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Inflorescence Mutant Effect on Seed Yield Components in Alfalfa (*Medicago sativa* L. Gannong No. 6)

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Abstract: Alfalfa (*Medicago sativa* L.) is one of the most important forage legumes worldwide. Breeding to improve seed yield will enhance the value of alfalfa. Using three alfalfa cultivars (*M. sativa* L. Gannong No. 6, *M. sativa* L. Gannong No. 1 and *M. sativa* L. Defl) a field study was conducted from 2004 to 2007 to evaluate the effects of an inflorescence mutant (Gannong No. 6) on seed yield. The hypothesis was that a mutant with a long inflorescence would improve seed yield by improving seed yield components such as the number of flowers, pods and seeds per inflorescence and 1,000 seed weight. Each of these factors were investigated to determine the relationship between inflorescence length and seed yield components and to identify the key factors involved in improving seed yield. The results were statistically analyzed and yielded four main conclusions. Firstly, the differences among cultivars had significant effects on inflorescence length and the number of flowers, pods and seeds per inflorescence. Secondly, in Gannong No. 6, the average number of flowers, pods and seeds per inflorescence was 75.40, 30.88 and 76.23, respectively with an average inflorescence length of 12.46 cm. Thirdly, in Gannong No. 6, the inflorescence length significantly affected some yield components such as the number of flowers, pods and seeds per inflorescence. Finally, Gannong No. 6 possessed high seed yield because of an increased inflorescence length.

Key words: Alfalfa, inflorescence mutant, inflorescence length, seed yield components, China

INTRODUCTION

Alfalfa (*Medicago sativa* L.) is the most important forage legume because of its high forage yields with excellent quality. It is cultivated primarily for forage production and its seed yield is not considered to be of agronomic value to producers. The seed yield of a cultivar determines its competitive selling price. Cultivars with higher seed yield have a higher selling price and are more likely to be selected by farmers for planting (Falcinelli, 1999).

Seed yield is significantly affected by the number of forage harvested before seed production and this effect is associated primarily with variation in the number of fertile tillers per plant and pods per raceme. There was a positive correlation between seed yield and the number of seeds per pod across 46 experimental conditions of various cultivars and environments (Hacquet, 1990). Genetic diversity of seed yield components has been described between and within populations (Bolanos-Aguilar *et al*., 2000). Seed weight per inflorescence is associated with variation in the number of seeds per pod and the number of pods per inflorescence.

Seed weight has a strong genetic association with seed yield in alfalfa (Bolanos-Aguilar *et al*., 2002). In addition, the number of inflorescence per seed weight is considered to depend on environmental conditions (Alan and Hakan, 2007).

With the increased number of dairy cows in China, alfalfa hay production needs to improve and expand. However, cultivars with a high seed yield are rare. The mean seed yield of alfalfa in China is low (201-915 kg ha^{-1}) (Zhizhong, 2002). The objective of this research was to identify the phenotypic effects induced by an inflorescence mutant, to investigate the determinants of seed yield and to understand the relationship between inflorescence length and seed yield in alfalfa. The hypothesis was that the inflorescence mutant Gannong No. 6 could improve seed yield due to changes in seed yield components such as the numbers of flowers, pods and seeds per inflorescence.

MATERIALS AND METHODS

Experimental materials: A new cultivar (*M. sativa* L. Gannong No. 6) bred by Zhizhong Cao and Wenxu Zhang

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was used for these studies (Fig. 1-4). The inflorescence length in Gannong No. 6 is >8 cm which is 2-3 fold longer than that of other alfalfa cultivars. Two other alfalfa cultivars were used as controls, Gannong No. 1 and Defl.

Experimental site: The field experiment was conducted from 2004 to 2007 at the Gansu Agricultural University Station located in Jingtai County, Northwestern China (latitude 35°33'N, longitude 103°33'E; elevation 1,274 m). The natural and soil conditions are presented in Table 1 and 2, respectively.

Fig. 1: Gannong No. 6 at the blooming period

Fig. 2: Gannong No. 6 at the pod stage

Fig. 3: Gannong No. 6 at the mature stage

Field methods: Randomized complete blocks were designed with three replicates. Each plot contained ten rows that were 5 m long and 30 cm apart with a space of 50 cm between adjacent plots and a space of 1 m between adjacent replicates. The seeding rate was 10 kg ha^{-1} for all cultivars. Chemical fertilizer was not used. Weeds were controlled by hand weeding when necessary. Plots were irrigated once during the re-greening stage according to the climatic conditions.

