Differential Gene Expression Patterns of Male Sterile Lines of Alfalfa Hybrids at Bud Differentiation Stage and Heterosis

Liyan Cai
Inner Mongolia Academy of Forestry Monitoring, China

Fengling Shi
Inner Mongolia Agricultural University, China

Cuiping Gao
Inner Mongolia Agricultural University, China

Fengyan Yi
Inner Mongolia Academy of Agricultural and Animal Husbandry Sciences, China

Xia Gao
Inner Mongolia Academy of Forestry Monitoring, China

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Cai Liyan[1], Shi Fengling[2]*, Gao Cuiping[2], Yi Fengyan[3], Gao Xiia[1]
[1]Inner Mongolia Academy of Forestry Monitoring, Hohhot, China
[2]Inner Mongolia Agriculture University, Hohhot, China
[3]Inner Mongolia Academy of Agricultural and Animal Husbandry Sciences, Hohhot, China
*Corresponding author e-mail: gcp1978@126.com

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Introduction

Alfalfa (*Medicago sativa* L.) is important legume forage that is widely cultivated in China and other countries. Alfalfa breeding can't meet the need of production now, highlighted in yield and resistance of current alfalfa cultivars (He *et al*., 2000; Wei *et al*., 2007). Increasing heterozygosity of hybrids and thus the heterosis is a way to breed alfalfa cultivars with high yield and resistance (Hong *et al*., 2009). The objective of this study was to investigate the correlations of various differential gene expression patterns with forage yield in order to better understand the role the alfalfa male sterile lines may play in the heterosis of their progeny.

Materials and Methods

Plant materials: An incomplete diallel cross (NC II) was used to investigate the performance of the hybrids and their correlations with gene expression patterns present, where eight male sterile lines of alfalfa as female and four alfalfa cultivars with high yield as male parents were crossed and 32 combinations were generated. Randomized block design was conducted to compare the yields of parents and hybrids produced. The forage yield was then measured in the following year, and the cross combinations were grouped according to their hay yield of hybrids and their respective heterosis-based performance. 2 superior and 2 less superior combinations were chosen differential gene expression analysis.

Primers and adaptors for cDNA-AFLP analysis: The primers and adaptors (Bachem *et al*., 1996) were synthesized by the Shanghai Bio-Engineering Co., Ltd.

Statistical analysis: All the differentially displayed cDNA bands between 100 and 1,000bp were scored. According to the presence of the band in the parents and/or F_1 progeny, differential expressions of a cDNA-AFLP marker were arranged to different patterns. Numbers of fragments in every differential pattern of each cross combination were calculated for statistics. For each differential pattern of all cross combinations, the set of numbers of fragments as a whole was used as one variety. Correlation analysis of each two differential patterns was evaluated with the average of performance and mid-parent heterosis of forage yield traits was carried out.

The mid-parent heterosis = [(F_1/ the average of parents)/ the average of parents] × 100%. The correlation analysis was analyzed by SAS 9.0.

Results and Discussion

Patterns of differentially expressed fragments: Six differential expression patterns were observed according to the presence of the band among F_1 and its two parents. They were defined as follow: (1) UNF_1: markers only expressed in F_1. (2) ABF_1: markers expressed in both parents but not in F_1. (3) UNP_1: markers expressed only in maternal parent. (4) UNP_2: markers expressed only in paternal parent. (5) DMP_1: markers expressed in only maternal parent and F_1. (6) DMP_2: markers expressed in only paternal parent and F_1.

Differentially expressed fragments in superior and less superior combinations: The best fourteen informative primer combinations out of the 64 (E_2M_1, E_2M_5, E_3M_1, E_3M_2, E_3M_3, E_3M_7, E_3M_8, E_4M_4, E_4M_7, E_5M_2, E_7M_2, E_7M_5, E_7M_6, E_7M_8) were used in selective amplification for cDNA-AFLP analysis. Approximately 2336 fragments with molecular weight of 100～1000bp were detected among the F_1s and parents in at least one cross. 2028 (or 86.82%) fragments were detectable

in both replicates, and 411(or 20.27%) were polymorphic. 65.21% polymorphic bands were observed in superior crosses while 34.79% in less-superior crosses.

Correlations between differential patterns and hybrid performance of forage yield traits: Correlation analysis showed that: (1) significant positive correlation was detected between UNF_1 and the hay yield, branch number, diameter of main stem and node number). (2) UNP_2 was only positively correlated to the plant height (r=0.9647). (3) DMP_1 was positively correlated to the branch number (r=0.9662). (4) ABF_1, UNP_1 and DMP_2 were not correlated to the performance of all forage yield related traits.

Correlations between differential patterns and mid-parent heterosis of forage yield traits: Correlation coefficients between 6 differential gene patterns and the mid-parent heterosis of forage yield traits were listed in Table 4. Correlation analysis showed that: (1) significant positive correlations were detected between $UNF_1$and hey yield, plant height as well as branch number. (2) significant positive correlations were observed between DMP_1 and plant height, branch number as well as node number. (3) significant positive correlations were detected between ABF_1, and node number. (4) UNP_1, UNP_2 and DMP_2 were not correlated to the any of the traits related to heterosis.

UNP_1 and DMP_2 were not correlated to the mid-parent heterosis based on the forage yield-related traits, while UNF_1 positively correlated to the mid-parents heterosis based on hay yield, and DMP_1 and UNF_1 positively correlated to the mid-parents heterosis based on branch number. It can be also speculated that alfalfa hay yields and branch number and the corresponding heterosis may be controlled by the same genes. However, the genes controlling plant height, diameter of main stem and node number may be different from the genes controlling their heterosis.

Conclusion

The results indicated that there were 411 differential gene expression fragments and 6 differential gene expression patterns by 14 informative primer combinations. Significant correlations between bands expressed in hybrids and the performance of forage yield were observed while lower correlations were found between bands expressed in only one parent as well as the F_1 and forage yield. Our study also showed that bands expressed only in male parent were in favor of the forage performance or heterosis.

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