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Full Length Article

Foliar Applied Calcium Improves Seed Yield and Yield Components of Alfalfa

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Abstract

A field experiment was carried out during 2009 to 2012. Calcium chloride ($CaCl_2$) at five levels (0, 0.2, 0.4, 0.6, 0.8 mg g^{-1}) was applied as foliar sprays at the initial and peak flowering of alfalfa (*Medicago sativa* L.) and data were recorded for seed yield and yield components. Results showed that the year, calcium (Ca) levels and the interaction affected significantly seed yield and yield components except seeds per pod. The highest seed yield was obtained in 2012 (746.82 kg ha^{-1}) and lowest in 2010 (116.08 kg ha^{-1}). With the Ca levels increasing from 0.2 to 0.8 mg g^{-1}, the highest seed yield (585.41 kg ha^{-1}) was obtained for the 0.4 mg g^{-1} treatment. Additionally, flowers per raceme ($r=0.56^{**}$) and pods per raceme ($r=0.61^{**}$) was significantly and positively correlated with alfalfa seed yield, respectively. Path analysis depicted that pods per raceme influenced positively and directly seed yield ($pc=0.60^{**}$) and flowers per raceme had a positively indirect effect via pods per raceme on seed yield ($pc=0.40^{**}$). Racemes per square meter negatively and directly affected seed yield (pc=-0.223), but racemes per square meter had a positively indirect effect via pods per raceme on seed yield (pc=0.23). It could be suggested that 0.4 mg g^{-1} Ca level was beneficial for improving alfalfa seed yield and pods per raceme as crucial yield component in alfalfa.

Keywords: Alfalfa; Spraying; Fertilization; Yield; Correlation

Introduction

For forage crop, seed yield is not part of the agronomic value. However, in alfalfa, the commercial success of a new variety depends not only on its forage production, but also seed attributes (Falcinelli, 1999). However, a large variations is observed in seed yield of alfalfa (Narinov and Kljuj, 1990), due to many factors including genotype, climatic conditions and agronomic management.

Genetics should be taken into consideration to attain high quality seeds (Barnes and Sheaffer, 1995). Genetic diversity of alfalfa for seed yield and yield components has been identified (Bolanos-Aguilar *et al*., 2000). Nevertheless, breeding for the actual seed yield enhancement has been restricted. Suitable environmental conditions are essential for seed production of alfalfa. In years with excessive precipitation, alfalfa plants tend to prolong the vegetative growth phase and lodge easily. In years with very low temperature, which affects plant growth and reduces insect activity, alfalfa seed yield will be poorly meager. Thus, favorable climate should be low relative humidity and moderate air temperature (Rincker *et al*., 1988).

Generally, a balanced supply of nutrients is essential for ideal seed yield and seed quality. Calcium (Ca) plays a pivotal role in plant growth, development and respond to external and internal signals (Reddy, 2001; Kudla *et al*., 2010). Lots of researches on the effect of Ca in crop plants are reported. For instance, Xu *et al*. (2013) reported that Ca could improve the photosynthetic rate of zoysiagrass (*Zoysia japonica* Steud.) under drought conditions. Ca deficiency restrains the growth of meristematic tissues and youngest leaves would become deformed and chlorotic (Marschner, 1995). Additionally, Ca supply can promote nitrogen absorption and increase nitrogen use efficiency to make plant active (Mahmood *et al*., 2009). Ca contributes to the growth and survival of symbiotic bacteria in legume, and could increase the yield of groundnut and soybean (Wang *et al*., 2004; Gashti *et al*., 2012). For alfalfa, it has been reported that exogenous Ca could accelerate the ability of alfalfa seedling to resist cold, drought and salt (You *et al*., 2003; Jiang *et al*., 2008; Liu *et al*., 2011). However, little is known about the efficiency of foliar applied Ca in improving alfalfa seed yield. Furthermore, a major problem with Ca nutrition is its low plant and soil mobility. Unlike most other nutrients, Ca cannot mobilize from older to younger tissues. Thus, the objectives of this

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study were to investigate whether foliar applied Ca could increase alfalfa seed yield during the consecutive growing year and determine the optimum concentration, in order to provide the information for fertilization management of alfalfa seed production.

