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Identification Gene Fertility *Rf1* in Collection Samples of *Sorghum bicolor* (L.) Moench on Southern Russia †

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Abstract: Grain sorghum (*Sorghum bicolor* (L.) Moench) is one of the major crops used for various purposes, including animal and human nutrition. The most relevant strategy for create new sorghum hybrids is the use of lines with cytoplasmic male sterility (CMS). However, the process of creating sterile lines and lines that restore fertility is very laborious and time-consuming. The breeders need to know the genotype of the original parental forms of sorghum by the presence of the main genes that control fertility to speed up breeding work. One of these is the Rf1 gene. Our study aimed to identify alleles of the Rf1 gene in collection samples of grain sorghum (*Sorghum bicolor* (L.) Moench) adapted to the arid conditions of southern Russia. The studies carried out on southern Russia (FSBSI "ARC "Donskoy", Zernograd, Russia) in 2018–2019. The presence of alleles of the Rf1 fertility gene using the Xtxp18 SSR marker by PCR analysis in collection samples of grain sorghum (313 pcs.) was studied. A crossed some samples with two sterile lines—"Demetra S" and "Dzhetta S" (developed in FSBSI "ARC "Donskoy") were doing in 2019. The assessment of the fertility of self-pollinated lines was carried out in the field using a 3-point scale. The polymorphism of the Xtxp18 marker, a wide allelic diversity of the Rf1 gene in collection samples of sorghum and the association of the identified Rf1 alleles with the fertility and sterility of self-pollinated hybrids of grain sorghum as a result of the study was performed. This result will make it possible to deepen understanding of the influence of the Rf1 gene alleles on the level of fertility of sorghum plants in the future, as well as to accelerate the breeding process to create sterile lines and their fertile analogues for further obtaining commercial hybrids.

Keywords: *Sorghum bicolor*; Rf1; fertility; PCR; allele identification

1. Introduction

Grain sorghum (*Sorghum bicolor* (L.) Moench) is one of the major crops used for various purposes, including animal and human nutrition [1]. The most relevant strategy for create new sorghum hybrids is the use of lines with cytoplasmic male sterility (CMS) [2]. However, the process of creating sterile lines and lines that restore fertility is very laborious and time-consuming [3]. The breeders need to know the genotype of the original parental forms of sorghum by the presence of the main genes that control fertility to speed up breeding work. One of these is the Rf1 gene [4].

The genetic background of our collection samples of grain sorghum adapted to the arid climate of southern Russia has not been studied before.

Our study aimed to identify alleles of the Rf1 gene in collection samples of grain sorghum (*Sorghum bicolor* (L.) Moench) adapted to the arid conditions of southern Russia.

2. Experiments

The studies carried out on southern Russia (FSBSI "ARC "Donskoy", Zernograd, Russia) in 2018-2019 (46°50′42″ N, 40°18′30″ E).

We studied 313 collection samples of grain sorghum (*Sorghum bicolor* (L.) Moench) in laboratory conditions, and 106 hybrid combinations (53 combinations each with sterile lines of Demetra S and Dzhetta S) in the field in two repetitions.

The genomic DNA was isolated from the leaves and grains *Sorghum bicolor* using the modified CTAB protocol [5].

The homogenization of grains and leaves of sorghum was using a Bertin Precellys24 homogenizer (France) in 2 mL test tubes with the addition 6 pcs. of 2.8 mm zirconium oxide beads, in the presence of 200–250 μL of DNA isolation buffer with program: 6500 rpm—30 s, pause 5 s, 6500 rpm—30 s.

The presence of alleles of the Rf1 fertility gene was using the Xtxp18 SSR marker by PCR analysis [6]. The estimation of the allele size of the Rf1 gene was carried out using electrophoresis on 2% agarose gel, a Bio-Rad GelDoc XR+ device and the Bio-Rad ImageLab 6.0.1 software.

A crossed 53 samples with two sterile lines—"Demetra S" and "Dzhetta S" (developed in FSBSI "ARC "Donskoy") were doing in 2018 and 2019. The assessment of the fertility of self-pollinated lines was carried out in the field using a 3-point scale: 0—sterile line, 1—semi-sterile line, 2—fertile line. Data analyses was carried out in Microsoft Excel.

3. Results

As a result of the assessment of the collection samples of grain sorghum for the alleles of the Rf1 gene, a wide genetic diversity was revealed in them and many different alleles were identified. The percentage distribution of grain sorghum samples by the presence of Rf1 gene alleles is shown in Figure 1.

