2016).

Plots were harvested when 75% of the pods turned blackish brown. Seed yields were determined from a random two 1 m^2 sample from each plot harvested by hand. The three seed yield components included racemes per stem, pods per raceme, and seeds per pod. The average numbers of racemes per stem, pods per raceme, and seeds per pod were determined from random samples of 10 stems, 20 racemes, and 20 pods.

The statistical analysis was performed using the SAS general linear model procedure (SAS Institute, 1998). The means were compared using LSD test at the 0.05 probability level.

RESULTS AND DISCUSSION

Seed Yield

The combined variance analysis results of the 4-year research results are given in Table 2. According to the 4-year average results, the effects of year, plant growth regulator (PGR) and row spacing (RS) on alfalfa seed yield were found to be significant at the 0.01 level.

Table 2. Source of variation and mean squares for seed yield, racemes per stem, pods per raceme and seeds per pod under plant growth regulator (PGR) and row spacing treatments in 2020, 2021, 2022, and 2023

Sources of variation	Df	Seed yield	Biomass yield	Harvest index	Racemes per stem	Pods per raceme	Seeds per pod
year (y)	3	216800**	9340444**	15.21**	4.73**	1.19**	3.61**
Rep. x (y)	8	142	163951*	0.886	0.41	0.042	0.06
PGR	1	21600**	952813**	28.48**	1.50*	0.345**	0.78**
Y x PRG	3	272	30920	0.07	0.017	0.01	0.01
Error 1	8	475	69028	0.961	0.199	0.03	0.02
RS	3	17149**	1509460**	19.29**	2.19**	0.84**	0.91**
y x RS	9	83	19774	0.16	0.048	0.01	0.03
PGR x RS	3	227	4466	0.096	0.047	0.04	0.07
y x PGR x RS	9	294	10486	0.256	0.041	0.03	0.02
Error 2	48	309	61935	0.588	0.292	0.05	0.05

*,** significant at the 0.05 and 0.01 probability levels, respectively. Df degree of freedom.
PGR: Plant growth regulator, RS: Row spacing.

In addition, variance analysis was performed separately for all years. Since the PGR x RS effect was insignificant, letter grouping was performed for the values found to be statistically significant for RS, with and without PGR. (This analysis was conducted separately for all components).

In all years of the study and in the average of the years, the highest seed yield was obtained in PGR application. The highest seed yield in all years was 52.5 cm row spacing. In the study, the highest seed yield was achieved in the 2^{nd} year, followed by the 1^{st} and 3^{rd} years, and the lowest seed yield was determined in the last year (Table 3). The highest seed yield (623 kg) was found in the 52.5 cm RS with PGR applied in the 2nd year, and the lowest seed yield (328 kg) was found in the 17.5 cm RS without PGR in the 4th year.

Table 3. Average values for seed yield (kg ha^{-1}) under plant growth regulator (PGR) and row spacing treatments in 2020, 2021, 2022, and 2023

Row spacing (cm)	2020		2021		2022		2023		Mean	
	Non-PGR	PGR	Non-PGR	PGR	Non-PGR	PGR	Non-PGR	PGR	Non-PGR	PGR
17.5	511 b	548 c	534bc	551 b	461 b	484 c	328b	338b	458 c	480 c
35.0	523ab	574 b	547 b	579ab	488ab	508 b	337 b	366b	474 b	507 b
52.5	572 a	603 a	591 a	623 a	507 a	567 a	381 a	402a	513 a	549 a
70.0	527ab	547 c	523 c	572 b	473ab	501bc	341 b	361b	466 c	495 c
Mean	533 B	568 A	549 B	581 A	482 B	515 A	347 B	367 A	478B	508A
Level of significance										
PGR	**		**		**		**		*	
RS	**		**		**		**		**	
PGRxRS	ns		ns		ns		ns		ns	

*,** significant at the 0.05 and 0.01 probability levels, respectively.
Means followed by the same letter(s) and column(s) are not significantly different at the p = 0.05 level.
PGR: Plant growth regulator, RS: Row spacing.