Materials and Methods

Experimental Site

The experiment was carried out at the Etuoke Banner, Inner Mongolia Autonomous Region, China (latitude, 39°12′N; longitude, 106°95′E; elevation, 1150 m) from 2009 to 2012. The soil type (0–30 cm) was gray meadow with pH of 8.98; organic matter, 9.71 g kg^{-1}; alkaline-hydrolysable nitrogen, 72.7 mg kg^{-1}; phosphorus, 7.24 mg kg^{-1}; potassium, 74 mg kg^{-1}; and Ca, 93 mg kg^{-1}. Weather data was obtained from local observing station.

Experimental Materials

The sowing seed of the alfalfa (*Medicago sativa* L. var. Zhongmu No. 2) was provided by the Institute of Animal Science, Chinese Academy of Agricultural Sciences.

Field Trials Design

The field trial was established on April 10, 2008 at the seed rate of 10 kg ha^{-1}. The experimental plot was 36 m^2 (4.5 m × 8 m). Randomized complete blocks were designed with three replicates. The crop was sprayed with $CaCl_2$, at five concentrations (0, 0.2, 0.4, 0.6, 0.8 mg g^{-1}) @ 1000 L ha^{-1}, at the initial and peak flowering stage respectively. P_2O_5 (750 kg ha^{-1}) and K_2O (180 kg ha^{-1}) were applied as base fertilizers. Irrigation was performed according to the climate conditions during the returning green period.

Methods and Data Collection

The first year was considered as the establishment year. Measurements for data collection were performed during the subsequent 4 years. During 2009 to 2012, the four yield components recorded were racemes per square meter, flowers per raceme, pods per raceme, and seeds per pod. To measure racemes per square meter at the peak flowering. Thirty racemes and pods each were stochastically selected from each sampling unit to measure the average number of flowers per raceme, pods per raceme, and seeds per pod. Seed yield per unit area was obtained from the weight of seeds of each plot.

Statistical Analysis

The experiments were conducted for 4 consecutive years (from 2009 to 2012) at the same location. Years, Ca treatments (0, 0.2, 0.4, 0.6 and 0.8 mg g^{-1}), and their interactions were examined with a standard *F*-test. The data were analyzed statistically using completely randomized block design. Means of each treatment were compared by Fisher's protected LSD test with a significant level of 0.05. Pearson correlation analysis was analyzed among seed yield and yield components. Path-coefficient was used for determining relationships among components of alfalfa seed yield. These analysis were performed using SAS (version 8.0).

Results

Effect of Climate on Seed Yield and Yield Components

During the four growing years, environmental conditions were quite variable, especially during flowering and pod formation (June and July), due to the change in temperature and rainfall (Table 1). The growing season in 2012 was warm and dry, but in 2010 the spring was quite humid especially in May (82.0 mm) at flowering and fertilization. Meanwhile, lowest monthly temperature (0.4°C) and mean monthly temperature (6.3°C) were lower in April 2010 than other experimental years, which coincident with the beginning of plant reviving. Furthermore, the total sunny hours in May 2010 were fewer than other experimental years resulting in lower products of photosynthesis.

Results of compound variance analysis presented meaningful differences (P<0.01) among experimental years for seed yield components and seed yield (Table 2). The climate change was a key factor to affect seed yield components and seed yield among years. Results obtained from 2009 to 2012 indicated meaningful differences (P<0.05) for seed yield among the consecutive years, the highest seed yield of alfalfa was attained in 2012 (746.82 kg ha^{-1}) and the lowest in 2010 (116.08 kg ha^{-1}, Table 3). Similarly, flowers per raceme and pods per raceme were significantly (P<0.05) higher in 2012 than other experimental years. Because of more rainfall (82.0 mm) and less sunny hours (253.9 h) in May 2010, flowers per raceme and seed yield were both meaningfully (P<0.05) lower than other experimental years.

Effects on Seed Yield Components

Number of racemes per square meter: A decrease with increase in concentration from 0.2 to 0.8 mg g^{-1} was observed and higher racemes per square meter significantly (P<0.05) than CK (0.0 mg g^{-1}) (Table 4). At 0.2 and 0.4 mg g^{-1} Ca supply, racemes per square meter were highest with no significant difference (P>0.05) between these two treatments as compared to others.

