Development of Molecular Markers for Characteristic 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 ventricosus Pers.) by protein and DNA markers has been developed. Hybrids were developed by method of young embryo in vitro. Forms with stable seed production and provides 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 inter-specific hybrids of foxtail has been revealed. Analysis of hybrids by DNA markers by ISSR-PCR method has revealed high polymorphism of samples.

Key words: Foxtail • Inter-specific hybrids • Molecular markers • ISSR-PCR • Protein markers • DNA polymorphism

INTRODUCTION

Biotechnological methods and approaches play exceptional role in today plant selection. They allow not only significantly improve the rates of development of new highly productive and resistant cultivars of the most important agricultural plants but 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, with shade tolerance; high competitiveness in multi-component grasses; stable seed production [4].

Foxtail is valuable fodder grass for grass mixed grass crop for mowing and grazing usage of 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 in the second hay-crop – only extended innovation shoots. 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 but it has low seed production due to irregularity of seed maturing and their high fall. Ventricose foxtail (Alopecurus ventricosus) has high seed production but it characterized by low fodder quality.

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We have developed inter-specific hybrids of foxtail with high fodder quality and stable seed production by method of cultivating of young embryo in vitro [8]. Sorts of meadow foxtail Rassvet, Brudzynska, Donskoi-20, Poiret, 4-RA local, Alatyan were used as maternal ford and ventricose foxtail (Alopecurus 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 studying inter-specific and intra-specific polymorphism, identifying and certifying different types of cultivated plants. ISSR-PCR is the most perspective methods of revealing intra-specific polymorphism. It has been successfully applied for genetic typing, marking and phylogenetic plants' research [13, 14, 15].

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

MATERIALS AND METHODS

Parental sorts of meadow foxtail (Alopecurus platensis L.) and ventricose foxtail (Alopecurus 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-caa-cac-caa-cg
IS3: gag-agag-agag-agag-ac
IS5: cac-caa-caa-caa-car-c

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

Main Body: Plants of meadow foxtail Rassvet, Rg-782, Brudzynska, Krinichni, Puszavan, 4-RA local, Donskoi 20, Poirot, Obski were used as maternal forms for analysis of inter-specific hybrids and ventricose foxtail Dovskoi was used as paternal form.

Total protein content in leaves of hybrid plants of foxtail and parental forms in three hay-crops was defined with thrice-repeated repeatability. Soluble carbohydrates content was defined with fourfold repeatability. In general the leaves of hybrid plants of foxtail have close measurements of protein, the greatest protein content was observed in hybrid plants 3/1, 3/2 and 3/3 and hybrid plant 1/3 had the lowest protein level (Fig. 1). Hybrid plants significantly differ in soluble carbohydrates content. Soluble carbohydrates content of hybrid plants 3/1, 3/2, 3/3, of three maternal forms and one parental form is 2-3 times higher that of hybrid plant 1/1 – 2/3.

There was also carried out biochemical analysis of hybrid plants of foxtail by electrophoretic spectrum of crude proteins of seeds and it was determined that the
Fig. 2: Electrophoretic division of crude soluble proteins of foxtail seeds:

Fig. 3: Analysis of DNA of inter-specific hybrids of foxtail with ISSR-PCR method

Fig. 3A, 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.

Fig. 3a – DNA from parent and hybrid seeds: 1- Rasvet ð Dovski; 2 - Poiret x Dovski;
3 – Poiret; 4 – Brudzynska; 5 – Obski x Dovski; 6 – Alattijini; 7 – Donskoi x Dovski;
8- Donskoi xDovski; 9- Rasvet; 10 – Obski x Dovski; 11 – Alattijini x Dovski;
12 – Poiret x Dovski; 13 – 4AR mestnu x Dovski; 14- 4AR mestnu; 15 – Brudzynska

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 (Fig. 2).
A(1-2) third cross-breeding combination, B (3-5) maternal forms Donskoi 20, Pioreset, Obski, (6) – parental form Dovski; C (7-14) – first cross-breeding combination; D (15-16) – second cross-breeding combination.

Comparison of intra-specific 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%. The lowest changeability is characteristics for the second cross-breeding combination - 86%, in third combination it equals 74%.

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

CONCLUSION

Inter-specific hybrid plants resulting from cross-breeding of meadow foxtail *Alopecurus pratensis* L.) with ventricose foxtail (*Alopecurus 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 inter-specific hybrids by DNA markers by ISSR-PCR method allowed revealing high polymorphism of samples.

**Resume:** Developed methods of molecular marking with protein and DNA markers may be used for characterizing inter-specific hybrids of foxtail.

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**REFERENCES**


