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Analysis of Main Characters of Rice Progenies by Injecting Sorghum DNA into Rice

Guangxin Li, Guangyuan Wang, Xiaohui Yu, Qing Mei, Guopeng Wang
Institute of Crop Science, Shanxi Academy of Agricultural Sciences, Taiyuan, 030031, China
Corresponding author email: nkywgy@sohu.com

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Abstract In this study, the whole genome DNA of sorghum apomixes line SSA-1 (Shanxi sorghum apomict-1) was injected into two rice varieties of 'Liaoyan 28' and 'Liaoyan 6' using the pollen tube pathway method. The genetic characters of the progeny changed obviously. By self-selection and identification, 27 phenotypic stable D_4 generation lines were obtained. Through field planting, the phenotypes of agronomic traits such as heading date, tiller number, plant height, and 1 000-grain weight of the introduced lines were statistically analyzed. The results showed that the agronomic traits of the D_4 generation lines were widely varied, some D_4 generation lines were more inclined to donor sorghum in culm and panicle color, which indicated that the whole genome DNA of sorghum was successfully introduced into the receptor cells, and the expression of the receptor gene was affected. Compared with the recipient parents, the introduced lines showed significant variations in the main traits such as heading date, tiller number, plant height, grain number per ear, grain weight per ear, ear length, and thousand grain weights. Further analysis and selection of mutant lines is helpful to discover new germplasm materials and provide abundant germplasm resources for breeding new rice varieties.

Keywords Sorghum apomixes line; Pollen tube pathway method; Main characters; Genetic variation

Rice is one of the three major food crops in the world, and also the most important food crop in China. Since entering the 21st century, the average annual planting area of rice in China accounts for 1/5 of the world's rice planting area. Therefore, its production and development play a crucial role in the food security of China and even the world (Peng et al., 2009; Liu et al., 2018). Dwarf breeding and heterosis utilization are two major changes in rice breeding, both of which are completed on the basis of the discovery and utilization of specific germplasm resources, which shows that rice seed resources play a very important role in the process of rice breeding (Wu et al., 2018). Since the introduction of hybrid rice in the 1970s, China's rice yield has been improved by leaps and leaps, but the subsequent rice breeding rate is relatively slow, mainly because of the lack of breakthrough progress in valuable new germplasm resources (Zhang et al., 2019). Remote hybridization is one of the most commonly used methods to create new germplasm in conventional breeding. The application of molecular biology, which is constantly improved in knowledge system and technology, in the field of crop breeding will introduce favorable foreign genes into rice, which will play an important role in improving the yield, quality and resistance of rice. It has become an effective means to innovate rice germplasm resources (Wang et al., 1999; Chen et al., 2018).

The pollen tube channel method proposed by Chinese scientist Zhou Guangyu in the 1970s has been verified on soybean, cotton, wheat, rice and other crops (Kong et al., 2005; Wang et al., 2014). So far, researchers have introduced the DNA of Cili maize into the receptors of rice 'Xiangzaoxian 8' and 'Xiangzaoxian 4' by using the pollen tube channel method, and their offspring have obtained extensive variation, mainly reflected in the yield components such as panicle length, grains per panicle and 1000-grain weight (Wan et al., 1992, Hunan Agricultural Sciences, (3): 6-7; Xiao and Deng, 2006). Through the pollen tube method, the DNA of Bicao (*Echinochloa crus-galli* (L.) P. Beauv.) was introduced into the recipient rice 'Xiangwanxian 5' and a new late indica line 'Late HK7' was bred from its mutant offspring (He et al., 2002, Crop Research, (2): 100-101; Xiao and Deng, 2006). There are many rice mutant progeny with extensive plant morphological variation, but these variations usually do not appear in interbreed hybrid progeny, and these variations are achieved by pollen tube channel transgenic technology (Fu et al., 1992; Xiao and Deng, 2006). Many experiments have proved that the imported lines obtained by pollen tube channel method can greatly shorten the selection generation, its variation is wide, some inserted DNA fragments are easy to homozygous, and it is easy to obtain the population with the same genetic background. This method can not only supplement the deficiency of some genetic resources, improve the diversity of gene pool, but also overcome the incompatibility of distant hybridization, and improve the goal and accuracy of conventional breeding in field (Yang et al., 1993; Xiao and Deng, 2006; Sun et al., 2009; Huang et al., 2015; Luo et al., 2016). The application of this method in the creation of rice germplasm resources has been mature. Through the wide availability of genetic variation, many new rice germplasm with good resistance, high yield and quality have been bred (Xiao and Deng, 2006; Wang et al., 2011).