Table 1: High, low, mean monthly temperature, sunshine hours and rainfall in 2009–2012

Years	Low, high, and mean temperature (ºC)														
	April			May			June			July			August		
	L	H	M	L	H	M	L	H	M	L	H	M	L	H	M
2009	4.4	18.8	11.6	9.1	23.3	16.3	15.9	31.3	22.4	17.3	30.3	23.6	14.1	25.8	19.8
2010	0.4	13.3	6.3	9.4	22.0	15.7	15.0	27.7	21.6	18.0	31.2	24.7	15.8	28.2	21.6
2011	2.9	17.4	10.1	8.4	22.2	15.4	15.7	29.5	22.7	16.2	30.0	23.0	16.2	28.8	21.8
2012	5.5	18.2	10.4	8.7	24.5	17.0	13.1	27.2	20.3	16.3	29.1	22.4	14.9	27.8	21.3
		Total rainfall (mm)													
2009		0.3			36.0			0.8			49.9			50.7	
2010		10.0			82.0			59.3			41.7			16.1	
2011		5.9			4.4			3.0			38.4			37.1	
2012		6.4			28.7			60.6			124.3			9.1	
		Total sunny hours (h)													
2009		268.7			297.9			328.0			255.3			236.1	
2010		262.5			253.9			283.0			289.5			243.4	
2011		274.0			269.3			265.1			264.3			249.0	
2012		261.0			279.9			274.1			240.7			262.7	

L, lowest monthly temperature; H, highest monthly temperature; M, mean monthly temperature

Table 2: F tests of year, Ca and their interactions on seed yield and yield components of alfalfa

Source	df	Mean square				
		Racemes per square meter	Flowers per raceme	Pods per raceme	Seeds per pod	Seed yield
Ca	4	**	*	**	NS	**
Year	3	**	**	**	**	**
Ca × year	12	**	**	**	*	**

* P< 0.05, **P< 0.01, NS, not significant

Table 3: Trend response to seed yield and yield components of alfalfa from 2009 to 2012

Years	Racemes per square meter	Flowers per raceme	Pods per raceme	Seeds per pod	Seed yield (kg ha^{-1})
2009	5429.3 d	22.13 b	7.86 c	4.89 b	517.74 c
2010	12083.0 b	19.97 c	7.93 c	5.27 a	116.08 d
2011	13022.7 a	20.32 c	8.60 b	5.46 a	578.34 b
2012	11474.0 c	24.91 a	10.34 a	5.33 a	746.82 a

Means in the same column with different letters are significantly different at the 0.05 level

Table 4: Effect of Ca treatments on seed yield and yield components of alfalfa from 2009 to 2012

Ca concentrations (mg g^{-1})	Racemes per square meter	Flowers per raceme	Pods per raceme	Seeds per pod	Seed yield (kg ha^{-1})
0 (CK)	9446.60 c	21.76 ab	8.51 b	5.27 ab	393.81 d
0.1	11132.64 a	22.08 a	8.55 b	5.06 b	517.80 b
0.2	11305.60 a	22.06 a	8.78 a	5.41 a	585.41 a
0.3	10359.30 b	21.63 b	8.82 a	5.34 ab	476.00 c
0.4	10267.30 b	21.63 b	8.75 a	5.10 b	475.71 c

Means in the same column with different letters are significantly different at the 0.05 level

Number of flowers per raceme: Number of flowers per raceme also showed significant (P<0.05) influence of Ca treatments (Table 2). Flowers per raceme decreased with the increasing Ca level, but there were no significant differences (P>0.05) between Ca treatments and CK (Table 4). Flowers per raceme with 0.2 and 0.4 mg g^{-1} Ca were significantly (P<0.05) higher than other treatments. The maximum number of flowers per raceme was recorded with 0.2 mg g^{-1} Ca treatment.

Number of pods per raceme: Pods per raceme increased with the increasing Ca level, but no significant difference (P>0.05) was observed between 0.2 mg g^{-1} Ca treatment and CK (Table 4). Likely, no significant difference (P>0.05) was found for pods per raceme among 0.4, 0.6 and 0.8 mg g^{-1} Ca treatments, and all significantly (P<0.05) higher than CK and 0.2 mg g^{-1} Ca treatment.