Observation and data collection: The 1st year was considered as an establishment year and experimental measurements were taken during the subsequent 3 years. From 2005 to 2007, the inflorescence length of 30 samples taken randomly from each plot was measured using a meter ruler. The number of inflorescences per square meter, the number of flowers, pods and seeds per inflorescence and the 1,000 seed weight were determined by measuring the 30 random samples taken from each plot. Actual seed yields were determined by hand harvesting from each plot when 75% of the pods had turned blackish-brown. The seeds harvested from each plot were dried, threshed, cleaned, sieved, weighed and stored in paper bags before laboratory testing. Seed yield was calculated with seeds at 13% standard moisture content (kg ha^{-1}).

Statistical analyses. Data were analyzed statistically using randomized complete block design and Duncan's multiple range tests at 1% probability to compare the means of various measurements following different treatments. Computational analyses were performed with SPSS 13.0 (SPSS, Inc., Chicago, IL, USA).

Fig. 4: Gannong No. 6 inflorescence at the mature stage

Table 1: Natural conditions of the experimental fields

Climate	Annual rainfall (mm)	Annual transpiration (mm)	Annual temperature (°C)	Frost-free season (day)
Continental monsoon climate	185	3,000	9	141

Values are the means of 3 experimental years (2005, 2006 and 2007)

Table 2: Soil nutrients of the experimental fields (mg L^{-1})

Soil type	pH	OM	Ca	K	NH_4^+	P	Mg
Sierozem	8.6	0.25	841.1	103.7	5.4	14.25	207.4

Values are the means of 3 experimental years (2005, 2006 and 2007)

RESULTS AND DISCUSSION

In this study, the effects of inflorescence length on seed yield and seed yield components of alfalfa were investigated. The salient components of seed yield such as the number of inflorescences per square meter; inflorescence length; the number of flowers and pods per inflorescence and the 1,000 seed weight were studied to analyze the effect of inflorescence length on seed yield in alfalfa.

Number of inflorescences per square meter: The number of inflorescences per square meter was significantly different between different varieties of alfalfa ($p<0.01$; Table 3). The inflorescence number per plant is the main determinant of alfalfa seed yield (Taylor and Marble, 1986). The number of inflorescences per square meter was highest (12,277.92) in Gannong No. 6 and lowest (8,376.05) in Gannong No. 1 (Table 4). There was no significant difference ($p>0.01$) in the number of inflorescences per square meter between Gannong No. 6 and Defl (Table 4). However, the number of inflorescences per square meter was significantly higher ($p<0.01$) in Gannong No. 6 than in Gannong No. 1 (Table 4). However, the seed yield results showed that a low number of inflorescences per square meter does not necessarily correlate with a low seed yield as is observed in Gannong No. 1.

Inflorescence length: The results indicated that differences among cultivars have a significant effect ($p<0.01$) on the inflorescence length of alfalfa (Table 5). A similar result was observed in 18 progenies of synthetic populations of alfalfa (Uzik, 1996a).

During this 3 years investigation, the longest inflorescence (12.46 cm) was observed in Gannong No. 6 and the shortest (2.31 cm) was measured in Defl (Table 6). The inflorescence length of alfalfa generally ranges from 2-4 cm (Ukic, 1994). Further studies revealed that inflorescence length could be classified into three phenotypes: long racemes (>4 cm long), panicle inflorescences that are 2-4 cm in length and racemes that are approximately 2 cm long (Wyrzykowska *et al*., 2007). Gannong No. 6 belongs to the category of long racemes. Seed yield per plant is positively correlated with raceme length ($r = 0.31^*$) (Bodzon, 2004; Liatukiene *et al*., 2009). Consequently, Gannong No. 6 was predicted to have a high seed yield.

Table 3: Analysis of variance on seed yield and seed yield components of alfalfa in different cultivars

Source of variation	df	NISM	NFI	NPI	NSI	TSW	SYH
Replication	2	NS	NS	NS	NS	NS	NS
Treatment	2	**	**	**	**	**	**
Error	4	NS	NS	NS	NS	NS	NS
CV	8	NS	NS	NS	NS	NS	NS

**Significant at the 0.01 probability level. NS: Not Significant. Values are the means of 3 experimental years (2005, 2006 and 2007); NISM: Number of Inflorescences per Square Meter; NFI: Number of Flowers per Inflorescence; NPI: Number of Pods per Inflorescence; NSI; Number of Seeds per Inflorescence; TSW, 1,000 Seed Weight (g); SYH: Seed Yield per Hectare (kg ha^{-1})

Inflorescence length in Gannong No. 6 was significantly higher ($p<0.01$) than in Gannong No. 1 or Defl however, there was no significant difference ($p>0.01$) in inflorescence length between Gannong No. 1 and Defl (Table 6). This is probably because Gannong No. 1 and Defl belong to panicle inflorescences whereas Gannong No. 6 belongs to the category of long racemes (Wyrzykowska *et al*., 2007).