Biomass Yield

According to the four-year average results, the effects of year, plant growth regulator (PGR) and row spacing (RSP) on alfalfa biomass yield were found to be significant at the 0.01 level (Table 4). The effects of PGR and RS on biomass yield were found to be significant in all years except the first year.

Table 4. Average values for biomass yield (kg ha^{-1}) under plant growth regulator (PGR) and row spacing treatments in 2020, 2021, 2022, and 2023

Row spacing (cm)	2020		2021		2022		2023		Mean	
	Non-PGR	PGR	Non-PGR	PGR	Non-PGR	PGR	Non-PGR	PGR	Non-PGR	PGR
17.5	5285	5145	5750a	5450 a	5315a	5075a	4210a	3980a	5140 a	4912 a
35.0	4920	4850	5365b	5200ab	5120a	4845b	3985b	3718b	4887b	4653 b
52.5	4840	4765	5240b	5110bc	4950b	4715b	3860b	3645b	4722b	4558 b
70.0	4756	4625	5100b	4845c	4510c	4450c	3750b	3350b	4529 c	4317 c
Mean	4950	4846	5633A	5151B	4913A	4771B	3915A	3673B	4809A	4610B
Level of significance										
PGR	ns		**		*		*		**	
RS	ns		**		**		*		**	
PGRxRS	ns		ns		ns		ns		ns	

*,** significant at the 0.05 and 0.01 probability levels, respectively.
Means followed by the same letter(s) and column(s) are not significantly different at the p = 0.05 level.
PGR: Plant growth regulator, RS: Row spacing.

The highest biomass yield was determined in the 17.5 cm RS (5750 kg) in which PGR was not applied in the 2nd year, and the lowest biomass yield was found in the 70 cm RS (3350 kg) in which PGR was applied in the 4th year (Table 4).

Table 5. Average values for harvest index under plant growth regulator (PGR) and row spacing treatments in 2020, 2021, 2022, and 2023

Row spacing (cm)	2020		2021		2022		2023		Mean	
	Non-PGR	PGR	Non-PGR	PGR	Non-PGR	PGR	Non-PGR	PGR	Non-PGR	PGR
17.5	9.6 b	10.8	9.3c	10.1c	8.9	9.6c	7.8b	8.5b	8.8c	9.8b
35.0	10.6ab	11.8	10.2b	11.1b	9.5	10.5b	8.5b	9.8ab	9.7b	10.4ab
52.5	11.8a	12.7	11.3a	12.2a	10.3	12.1a	9.9a	11.1a	10.8a	12.0a
70.0	11.1ab	11.8	10.2b	11.8ab	10.6	11.3ab	9.1ab	10.8a	10.3a	11.4a
Mean	10.8B	11.8A	10.3B	11.3A	9.8	10.9	8.8B	10.1A	9.9B	11.0A
Level of significance										
PGR	*		**		**		**		**	
RS	*		**		**		**		**	
PGRxRS	ns		ns		ns		ns		ns	

*,** significant at the 0.05 and 0.01 probability levels, respectively.
Means followed by the same letter(s) and column(s) are not significantly different at the p = 0.05 level.
PGR: Plant growth regulator, RS: Row spacing.

Harvest İndex

The results of variance analysis showed that the effects of year, PGR and RS on harvest index (HI) were significant at 0.01 probability level (Table 5). It was observed that PGR application increased the HI values in all years. Also, the average HI values of 52.5 and 70 cm RS were found to be higher than 17.5 and 35 cm (Table 5).

Racemes per Stem

The significant probability level for year and RS were 0.01, and PGR was 0.05 in racemes per stem (Table 6). No effect of RS and PGR on racemes was detected in all years of the study. In the average of 4 years, it was observed that PGR application slightly increased the number of racemes at 52.5 cm RS (Table 6).

Pods per Raceme

According to the 4-year average results, the effects of year, PGR and RS on pods per raceme value were found to be significant at the 0.01 level (Table 7). PGR application increased the pods per raceme value in all row spacing in all years. The highest pods per raceme value was determined in 52.5 cm RS (6.11) where PGR was applied in the 2nd year of the present study (Table 7).