Number of seeds per pod: The results showed that seeds per pod increased and then decreased with increasing Ca concentration, but no significant differences (P>0.05) between Ca application and CK was found (Table 4). Among the Ca treatments, seeds per pod were highest in 0.4 mg g^{-1} and significant differences (P<0.05) was observed between 0.4 mg g^{-1} and 0.2 mg g^{-1} or 0.8 mg g^{-1} treatment.

Effects of Ca Application on Seed Yield

With the Ca level, increasing from 0.2 to 0.8 mg g^{-1}, the seed yield increased primarily and then decreased, and for each Ca treatment, it was higher significantly (P<0.05) than CK (Table 4). The highest seed yield of 585.41 kg ha^{-1} was obtained in the 0.4 mg g^{-1} application treatment. There was significant difference between seed yield in 0.2 and 0.4 mg g^{-1} Ca treatment and higher significantly (P<0.05) than 0.6 and 0.8 mg g^{-1}.

Correlation and Path-coefficient Analysis between Seed Yield and Yield Components

Correlation between seed yield and yield components, flowers per raceme (r=0.56**) and pods per raceme (r=0.61**) were found significantly with seed yield over years and Ca treatments (Table 5). Correlation of raceme per square meter (r=0.01) and seeds per pod (r=0.09) was insignificantly with seed yield, respectively. There was also a significantly positive relationship between pods per raceme and flowers per raceme (r=0.67**). Significant correlation between raceme per square meter and pods per raceme (r=0.38**) or seeds per pod (r=0.41**) was found.

Path-coefficient (pc) analysis indicated non-significant direct effects of flowers per raceme and seeds per pod on alfalfa seed yield (Table 6). However, pods per raceme (pc=0.60**) and flowers per raceme (pc= 0.40**) had significant and positive highest-direct effect on seed yield. Racemes per square meter had a negative and marginal direct effect on yield (pc= -0.22, P=0.1), but a positively indirect effect via pods per raceme resulted in the positive genetic correlation (pc=0.23) with seed yield (Table 6).

Discussion

The weather conditions showed significant variations during the consecutive 4 years. The favorable climate conditions in 2012 were beneficial to alfalfa seed production, nevertheless the unfavorable conditions during March–May accounted for the lower seed yield attained in 2010. Medeiros *et al.* (1995) found that too much rainfall during anthesis probably prevented insect fertilization resulting in losses of fertilized flowers and embryo abortion. Furthermore, alfalfa plants were lush and lodged easily with high rainfall (Karagić *et al*., 2009). Lodging reduced the number of viable flowers and stimulated regrowth from the crown or old branches, decreasing nutrient partitioning to the developing seed. Thus, the differences of climate conditions during cropping years could lead to development variations of perennial plants.

Differential response of seed yield components was found to foliar application of Ca. Racemes per square meter and flowers per raceme decreased, but pods per raceme increased with Ca application concentration increasing from 0.2 to 0.8 mg g^{-1}. Ca application also increased racemes per square meter, flowers per raceme decreased and pods per raceme compared with the CK. Taylor and Mirabel (1986) reported that racemes per square meter was a main seed yield component of alfalfa. However, Picchioni *et al*. (2002) delivered reverse conclusion that additional Ca treatment did not influence racemes per plant in *Lupinus havardii* after adding 4 concentrations of Ca (0, 2.5, 5.0, 10.0 mM) to nutrient culture solution. Lamaire *et al.* (1995) reported that flower development was affected by Ca supply. The strongly positive correlation of alfalfa seed yield per plant with flowers per raceme was found by Mrázkov and Vacek (1981). The results in this experiment showed that 0.2 and 0.4 mg g^{-1} Ca application could increase flowers per raceme in alfalfa. Kazemi (2013) reported that foliar application of salicylic acid and Ca had significant influence on vegetative and reproductive growth of strawberry (*Fragaria×ananassa* Duch.) and could increase the number of flowers. Similarly, results of Tang *et al.* (2007) showed that Ca supply through foliar spray could significantly increase the flower numbers of cyclamen (*Cyclamen persicum* L.). Abdoli *et al.* (2004) reported that pods per raceme was also an important component. Meena *et al*. (2007) concluded that Ca insufficiency resulted in groundnut pegs and pods to abort and reduced yield. Because of poor liquidity from older