Number of flowers per inflorescence: Inflorescence length significantly affected the number of flowers per inflorescence ($p<0.01$; Table 3). Similar results were observed in 18 progenies of synthetic populations of alfalfa (Uzik, 1996a).

The number of flowers per inflorescence was highest (75.40) in Gannong No. 6 and lowest (20.55) in Gannong No. 1 (Table 4). The number of flowers per inflorescence ranges from 8-20 in alfalfa and the average number of flowers per inflorescence in synthetic populations of alfalfa is 26.6 (Ukic, 1994). The number of flowers per inflorescence in Gannong No. 6 was significantly higher ($p<0.01$) than in Gannong No. 1 and Defl (Table 4). There was no significant difference in the number of flowers per inflorescence between Gannong No. 1 and Defl (Table 4). The average number of flowers per inflorescence was significantly higher in Gannong No. 6 than in any of the other varieties because of its long inflorescence.

Seed yield per plant is positively correlated with the number of flowers per raceme ($r = 0.61^*$) (Bodzon, 2004; Liatukiene *et al*., 2009). Therefore, it is reasonable to predict that the seed yield of Gannong No. 6 would be higher than that of the other varieties.

Number of pods per inflorescence: Inflorescence length significantly affected the number of pods per inflorescence in alfalfa ($p<0.01$; Table 3). The number of

Table 4: Means of the yield determinants and seed yields of three alfalfa cultivars

Cultivars	NISM	NFI	NPI	NSI	TSW	SYH
Gannong No. 6	12,277.92±640.06^{A}	75.40±10.41^{A}	30.88±0.98^{A}	76.23±6.90^{A}	2.03±0.00^{C}	859.17±20.56^{A}
Gannong No. 1	8,376.05±179.94^{B}	20.55±1.02^{B}	13.61±0.21^{B}	46.73±1.31^{B}	2.31±0.00^{B}	447.78±45.35^{B}
Defl	11,049.31±852.38^{A}	21.15±0.78^{B}	12.18±1.12^{B}	40.44±4.09^{B}	2.47±0.01^{A}	290.16±48.73^{B}

Means in the same column with different letters are significantly different (p = 0.01). Values are the means±SE of 3 experimental years (2005, 2006 and 2007). NISM: Number of Inflorescences per Square Meter; NFI: Number of Flowers per Inflorescence; NPI: Number of Pods per Inflorescence; NSI: Number of Seeds per Inflorescence; TSW: 1,000 Seed Weight (g); SYH: Seed Yield per Hectare (kg ha^{-1})

Table 5: Analysis of variance of inflorescence length in different cultivars

		Inflorescence length			
Source of variation	df	2005	2006	2007	Mean
Replication	2	NS	NS	NS	NS
Treatment	2	**	**	**	**
Error	4	NS	NS	NS	NS
CV	8	NS	NS	NS	NS

**Significant at the 0.01 probability level; NS: Not Significant

Table 6: Inflorescence length in different cultivars (Mean±SE; cm)

Cultivar	2005	2006	2007	Mean
Gannong No.6	12.13±0.52^{A}	14.42±0.69^{A}	10.84±0.41^{A}	12.46±1.05^{A}
Gannong No.1	2.70±0.32^{B}	1.80±0.08^{B}	2.69±0.41^{B}	2.40±0.30^{B}
Defl	2.35±0.38^{B}	2.02±0.11^{B}	2.57±0.36^{B}	2.31±0.16^{B}

Means in the same column with different letters are significantly different (p≤0.01)

pods per inflorescence was highest (30.88) in Gannong No. 6 and lowest (12.18) in Defl (Table 4). The earlier reported average number of pods per inflorescence of alfalfa are 10.03 (Bolanos-Aguilar *et al*., 2001) and 17.25 (Shebl *et al*., 2008).

The number of pods per inflorescence was significantly higher (p<0.01) in Gannong No. 6 than in Gannong No. 1 or Defl (Table 4). The number of pods per inflorescence was not significantly different (p>0.01) between Gannong No. 1 and Defl (Table 4). The number of pods per inflorescence is not significantly different (p>0.01) in any other varieties of alfalfa except Gannong No. 6. Furthermore, seed yield is positively correlated with the number of pods per raceme (r = 0.42*) (Uzik, 1996a, b; Liatukiene *et al*., 2009). These results suggest that the average number of pods per inflorescence increased to 30.88 in Gannong No. 6 because of its long inflorescence.

Number of seeds per inflorescence: Inflorescence length significantly affected (p<0.01) the number of seeds per inflorescence (Table 3). The number of seeds per inflorescence was highest (76.23) in Gannong No. 6 and lowest (40.44) in Defl (Table 4). Similar results were observed in other varieties of alfalfa (Uzik, 1996a). The earlier reported average number of seeds per inflorescence are 34.57 (Bolanos-Aguilar *et al*., 2000) and 25.71 (Ukic, 1994).