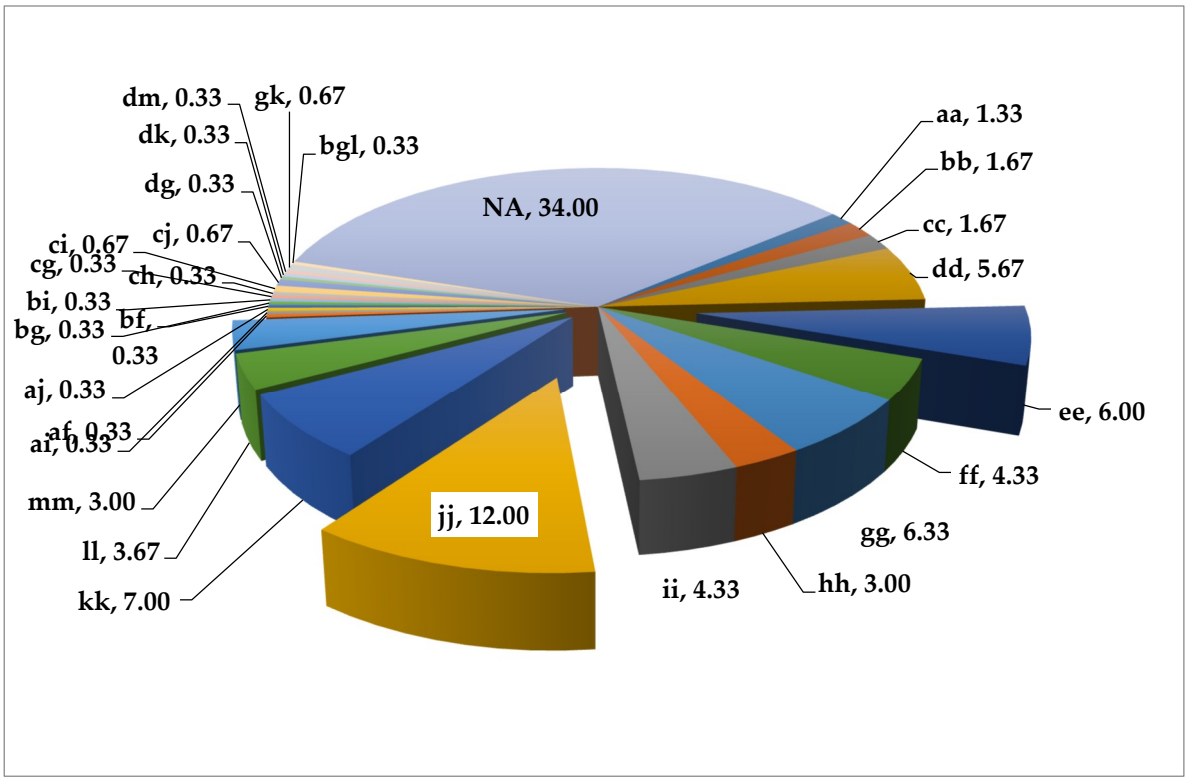

Figure 1. Percentage distribution of grain sorghum samples by the presence of Rf1 gene alleles: **aa**—266 bp, **bb**—262 bp, **cc**—258 bp, **dd**—248 bp, **ee**—238 bp (associated with Rf1), **ff**—236 bp, **gg**—232 bp, **hh**—230 bp, **ii**—228 bp, **jj**—220 bp (associated with Rf1), **kk**—210 bp, **ll**—200 bp,

mm—190 bp, **af**—266 bp + 236 bp, **ai**—266 bp + 228 bp, **aj**—266 bp + 220 bp, **bf**—262 bp + 236 bp, **bg**—262 bp + 232 bp, **bi**—262 bp + 228 bp, **cg**—258 bp + 232 bp, **ch**—258 bp + 230 bp, **ci**—258 bp + 228 bp, **cj**—258 bp + 220 bp, **dg**—248 bp + 232 bp, **dk**—248 bp + 210 bp, **dm**—248 bp + 190 bp, **gk**—232 bp + 210 bp, **bgl**—262 bp + 232 bp + 200 bp, **NA**—null-allele.

We identified 36 samples of grain sorghum with the j allele associated with fertility, 18 samples with the e allele associated with sterility, as well as samples with previously unknown alleles, which we temporarily named k—22 samples, l—11 samples, and m—9 samples. All identified genotypes are presented in Table 1.

Table 1. Identified alleles in collection samples of grain sorghum.

