Identification of artificial interspecific hybrids in the genus *Elymus* (Poaceae) by using ISSR-markers

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Abstract. Reproductive relationships between species of the genus *Elymus* (Poaceae) from mixed populations of the Southern Urals were studied. The true hybrids were identified by using ISSR-markers in comparison with parental biotypes. The presence in the true hybrids of species-specific ISSR-bands from both parental forms is provided by high level of homozygosity of species in genus *Elymus*, as a result of predominant self-pollination.

1 Introduction

The genus *Elymus* L. consists of polyploid perennial species of the tribe Triticeae Dum. (Poaceae). Taxa of the genus have haplomes “St”, “H”, “Y”, “P”, “W”, which originated from other genera of tribe Triticeae in different combinations, but “St” haplome presents in all *Elymus* species [1]. “St” haplome descended from *Pseudoroegneria* genus, “H” haplome – from *Hordeum* genus, “P” haplome – from *Agropyron* genus, “W” haplome – from *Australopyrum* Löve; the origination of “Y” haplome was not determined exactly yet. The genome constitution of *E. mutabilis* (Drob.) Tzvel. and *E. caninus* (L.) L. was identified as StStHH (2n=4x=28) [1]. In *E. uralensis* (Nevski) Tzvelev and *E. viridiglumis* (Nevski) Czer. the chromosome number 2n=28 was determined too [2], but genome formula was not identified conclusively.

Previously by using six ISSR-primers we showed the existence of genetic distances between biotypes of *E. uralensis*, *E. viridiglumis*, *E. mutabilis* and *E. caninus* growing on the closely located areas of Southern Urals, and significant distinctions between groups of Ural and Siberian species were revealed (Fig. 1).

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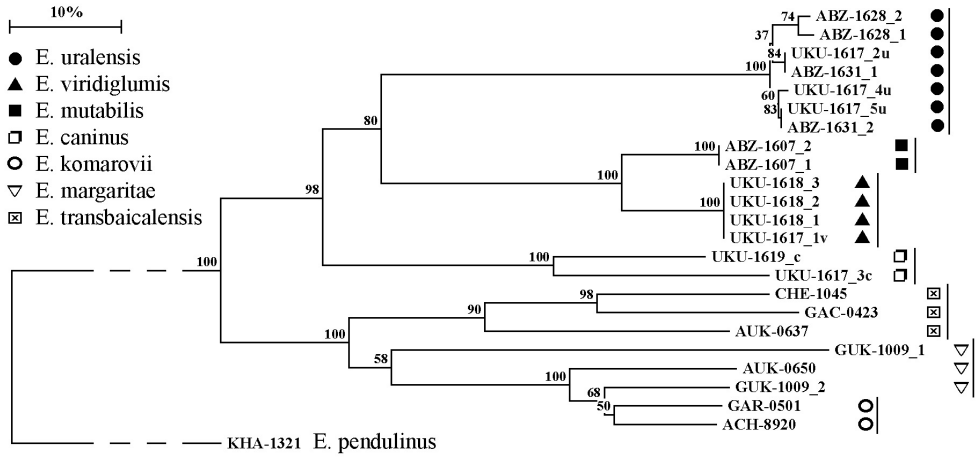

Fig. 1. Neighbour-Joining consensus tree with bootstrap support values based on results of the use of six ISSR-primers.

The multiple reticulate microevolution (which includes periodic hybridization and introgression), as a result of these species growing together during some generations, was suggested. For the study of reproductive compatibility of these species we created a series of hybrid plants between selective biotypes from mixed populations. The purpose of this work was identification of hybrid F_1 plants between *E. uralensis*, *E. viridiglumis*, *E. mutabilis* and *E. caninus* based on their specificity on ISSR-markers.

2 Materials and Methods

F_1 hybrids were created on the experimental plot of CSBG SB RAS (summer vegetation of 2017) by using the previously developed method [3]. These F_1 plants were grown in climate chamber of CSBG SB RAS. DNA was isolated from dried leaves by using kit for isolation of genomic DNA NucleoSpin Plant II (Macherey-Nagel, Germany). The PCR was set with ISSR-primers HB12 ($(CAC)_3GC$), M2 ($(AC)_8(C/T)G$) and M11 ($(CA)_6(G/A)$). The 15 µl mix of PCR contained 10 mM primer, 25 mM $MgCl_2$, 5 mM deoxynucleotides, 1x PCR-buffer, 0,9 units of Taq DNA polymerase and 2 µl of template DNA. PCR was carried out in C 1000 Thermal Cycler (BioRad Laboratories, USA). Annealing temperature for HB12 and M11 primers was 51°C, for M2 primer – 56°C. The obtained PCR-products were stained by SYBR-Green dye and separated by 1,5% agarose gel electrophoresis in 1x TBE buffer. The size of ISSR-fragments was determined according to 1 kb DNA ladder. Results of PCR were visualized by Gel Doc XR+ gel documentation system (BioRad Laboratories, USA).

3 Results and Discussion

We have obtained 20 plants in 7 cross combinations among *E. uralensis*, *E. viridiglumis*, *E. mutabilis* and *E. caninus*. Results of ISSR-analyses of assumed hybrids are shown in Fig. 2 in comparison with parental biotypes.