As a metamorphosis sexual reproduction mode, apomiesis avoids the obvious alternation between gametophyte generation and sporophyte generation in most higher organisms, and its fixed heterosis is more stable and can save breeding time and cost (Ping et al., 2009). By spik-stem injection in rice line ‘Gui 99’, the researchers introduced apomictic Dashu (*Panicum maximum* Jacq.) DNA and obtained female sterile mutant offspring. This method can cause the offspring to exhibit similar sexual embryo sac abortion as *Panicum maximum* Jacq.. The autoradiography was used for analysis, and the results showed that foreign DNA could be transferred into the flower apparatus through the vascular tube (Zhao et al., 1998; Meng et al., 2006). Two years later, they again performed RAPD analysis on the mutant lines of wild rice DNA injected into the stems of rice at booting stage, and the results showed that wild rice DNA was indeed introduced into the mutant lines (Zhao et al., 2000; Meng et al., 2006). In our previous studies, the whole genome DNA of sorghum germline SSA-1 was successfully imported into two rice varieties ‘Liaoyan 28’ and ‘Liaoyan 6’ by pollen tube channel method, and isozyme analysis was conducted on the progeny material of the receptor. In some of the progeny of the DNA, heterozygase bands with mobility rate RF =0.51 were found. It showed obvious heterosis in its offspring. Some strains also showed an enzyme band absent from the mother, which was similar to the band on the donor paternal parent. The appearance of the isozyme band was the result of gene expression, indicating that the sorghum genomic DNA was rejected, separated and finally stabilized after entering rice, but the agronomic traits of the strains have not been identified in detail (Li et al., 2008).

In this study, agricultural traits such as heading time, tiller number, plant height and 1 000-grain weight of the offspring lines of D4 rice with the same phenotype and stable heredity were identified. It is hoped that materials with excellent agronomic traits or unique variation could be obtained through identification and screening, so as to enrich rice seed resources and provide help for the preparation of good combinations and variety breeding.

1 Results and Analysis

1.1 The significance of trait differences between acceptor and introducer lines

Table 1 shows that the main traits of ‘Liaoyan 28’ and 15 imported lines, ‘Liaoyan 6’ and 12 imported lines were significantly different, indicating that the whole genome DNA of sorghum apomixous germline SSA-1 has successfully imported two rice varieties, ‘Liaoyan 28’ and ‘Liaoyan 6’, which can be further analyzed and studied.

1.2 Variation of agronomic traits

Through the investigation and screening of agronomic characters of 27 imported lines in the field, the mutant plants were found. These plants showed the unique agronomic traits of sorghum, such as wider leaves, straight blade leaves, deeper leaf color, red glumes, and round grain deformation. At the same time, they also showed certain changes in the common agronomic traits of panicle length, heading time, number of tillers and plant height, which were significantly different from ‘Liaoyan 28’ and ‘Liaoyan 6’. For example, among the 27 imported lines in the field, the heading date of 12 lines did not change significantly compared with their receptors, but 8 of them had an earlier heading date and 7 had a later heading date. In the planting experiment, the first heading date of the control variety ‘Liaoyan 28’ was August 2. D_4-13 and D_4-27 were the earliest lines of the imported line heading, and their heading date was July 27, 5 d earlier than that of the receptor ‘Liaoyan 28’. The other lines whose heading date was around July 30 (‘Liaoyan 6’ was on July 29) headed earlier. In addition, the plant height of the receptor was significantly lower than that of some of the importing lines. For example, the average plant height of the importing line D_4-l1 was 109.6 cm, the plant height of its receptor ‘Liaoyan 28’ was 90.3 cm, and the plant height of D_4-11 was 21.4% higher than that of its receptor. However, the plant height of the imported line of ‘Liaoyan 6’ was generally lower than that of its receptor. The plant height of lines D4-57, D4-66 and D4-85 was 89.7 cm, 101.0 cm and 93.2 cm, while the average plant height of ‘Liaoyan 6’ was 107.4 cm. The plant height ratio of the three imported lines decreased by 19.7%, 6.3% and 15.2%, respectively. The panicle length of imported lines also varied. For example, the average panicle length of D4-66 was 20.0 cm, while the panicle length of its receptor ‘Liaoyan 6’ was 19.5 cm, and the panicle length of D4-66 increased by 2.6%.

