

Exploration, Genetic Analysis, & Breeding Application of Multifoliate Traits on Alfalfa

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Question: Manipulation of the multifoliate trait of alfalfa (*Medicago sativa* L.) is an effective strategy for increasing alfalfa hay forage yields and protein content.

Target: Our research aims to identify the multifoliate trait of alfalfa and to breed new alfalfa cultivars. As a common legume in southern China, Alfalfa germplasm is an important resource in the Grassland-crops agricultural ecosystem.

Methods: Backcross breeding was used to select multifoliate alfalfa varieties that are suitable for the Jianghuai area of China and have higher yields and improved quality. Huaiyin alfalfa was used as a recurrent parent and multifoliate alfalfa (PL34HQ) as a non-recurrent parent, and breeding materials to meet backcross breeding requirements were obtained.

Results: When the agronomic traits of the BC₁ population were compared to those of the F₁ population, the multifoliate rate of the BC₁ population was higher ($p > 0.01$). Single plant multifoliate rates averaged 71.19 % and 26.65 %, respectively. The multifoliate rate of a single plant in the BC₁ population nearly changed from increasing to decreasing as it grew. The multifoliate expression of alfalfa leaves was characterized by cytoplasmic inheritance, and multifoliate expression was different before budding, most likely due to genetic and environmental factors.

The agronomic traits of the BC₁ population were compared to those of the F₁ population, and the genetic diversity of agronomic traits was studied at the molecular level. This report screened 425 pairs of SSR, including 100 pairs of EST-SSR and 325 pairs of SSR from *Medicago truncatula*. The results showed that the BC₁ population's agronomic traits were more similar to those of its parents than those of the F₁ population, and the BC₁ population's trait diversity was high. A total of 148 alleles variations were detected by 25 pairs of SSR markers, and an average of 5.92 per primer, the average ratio of polymorphism was 82.16%. Analysis of population structure showed that K had a maximum value when $K = 4$, the BC₁ could be divided into 4 groups, of which 84.27% of individual plants were with single genetic components, and the rest 15.37% were with a mixed genetic component. The genetic diversity of the BC₁ population was abundant, and the division of population structure was not completely related to the genetic relationship.

Conclusion: In this study, using hybridization, backcross breeding, SSR molecular marker-assisted selection, and population structure study, the target types with good parental traits were selected, which provided abundant materials for subsequent multifoliate alfalfa breeding. The genetic structure of the alfalfa population was revealed and the genetic characteristics of the alfalfa population were evaluated.

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