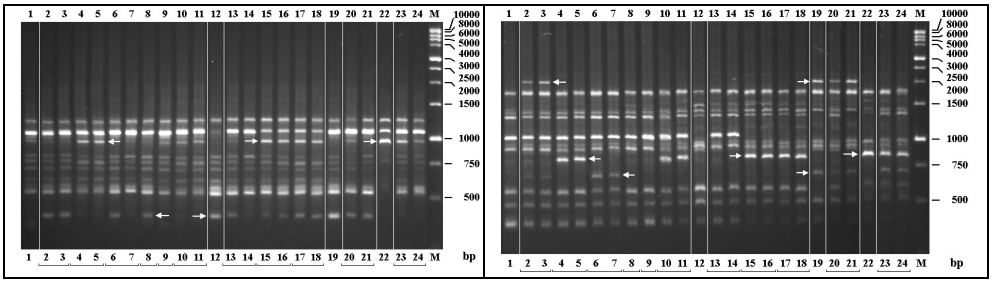

Fig. 2. ISSR-analysis of *E. uralensis*, *E. viridiglumis*, *E. mutabilis and E. caninus* and of hybrids between them with primers HB12 (left) and M11 (right). **1**. *E. uralensis* UKU-1617-2u; **2, 3**. uralen ABZ-1634 × mutab ABZ-1607; **4, 5**. uralen UKU-1617-2u × canin UKU-1613; **6, 7**. uralen UKU-1618 × mutab ABZ-1665; **8**. uralen UKU-1617-4u × viridi UKU-1618; **9**. uralen ABZ-1631 × viridi UKU-1618; **10, 11**. uralen UKU-1617-2 × canin UKU-1617-3c; **12**. *E. viridiglumis* UKU-1618; **13, 14**. viridi UKU-1618 × uralens ABZ-1634; **15-18**. viridi UKU-1618 × canin UKU-1613; **19**. *E. mutabilis* ABZ-1607; **20, 21**. mutab ABZ-1607 × uralens ABZ-1634; **22**. *E. caninus* UKU-1613; **23**. canin UKU-1613 × mutab ABZ-1665; **24**. canin ABZ-1654 × mutab ABZ-1665.

1. In the combination ***ural* × *mutab*** (Fig. 2, tracks 2, 3, 6, 7) 4 plants were obtained. With the use of M11 primer 2 of 4 plants had paternal ~ 2000 b.p. band, and 2 other plants had paternal ~ 700 b.p. band (shown by arrows). Based on these results, we can conclude, that all 4 obtained plants are hybridous.
2. In the combination ***ural* × *canin*** (Fig. 2, tracks 4, 5, 10, 11) also 4 plants were obtained. With HB12 and M11 primers all 4 plants had fragments, specific for paternal form *E. caninus*, ~ 900 b.p. and ~ 800 b.p., respectively (shown by arrows). Hence, all 4 plants are hybridous.
3. In the combination ***ural* × *viridi*** (Fig. 2, tracks 8, 9) 2 plants were obtained. With M2 primer, the first plant had paternal ~ 850 b.p. band, and with HB12 primer it had paternal ~ 200 b.p. band (shown by arrows). With M11 primer both plants did not differ from the maternal form. We can conclude that the first plant is a hybrid, and the second one – is non-hybridous.
4. In the combination ***viridi* × *ural*** (Fig. 2, tracks 13, 14) all 2 obtained plants with primers HB12, M2 and M11 had amplicons, specific for paternal form *E. uralensis*, ~ 1100 b.p., ~ 600 b.p. and ~ 1200 b.p., respectively, what verifies that they are hybridous.
5. In the combination ***viridi* × *canin*** (Fig. 2, tracks 15-18) all 4 obtained plants with primers HB12 and M11 had specific paternal bands ~ 900 b.p. and ~ 800 b.p., respectively. With M2 primer paternal band in all plants coincides in size with the maternal one, so plants did not differ from the maternal form, but we can surely say, that all 4 plants are hybridous.
6. In the combination ***mutab* × *ural*** (Fig. 2, tracks 20, 21) 2 plants were obtained. With M2 and M11 primers these plants do not differ from maternal forms and do not have specific paternal amplicons. With HB12 one plant had double ~ 1000 b.p. fragment as in the maternal form, while the other plant does not have it. Based on a molecular data, we can assume that these plants are non-hybridous.
7. In the combination ***canin* × *mutab*** (Fig. 2, tracks 23, 24) all 2 obtained plants with primers HB12 and M2 had specific paternal bands ~ 600 b.p. and ~ 850 b.p., respectively. With M11 primer these plants had no clear paternal bands sized 2000 b.p., but they had paternal bands ~ 700 b.p. It all points that these plants are hybridous.

We have obtained hybrid F_1 plants in crosses of *E. uralensis*, *E. viridiglumis*, *E. mutabilis* and *E. caninus* in different combinations, except for the pair *E. mutabilis* – *E. viridiglumis*. The presence in hybrids of species-specific ISSR-bands from both parental forms is provided by high level of homozygosity of species in the genus *Elymus*, as a result of predominant self-pollination. Hence, the present study shows the possibility to determine the hybridity of plants at early ontogenesis stages by means of ISSR-markers. Subsequently, based on the results of the analysis of anthers condition and seed fertility of these hybrids, the conclusion will be drawn about the presence or absence of reproductive isolation and introgressive processes between species.

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