Allele	Samples	Number of Samples
a	Zernogradskoe 204, ZSK 1579/17, ZSK 134/17, ZSK 287/17	4
b	Luch 6, i. o. V-169, 871/17, ZSK 600/15, ZSK 2010,	5
c	Svetloe, LBK-28, Belozyornoe 100, Nizkorosloe 815/17, Belozyornoe 818/17	5
d	Sostav, Genicheskoe 209, H.S. 21, 03-3005, R-583, N-16, Sb-121/5, F-5096, Belyj luch, Kazachyok, 086c x RF 10, Sorgo prosovoe, Ataman, Nord 2, ZSK 444/16, ZSK 162, ZSK 121/17	17
e	Volzhskoe 44, Volzhskoe 615, Kubanskoe 126/01, 06-2063, 06-2177, 06-2199 K, 31067, Nast 76/07, Snezhok 55, Druzhba, Svetloe (2018-1360), NK-4004, Caprock 7000, Orlovskoe, Sb-126/4, Rod 974/17, ZSK 106/17, ZSK 109/17	18
f	Ayushka, Volzhskoe 4, Kamelik, Pioner 412/Milovskoe 6, №2-13, Kafrskoe beloe, Hegari Avandale, RSG 3-2-2000, 32177, Luch 1, Arena, Snezhok 58, ZSK 296/17	13
g	Slavyanka, Kinel'skoe 63, Krusta, NK 222/Odnoplodnoe, №13-13, O.O. Shallu 4E, Urus-Dzhugara, Martin milo B, Antej, Krasnozyornoe 79, SPZS-16, G-167, Belozyornoe 53, Krupinka 99, Krupinka 23, Whete Naggon, PSH, SPZS-11, Nizkorosloe 869/17	19
h	Pishchevoe 35, №8-13, №26-14, №30-14, KKH№7, 06-2029, NK-90, Gassabi, Lazurit 17	9
i	Pioner 88 h 412 № 26, Troistoe, Genicheskoe 126-6/ON-59, №8 Otbor, 06. VI. 12. RB, i. o. Gelya, Akkord, L-95, i. o. Zernogradskoe 54, ZSK-4, Otbor 100, SPZS-16, ZSK 216/17, ZSK 404/17	14
j	Demetra F, Kamyshinskoe 64, Prem'era, Naran, Avans, Zenit, Kremovoe, Pioner 88 h 41IS51-63, ON-35f, K-10989, KS-3 karlikovoe, KKH№10, KKH№12, Kitajskoe 8, Kitajskoe 10, Genicheskoe 130, CS-175, Vu 112, F3 Nizkorosloe 93 h Grand, Pop Sorghum, Kubanskoe 129 (M-1), Kubanskoe krasnoe 1677, Urozhajnoe 8, M-61134, Indijskoe 233 K, Indijskoe 84, Krupinka rozovaya, Earli Hegari, Velikan, ZSK 145/17, Zernogradskoe 54 zheltoe, Hazine 4/517, Nizkorosloe 81/89, Rod 914/17, ZSK 176/17, SPZS-6	36
k	Vostorg, KKH№6, Kitajskoe 6, Odesskoe 20f, S kafrskoe, S bicolor IS 2341 SPV, KS-2 rannij, 06.VI. 18. RG, 06-2199, M-60887, Lazurit, Persis 55, Pishchevoe 227, Avrora, i. o. ZHemchug № 56, Sunrise, 144 f/8, Genicheskoe 130 (k-10912), Rod 906/17, K-927/07, ZSK 838/17, Seso 3	22
l	Majlo karlikovoe, 2477S V-V, 06.VI.12. RK, 06. VI. 25. R, 06-2063, Zersta 97, Svetloe x Sarli-R-Line, R-8S, Zernogradskoe 53, ZSK 176/16, ZSK 242/17	11
m	Zine E x F3 IS12609, M-60938, Kitajskoe 1, Krupnozyornoe 2230, Lazurit 488/17, ZSK 2262/17, Zernogradskij zhemchug 56, Zernogradskij zhemchug 53, ZSK 161/17	9
af	Majlo 168/Combine	1
ai	O.O. Xaller Sooner	1
aj	Dzhetta F	1
bf	Luchistoe	1
bg	Hazine 28	1
bi	Kadeiba-3732	1
cg	№36-13	1
ch	Milovskoe 84	1
ci	Milovskoe 12-1, №50-13	2
cj	Start, Hegari 2259	2
dg	№53-13	1