Seeds per Pod

The effects of years, PGR and RS on Seeds per Pod were statistically significant at 0.0.1 level (Table 8). Also, only in the first year, both PGR and RS had an effect on Seeds per Pod (Table 8). As an average of 4 years, the highest seeds per pod value was detected in the 52.5 cm RS (4.78) where PGR was applied.

Table 6. Average values for racemes per stem under plant growth regulator (PGR) and row spacing treatments in 2020, 2021, 2022, and 2023

Row spacing (cm)	2020		2021		2022		2023		Mean	
	Non-PGR	PGR	Non-PGR	PGR	Non-PGR	PGR	Non-PGR	PGR	Non-PGR	PGR
17.5	15.3	15.5	15.9	16.2	15.5	15.8	14.4	15.1	15.2 c	15.7 b
35.0	15.6	15.8	16.2	16.5	15.8	16.2	15.3	15.4	15.7 b	15.9 b
52.5	15.9	16.1	16.5	16.9	16.3	16.4	15.7	15.8	16.1 a	16.3 a
70.0	15.7	15.8	16.1	16.3	15.8	16.1	15.2	15.3	15.7 b	15.9 b
Mean	15.6	15.8	16.1	16.4	15.8	16.1	15.1	15.4	15.7B	16.0A
Level of significance										
PGR	ns		ns		ns		ns		*	
RS	ns		ns		ns		ns		**	
PGRxRS	ns		ns		ns		ns		ns	

*,** significant at the 0.05 and 0.01 probability levels, respectively.
Means followed by the same letter(s) and column(s) are not significantly different at the p = 0.05 level.
PGR: Plant growth regulator, RS: Row spacing.

Table 7. Average values for pods per raceme under plant growth regulator (PGR) and row spacing treatments in 2020, 2021, 2022, and 2023

Row spacing (cm)	2020		2021		2022		2023		Mean	
	Non-PGR	PGR	Non-PGR	PGR	Non-PGR	PGR	Non-PGR	PGR	Non-PGR	PGR
17.5	5.21	5.36	5.54	5.71 b	5.43 b	5.59 b	4.98	5.14	5.29 c	5.45 c
35.0	5.33	5.41	5.63	5.83ab	5.54ab	5.64 b	5.13	5.22	5.41 b	5.53 b
52.5	5.74	5.88	5.98	6.11 a	5.81 a	5.93 a	5.44	5.49	5.74 a	5.85 a
70.0	5.44	5.49	5.55	5.75 b	5.41 b	5.57 b	5.27	5.23	5.42 b	5.51 b
Mean	5.43	5.54	5.67B	5.85A	5.48B	5.68A	5.20	5.27	5.46B	5.58A
Level of significance										
PGR	ns		*		*		ns		**	
RS	ns		*		**		ns		**	
PGRxRS	ns		ns		ns		ns		ns	

*,** significant at the 0.05 and 0.01 probability levels, respectively.
Means followed by the same letter(s) and column(s) are not significantly different at the p = 0.05 level.
PGR: Plant growth regulator, RS: Row spacing.

Table 8. Average values for seeds per pod under plant growth regulator (PGR) and row spacing treatments in 2020, 2021, 2022, and 2023

Row spacing (cm)	2020		2021		2022		2023		Mean	
	Non-PGR	PGR	Non-PGR	PGR	Non-PGR	PGR	Non-PGR	PGR	Non-PGR	PGR
17.5	4.22 c	4.36 c	4.55 b	4.61 b	4.34	4.43	3.45	3.69 b	4.14 c	4.27 c
35.0	4.38bc	4.85ab	4.49 b	4.89ab	4.41	4.56	3.75	3.96ab	4.28 b	4.57 b
52.5	4.75 a	5.01 a	4.87 a	5.11 a	4.53	4.75	4.01	4.23 a	4.54 a	4.78 a
70.0	4.53ab	4.48bc	4.65ab	4.53 b	4.26	4.37	3.67	3.91 b	4.27 b	4.32 c
Mean	4.47B	4.68A	4.64	4.78	4.39	4.52	3.72	3.95	4.30B	4.48A
Level of significance										
PGR	**		ns		ns		ns		**	
RS	**		*		ns		*		**	
PGRxRS	ns		ns		ns		ns		ns	

*,** significant at the 0.05 and 0.01 probability levels, respectively.
Means followed by the same letter(s) and column(s) are not significantly different at the p = 0.05 level.
PGR: Plant growth regulator, RS: Row spacing.