Table 5: Simple correlation coefficients between seed yield and yield components in alfalfa

Characters	Racemes per square meter	Flowers per raceme	Pods per raceme	Seeds per pod	Seed yield
Racemes per square meter	1				
Flowers per raceme	-0.179	1			
Pods per raceme	0.376**	0.673**	1		
Seeds per pod	0.409**	-0.122	0.207	1	
Seed yield	0.011	0.557**	0.613**	0.096	1

**P< 0.01

Table 6: Path-coefficient analysis for seed yield and yield components in alfalfa and direct effects (underlined) and indirect effects are shown for racemes per square meter and pods per raceme

Characters	Racemes per square meter	Flowers per raceme	Pods per raceme	Seeds per pod
Racemes per square meter	<u>-0.223</u>	-0.023	0.224	0.032
Flowers per raceme	0.040	<u>0.126</u>	0.401**	-0.010
Pods per raceme	-0.084	0.085	<u>0.596</u>**	0.016
Seeds per pod	-0.091	-0.015	0.123	<u>0.079</u>

**P< 0.01

tissues to younger tissues, exogenous Ca must be requisite for the developing pod (Skelton and Shear, 1971). Thus, Ca supply contributed to pods development, which could increase the amount of mature pods per plant (Kabir *et al*., 2013). The present study findings are in agreement with the result of these experiments that Ca supply could significantly increase pods per raceme (Table 4). In addition, some researchers reported that seeds per pod was a steady relatively component in lots of crops (Idris, 2008; Zhang *et al*., 2008). Similarly, in this experiment, Ca application had not significant effect on seeds per pod.

Legumes were considered to have a higher requirement for Ca in their tissues than grasses. According to the results of this experiment, Ca application treatments could significantly ($P < 0.05$) increased seed yield by 20.8–48.7% in alfalfa, but the highest seed yield was obtained for 0.4 mg g^{-1} Ca application. Arshadullah *et al*. (2013) found that grain yield in wheat would be improved by 43% through application of 150 kg ha^{-1} Ca sulfate. Slack and Morrill (1972) pointed out that Ca was the most vital element in growth and development of peanut seeds and main limiting of the peanut production. Also Ca element was required by peanut plant from the time when pegs began to appear, fruit formation, until the pods were mature (Walker, 1975). Christos (2009) reported that Ca application could increase the chlorophyll concentration of plant. In addition, Ca supply could promote leaf photosynthetic rate (Yang *et al*., 2012). Thus, Ca application contributed to more synthesis of photosynthetic product and increased seed yield. Furthermore, Mahmood *et al*. (2009) reported that Ca supply could promote plant absorb nitrogen and increased nitrogen use efficiency, which contributed to the enhancement of seed yield. Thus, Ca supply could promote crop development. According to findings of present study, Ca application contributed to reproductive growth of alfalfa and improved the amount of vegetative organs including racemes per square meter, flowers per raceme and pods per raceme.

Alfalfa seed yield was reported to be significantly correlated with yield components (Ilić and Đukić, 2006). Bodzon (2004) reported that flowers per raceme had a significant relation with seed yield. Du *et al*. (2009) and Abadouz *et al*. (2010) pointed out positive correlation between pods per raceme and seed yield. These findings agreed with the result of this experiment. Ilić and Đukić (2006) found that the correlation between seeds per pod and seed yield was not meaningful.

Path-coefficient analysis was used to determine the amount of direct and indirect effect of the causal components on the effect components. Among yield components, pods per raceme had the significantly and positively highest-direct effect ($pc=0.60^{**}$) on seed yield (Table 6), as reported by Suleyman (2006) in alfalfa and Murat and Çiftci (2007) in rape. These findings implied that pods per raceme could serve as a selection index to enhance seed yield in alfalfa.

Conclusion

Results of this experiment suggests that Ca application could make profitable seed harvest, and application of 0.4 mg g^{-1} Ca resulted in the best growth and seed yield of alfalfa. Among seed yield components, pods per raceme showed highest sensitivity to Ca application, which implied pods per raceme was a key yield component in alfalfa when supplying Ca.

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