The number of seeds per inflorescence was significantly higher in Gannong No. 6 (p<0.01) than in Gannong No. 1 or Defl. The number of seeds per inflorescence was not significantly different (p>0.01) between Gannong No. 1 and Defl (Table 4). The seed yield per plant is dependent on the average number of seeds per raceme (Wyrzykowska *et al*., 2007). The seed yield is positively correlated with the number of pods per raceme (r = 0.57*) (Uzik, 1996b; Liatukiene *et al*., 2009). These results suggest that the average number of seeds per inflorescence increased to 76.23 in Gannong No. 6 because of its long inflorescence.

Table 7: Simple correlation coefficients of seed yield components in three cultivars during the experimental years 2005, 2006 and 2007

Traits	IL	NISM	NFI	NPI	NSI	TSW
NISM	0.74^{NS}					
NFI	1.00**	0.75^{NS}				
NPI	1.00**	0.69^{NS}	1.00**			
NSI	0.99*	0.62^{NS}	0.98*	1.00**		
TWS	−0.94^{NS}	−0.45^{NS}	−0.93^{NS}	−0.96*	−0.98*	
SYH	0.97*	0.54^{NS}	0.96*	0.98*	0.99**	−1.00**

*Significant at the 0.05 probability level. **Significant at the 0.01 probability level. NS: Not Significant; IL: Inflorescence Length; NISM: Number of Inflorescences per Square Meter; NFI: Number of Flowers per Inflorescence; NPI: Number of Pods per Inflorescence; NSI: Number of Seeds per Inflorescence; TSW: 1,000 Seed Weight (g); SYH: Seed Yield per Hectare (kg ha^{-1})

Seed weight: The 1,000 seed weight was of significant difference between the various varieties of alfalfa (p<0.01; Table 3 and 4). The 1,000 seed weight was greatest (2.47 g) in Defl and lowest (2.03 g) in Gannong No. 6 (Table 4). Duncan's multiple range test analysis results showed that Gannong No. 6 had a low 1,000 seed weight but a high seed yield (Table 4).

Seed yield: Seed yield was statistically different between the various varieties of alfalfa (p<0.01; Table 3). The average range of seed yield for alfalfa in China is 201-915 kg ha^{-1}. Seed yield was highest (859.17 kg ha^{-1}) in Gannong No. 6 and lowest (290.16 kg ha^{-1}) in Defl (Table 4). Seed yield was significantly higher in Gannong No. 6 (p<0.01) than in Gannong No. 1 or Defl (Table 4). Seed yield was not significantly different (p>0.01) between Gannong No. 1 and Defl (Table 4).

Effect of inflorescence length on seed yield and seed yield components of alfalfa: The simple correlation coefficients calculated for the examined characteristics are shown in Table 7. Inflorescence length was positively correlated with all the examined traits, except 1,000 seed weight (r = -0.94). Little is known about the correlation of alfalfa

inflorescence length with seed yield and seed yield components. Inflorescence length was positively and significantly correlated with the number of flowers per inflorescence (r = 1.00**), the number of pods per inflorescence (r = 1.00**), the number of seeds per inflorescence (r = 0.99**) and seed yield (r = 0.97*). Seed yield was positively and significantly correlated with inflorescence length (r = 0.97*) and the number of flowers (r = 0.96*), pods (r = 0.98*) and seeds (r = 0.99**) per inflorescence. Seed yield was not significantly correlated with the number of inflorescences per square meter ($r = 0.54^{NS}$). But seed yield was negatively and significantly correlated with the 1,000 seed weight (r = -1.00**). Other studies have reported that the number of pods and seeds per inflorescence have the highest positive correlations with seed yield per plant (Reprove and Zolotarev, 1988; Hacquet, 1990; Rossellini *et al*., 1990; Bolanos-Aguilar *et al*., 2001, 2002). The current report shows that the number of seeds, pods and flowers per inflorescence and inflorescence length are key factors in determining alfalfa seed yield. The high seed yield of Gannong No. 6 is likely due to its increased inflorescence length and its greater number of flowers, pods and seeds per inflorescence compared to those of other cultivars.

CONCLUSION

Inflorescence length is the most important phenotypic characteristic of the inflorescence mutant (*M. sativa* L. Gannong No. 6) which has a high seed yield. The long inflorescence length of Gannong No. 6 significantly affected seed yield and the number of flowers, pods and seeds per inflorescence. The seed yield of Gannong No. 6 is significantly increased by its longer inflorescence length.

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