Table 1 Variance (ANOVA) of the main characters of transferred lines with their receptor

Material	Source	*df*	The main characters *MS*				
			Filled grains per panicle	1 000-grain weight (g)	Plant height (cm)	Panicle length (cm)	Duration from seeding to heading (d)
‘Liaoyan28’ and transferred lines	Block	1	5.28	0.005	6.3	1.4	3.13
	Material	15	511.34**	5.77 **	120.26 **	7.27**	456.97 **
	Error	15	2.28	0.74	4.25	0.69	0.26
	Total	31	248.7	3.15	60.45	3.9	221.34
‘Liaoyan6’ and transferred lines	Block	1	8.6538	0.0385	0.1112	0.0754	2.4615
	Material	12	2691.46 **	8.13 **	79.73 **	17.42**	252.54 **
	Error	12	3.49	0.24	2.28	0.64	0.63
	Total	25	1293.92	4.02	39.37	8.67	121.62

Note: **: Significant at 0.01 probability level

1.3 Variation of grain number per panicle

According to the statistics of the number of grains per panicle per plant, the results showed that the number of grains per panicle of the imported line had abundant variation, which was higher and lower than that of the recipient. The number of solid grains per panicle of ‘Liaoyan 28’ was 109, while the number of solid grains per panicle of D4-11, D4-22 and D4-33 was 112, 145 and 152, respectively, in the 15 imported lines using it as the control, and the number of solid grains per panicle was increased by 2.8%, 33.0% and 39.4% (Figure 1).

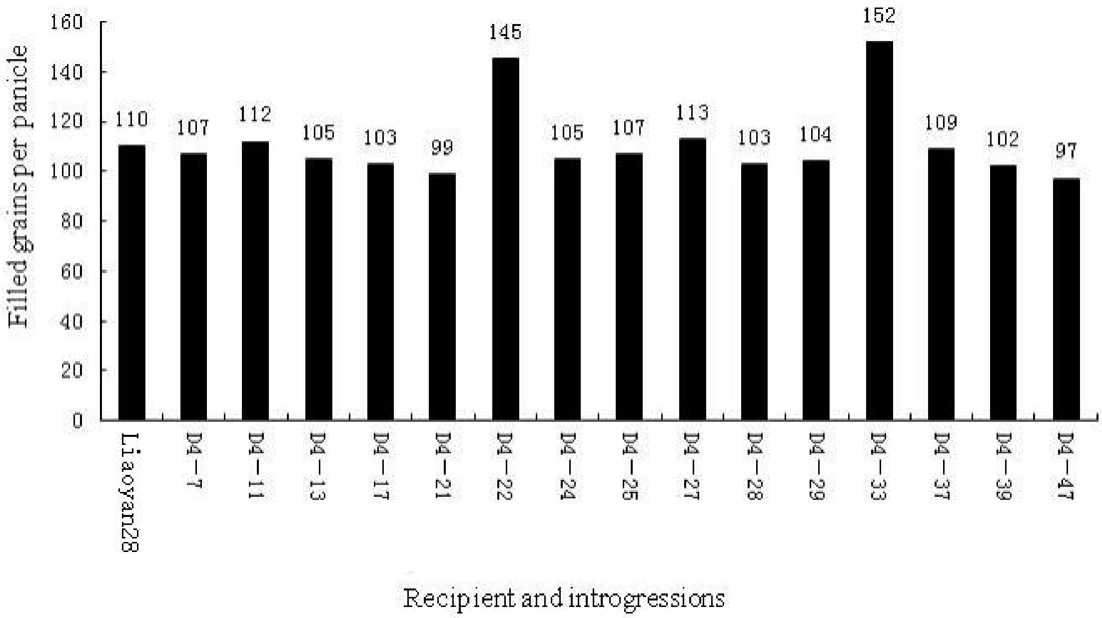

Figure 1 The comparison of filled grains per panicle of transferred lines with their receptor ‘Liaoyan28’