dk	Topaz	1
dm	ZSK 300/17	1
gk	Dzhugara karlikovaya, SPZS-2	2
bgl	Populyaciya 32	1
NA	Zernogradskoe 88, Demetra S, Dzhetta S, Krupinka 10, Kamyshinskoe 31, Kamyshinskoe 75, Ros', Zersta 97, Ogonyok, Perspektivnyj 1, Fakel, Pishchevoe 614, Merkurij, Azart, Sarmat, Pioner 878, Pink kaffir, 03-3003, V4B, 06. VI. 18. R, 06-2196, 31063, R-116, 1176/10, GOS-11, Indijskoe 233, Indijskoe 233 ZH, Krupnozyornoe, Krupnozyornoe 2233, Sandal, i. o. u-208, Redbajn 66, Aralba, 086 c x RF 10 ZH, Orlovskoe 2, i. o. Zernogradskoe 204, Krupinka zheltaya, Gabane, Kberksdorf Light Red, Potshefstroom, Feterita №834, Hegari, Abu Sabeen, Wad Farag, Feterita kadugli, Shendi 1, Yulum 3, ZSK 163/17, ZSK 196/17, Ring 28, Skorospeloe 461/17, ZSK 1568/17, ZSK 423/17, ZSK 449/17, ZSK 1530/15, ZSK 411, ZSK 500/16, Druzhba (sem 2018), ZSK 282/14, Lazurit 754/17, Skorospeloe 789/17, Zernogradskoe 54 krasnoe, ZSK 837/17, Belozyornoe 1249/09, Oranzhevoe 1249/09, Lazurit 874/17, Gelya 920/17, Akkord 924/17, Hazine 66/28, Zernogradskoe 204 (990/17), Snezhok 1686/6, CM-39 USK, Rod 910/17, Rod 938/17, Rod 954/17, Rod 974/17, ZSK 1476/17, ZSK 1514/17, Zernogradskoe 89, ZSK 1550/17, ZSK 1558/17, ZSK 1583/17, ZSK 148/17, ZSK 151/17, ZSK 155/17, ZSK 167/17, ZSK 205/17, ZSK 222/17, ZSK 243/17, ZSK 257/17, ZSK 271/17, Snezhok 1685/6, ZSK 283/17, ZSK 285/17, ZSK 289/17, ZSK 298/17, ZSK 299/17, ZSK 408/17, ZSK 425/17, ZSK 814/17, ZSK 828/17, ZSK 842/17, Lazurit 486/17, ZSK 231/16, Zernogradskoe 204/4, ZSK 34, Seso 1, Nqrosorg 1, Zernogradskoe 88 st, Nqrosorg 2, Nqrosorg 3, Nqrosorg 4, Epuripur	113

In the field, we assessed self-pollinated lines of grain sorghum on a 3-point scale—fertile, semi-sterile and sterile. The number of identified fertile and sterile hybrid combinations is presented in Table 2.

Table 2. The identified fertile and sterile hybrid combinations of grain sorghum.

♂ Alleles	Number of Combinations	Number of Fertile and Sterile Lines [1]	
		♀ Demetra S	♀ Dzhetta S
NA	33	31.5 F : 1 FS : 0.5 S	32 F : 1 S
b	2	2 F	2 F
c	3	1 F : 2 S	1 F : 2 S
d	3	3 F	3 F
e	2	1 F : 1 S	2 F
g	2	1.5 F : 0.5 S	2 F
i	1	1 F	1 F
j	3	2 F : 1 S	3 F
k	1	1 F	1 F
l	1	0.5 F : 0.5 S	1 F
m	2	2 F	2 F

[1] F—fertile lines, FS—semi-sterile line, S—sterile lines.

Most of the combinations are fertile. For paternal forms with a null allele (NA) and allele "e", this may be due to the influence of other genes that control fertility. One semi-sterile line of Demetra S/ZSK 163/17 has been identified. Sterility in 2 repetitions was revealed in crossing combinations Demetra S/ZSK 1530/15, Dzhetta S/Zernogradskoe 204w4, Demetra S/LBK 28, Demetra S//Svetloje/Belozernoje 100, Dzhetta S//Svetloje/Belozernoje 100, hence their paternal forms are sterility fixers and can be used in further breeding work.

4. Discussion

As a result of our studies, the genetic background of 313 collection samples of grain sorghum was identified, adapted to the arid climate of southern Russia by the presence of Rf1 gene alleles. The use of the Xtxp18 marker made it possible to identify a wide variety of samples, in particular to

identify those that carry alleles associated with sterility and fertility. This result is consistent with data obtained by Klein et al. [6]. In addition, we identified three new allelic variants, temporarily designated by us as k, l, and m, which were not reported by other researchers. These allelic variants may serve as a basis for future studies of mutations in grain sorghum.

Analysis of self-pollinated grain sorghum lines obtained from crossing with sterile lines Demetra S and Dzhetta S revealed 4 paternal samples that can be used in the breeding process as sterility fixers, and therefore further obtaining new commercial hybrids in the future.

This result will make it possible to deepen understanding of the influence of the Rf1 gene alleles on the level of fertility of sorghum plants in the future, as well as to accelerate the breeding process to create sterile lines and their fertile analogues for further obtaining commercial hybrids.

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Abbreviations

The following abbreviations are used in this manuscript:

FSBSI "ARC "Donskoy"	Federal State Budgetary Scientific Institution "Agricultural Research Center "Donskoy"
bp	base pairs

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