In our research, PGR application caused an increase in seed yield in all RS. Naphthalene acetic acid (NAA), used as a plant growth regulator, prevents premature flower and fruit shedding, promotes flowering, provides significant earliness and increases seed size and yield (Haliloğlu and Çoklu, 2024). Zhang et al. (2009) reported that NAA applied to alfalfa increased seed yield by 29%. Chen et al. (2016) found that the NAA application increased the seed yield of alfalfa by approximately 20% compared to the control application. It had been reported that other PGRs used for seed production in alfalfa also provided significant increases in seed yield (Wenhua et al., 2008; Pajcin et al., 2020; Tyshchenko et al., 2020).

According to the 4-year average results in our research, the highest seed yield was determined in 52.5 cm RS in both non-PGR- and PGR-applied plots, while 35 cm RS was in second place.

In a previous study, it was reported that wider row spacing and plant distance increased biomass of the plant by producing healthy plant parts by receiving maximum sun light for the photosynthesis (Çalışkan and Çalışkan, 2018).

In order to obtain high seed yield, the row spacing determined in the studies carried out on alfalfa varies. Askarian et al. (1995) 30-45 cm, Zhang et al. (2008) 60 cm, Bekoviç et al. (2016) 40 cm, Kavut (2016) 20 cm, Moga and Schitea (2005), Avcı et al. (2017) 25-50 cm, Pajcin et al. (2020) 60 cm, Wang et al. (2023) 45-60 cm row spacing were the highest seed yields.

It was reported that in addition to row spacing and different cultural practices, environmental factors such as temperature, rainfall and humidity were also very important and effective in obtaining high seed yields (Chen et al., 2016).

It was observed that PGR shortened the plant height and decreased the biomass yield, but increased the branch, flower and seed yield of the alfalfa. In contrast we conclude that HIs were increased by the PGRs as compared with untreated plants. According to the average results of all years and 4 years of our research, it was determined that the biomass yield of the plots without PGR application was higher than PGR plots. In our research, the findings that PGR application reduced biomass efficiency but increases HI value were consistent with Chen et al. (2016) and Zhang (2009).

In our study, while biomass yield in narrow RS (17.5 and 35 cm) was higher than in wide RS (52.5 and 70 cm), HI values were found to be higher in wide RS. Our research results were consistent with the findings of Avcı et al. (2017).

PGR application showed an increase in racemes per stem at 0.05 level. On the other hand, the effect of PGR application on pods per raceme and seeds per pod was at 0.01 level. Zhang et al. (2008) reported that PGRs caused slight increases in three of the yield components, including racemes per stem, pods per raceme, and seeds per pod compared with non-PGR during the 3 yr. These results partially support our research results. In present study, the higher seed yield components due to PGR treatments could be attributed to the increased assimilate supply to the reproductive tissue (Chen et al., 2016).

The effects of row spacing on 3 seed yield components were found to be significant. It was generally reported that the number of components racemes per stem, pods per raceme, and seeds per pod increased as row spacing increased (Bekovic et al., 2016; Avcı et al., 2017; Wang et al., 2023), and the researchers' findings were compatible with our results.

CONCLUSIONS

In the study conducted in Samsun-Bafra conditions in the north of Turkey for 5 years; the effects of plant growth regulator and different row spacing on alfalfa seed yield and yield components were investigated. According to the research results, 52.5 cm row spacing was found to be suitable for seed yield. It was also determined that the growth regulator had an increasing effect on seed yield. In addition, it is thought that it would be useful to investigate different PGR doses in future studies.

ACKNOWLEDGEMENTS

We would like to thank Ondokuz Mayıs University Agricultural Research Center Directorate for providing machinery, equipment and personnel support in this research.

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