There were 12 importing lines with 'Liaoyan 6' as the receptor, and 5 lines D4-59, D4-62, D4-71, D4-81 and D4-87 were all higher than the receptor. The maximum number of solid grains per panicle was 191 of D4-59, which was 73.6% higher than that of the receptor. For the other four imported lines, the increases were 8.2%, 39.1%, 66.4% and 57.3% (Figure 2).

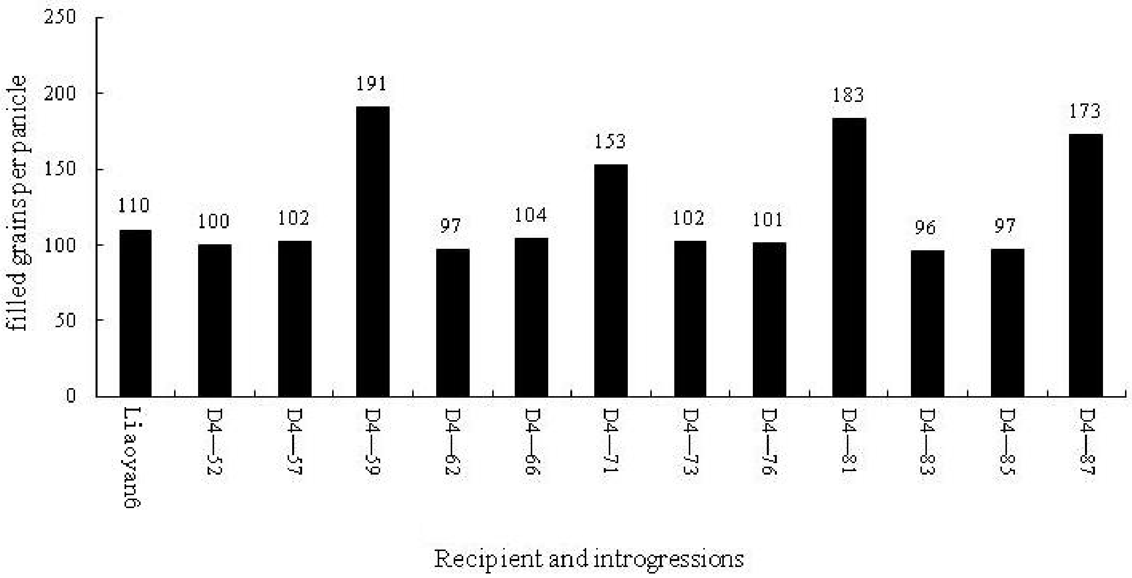

Figure 2 The comparison of filled grains per panicle of transferred lines with their receptor 'Liaoyan6'

1.4 Variation of 1000-grain weight

As an important basis for measuring yield in the field, the 1000-grain weight also changed obviously. For example, the 1000-grain weight of 'Liaoyan 28' was 24.7 g. Among the imported lines using it as the receptor, the 1000-grain weight of four lines increased compared with that of the receptor, and the 1000-grain weight of D4-22 and D4-33 increased the most significantly, which were 27.3 g and 29.7 g, respectively, increasing by 10.1% and 20.2% compared with that of the receptor (Figure 3).

