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Research Article
Development of molecular markers for characteristics of interspecific hybrids of foxtail

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Abstract
Methods of molecular and genetic characteristic of hybrids of meadow foxtail (Alopecurus pratensis L.) and ventricose foxtail (Alopecurus ventricorus Pers.) by protein and DNA markers has been developed. Hybrids were developed by method of cultivating young embryo in vitro. Forms with stable seed production and providing high quality fodders have been selected. Hybrid plants significantly differ in content of total protein and soluble carbohydrates. High changeability of polypeptide spectra of total protein extracted from interspecific hybrids of foxtail has been revealed. Analysis of hybrids by DNA markers by ISSR-PCR method has revealed high polymorphism of samples.

Keywords: Foxtail; interspecific hybrids; molecular markers; ISSR-PCR; protein markers; DNA polymorphism.

Introduction

Biotechnological methods and approaches play an exceptional role in plant selection today. They allow not only to significantly improve the rates of development of new highly productive and resistant cultivars of the most important agricultural plants but also in creating unique forms of plants with predetermined economically valuable characteristics with the help of bioengineering and cellular technologies [1,2].

Provisioning of reliable forage reserve for animal production has special importance for animal production development. New forms of permanent grasses are being developed for this purpose [3]. Selection of permanent grasses are oriented on development of genotypes with good regrowth and stability of yields; resistance to diseases, winter conditions, and with shade tolerance; high competitiveness in multi-component grasses; and stable seed production [4].

Foxtail is a valuable fodder grass of mixed grass crop for mowing and grazing usage in today rarely used cultures. It is undemanding to climate and soil conditions culture. We used two sorts of foxtails-meadow foxtail (Alopecurus pratensis) and ventricose foxtail (Alopecurus ventricosus) of 30 sorts of foxtails distributed in the territory of the former USSR. In first hay-crop these sorts forms 83% of reproductive shoots and only extended innovation shoots in the second hay-crop. These sorts are characterized by intensive regrowth and high quality of fodders on their base with the content of metabolizable energy equals 11-11.5 MJ/kg of dry matter and raw protein about 18-20% [5]. But both sorts have significant drawbacks that hamper their utilization in fodder production [6,7]. High quality fodder may be produced from the meadow foxtail (A. pratensis) but it has low seed production due to irregularity of seed maturing and their high fall. Ventricose foxtail (A. ventricosus) has high seed production but it was characterized by low fodder quality.

We have developed interspecific hybrids of foxtail with high fodder quality and stable seed production by the method of cultivating young embryo in vitro [8]. Sorts of meadow foxtail (A. pratensis) such as Rassvet, Brudzynska, Donskoj-20, Poiret, 4-RA local, and Alattyani were used as maternal ford and ventricose foxtail...
(A. ventricosus), Dovski was used as paternal form. Harvested hybrids were characterized by the following parameters: number of shoots and verdurous masses of one plant, bunch diameter, width and length of a leaf, seed production, % of dry matter. 19 genotypes were selected for further evaluation.

In modern selection methods of electrophoresis of different protein fractions [9] and DNA marking [10-12] are used for molecular and genetic evaluation of different genotypes. These methods allow for studying interspecific and intraspecific polymorphism, identifying and certifying different types of cultivated plants. ISSR-PCR is the most perspective method of revealing intraspecific polymorphism. It has been successfully applied for genetic typing, marking, and phylogenetic plants’ research [13-15].

The purpose of the present work is studying of genetic polymorphism of intraspecific hybrids of foxtail by protein and DNA markers.

Materials and Methods

Parental sorts of meadow foxtail (A. pratensis L.) and ventricose foxtail (A. ventricorus Pers.) were used as research object.

Laemmli analysis of protein polymorphism of albuminous fraction of foxtail hybrid plants’ seeds [9].

ISSR-PCR for revealing of polymorphism of DNA fragments was carried out according the methods described in FHN with the use of the following primers:

- IS2: aca-cac-aca-cac-aca-cg
- IS3: gag-aga-gag-aga-gag-ac
- IS5: cac-aca-cac-aca-car-c

PCR products were divided by electrophoresis in 2% agarose gel with ethidium bromide and analyzed with the system of gel-documentation GelDocXR (BioRad, USA).

Results and Discussion

Plants of meadow foxtail Rassvet, Rg-782, Brudzynska, Krinichni, Puszavan, 4-RA local, Donskoi 20, Piotret, and Obski were used as maternal forms for analysis of interspecific hybrids, and ventricose foxtail Dovski was used as paternal form.

Total protein content in leaves of hybrid plants of foxtail and parental forms in three haycrops were defined with threefold repeatability. Soluble carbohydrates content was defined with fourfold repeatability. In general the leaves of hybrid plants of foxtail have close measurements of protein, the highest protein content was observed in hybrid plants 3/1, 3/2, and 3/3; and hybrid plant 1/3 had the lowest protein level (see Figure 1). Hybrid plants significantly differ in soluble carbohydrates content. Soluble carbohydrates content of hybrid plants 3/1, 3/2, and 3/3, of three maternal forms and one parental form is 2-3 times higher than that of hybrid plant 1/1-2/3.

![Figure 1: Quantitative definition of total protein and soluble carbohydrates in leaves of hybrid plants and parental forms.](image-url)
Biochemical analysis carried out on hybrid plants of foxtail by electrophoretic spectrum of crude proteins of seeds and it was determined that the main part of polypeptides of hybrid foxtail plants and their parental forms are located in molecular mass range from 116.0 to 10.0 kDa. Spectra are highly variable and have components with individual distribution and the level of expression almost for each sample (see Figure 2).

Comparison of intraspecific changeability in three cross-breeding combinations showed that the highest protein polymorphism has manifested itself in first cross-breeding combination. Average quotient of similarity is 47-65%.

Figure 3: Analysis of DNA of interspecific hybrids of foxtail with ISSR-PCR method.
(A) Leaves’ DNA: 1-4 primer IS2, 5-8 primer 3, 9-12 primer IS5; Hybrids: 1, 5, 9 – Rasvet X Dovski; 2, 6, 10 – Donskoi X Dovski; 3, 7, 11 – Obski X Dovski; 4, 8, 12 – Brudzynska X Dovski. (B) DNA from parent and hybrid seeds: 1 – Rasvet X Dovski; 2 – Poiret X Dovski; 3 – Poiret; 4 – Brudzynska; 5 – Obski X Dovski; 6 – Alattijini; 7 – Donskoi X Dovski; 8 – Donskoi X Dovski; 9 – Rasvet; 10 – Obski X Dovski; 11 – Alattijini X Dovski; 12 – Poiret X Dovski; 13 – 4AR mestnu X Dovski; 14 X 4AR mestnu; 15 – Brudzynska.
The lowest changeability is characteristic for the second cross-breeding combination with 86%, and in the third combination is 74%.

ISSR-PCR analysis of DNA samples extracted from seeds and leaves of interspecific hybrids of foxtail showed that using primers IS2, IS3, and IS5 allowed obtaining relatively high polymorphism DNA that reflects genetic diversity of harvested hybrid forms (see Figure 3A and 3B).

Conclusion

Interspecific hybrid plants resulting from cross-breeding of meadow foxtail (A. pratensis L.) with ventricose foxtail (A. ventricorus Pers.) have been studied by total protein content and soluble carbohydrates content. High changeability of polypeptide spectra of total proteins between the hybrids has been observed. Analysis of interspecific hybrids by DNA markers by ISSR-PCR method allowed revealing high polymorphism of samples. Developed methods of molecular marking with protein and DNA markers may be used for characterizing interspecific hybrids of foxtail.

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References