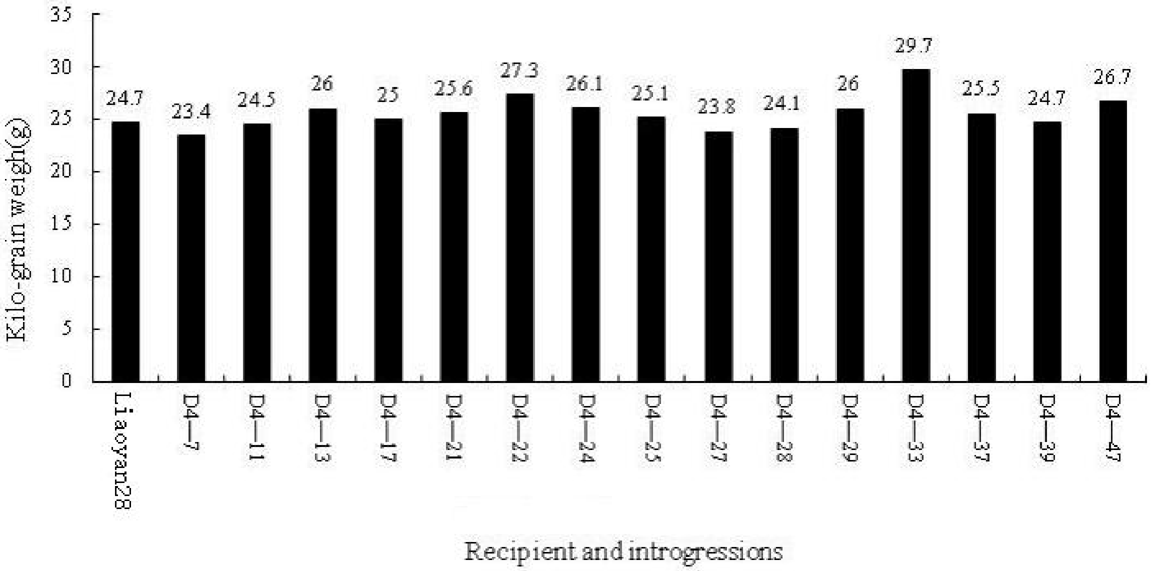

Figure 3 The comparison of Kilo-grain weight of transferred lines with their receptor 'Liaoyan28'

The 1000-grain weight of most imported lines with 'Liaoyan 6' as the receptor was higher than that of the receptor. For example, the 1000-grain weight of the imported lines D4-59, D4-81 and D4-85 were 29.3 g, 28.7 g and 29.5 g, respectively, with significant weight gain, which increased by 14.9%, 12.5% and 15.3% compared with that of the recipient (Figure 4).

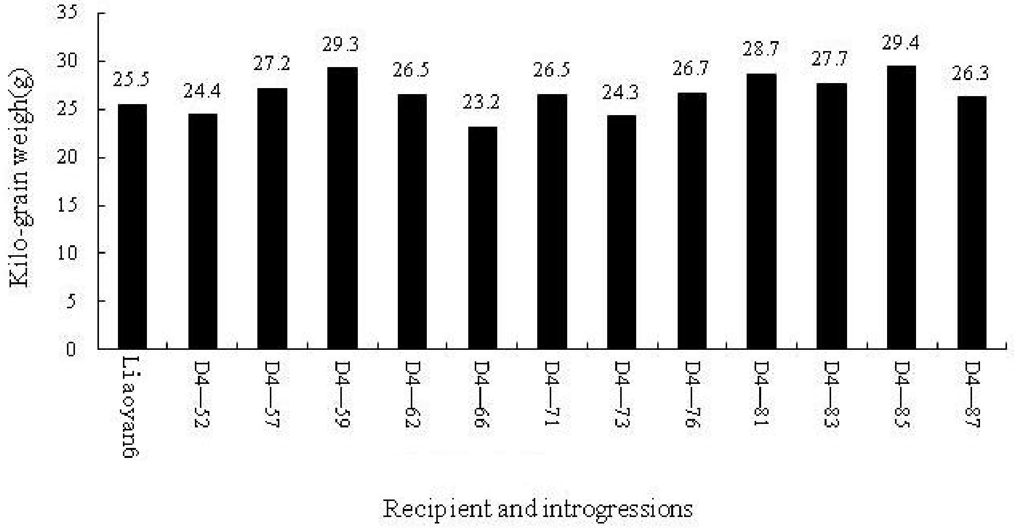

Figure 4 The comparison of Kilo-grain weight of transferred lines with their receptor 'Liaoyan6'

1.5 Variation of tillers

D4-81, whose receptor was 'Liaoyan 6', showed multiple tillers in 27 lines. The average tiller number of the receptor 'Liaoyan 6' was 9. Among the 10 individual plants of D_4-81, the number of tillers of the 2 individual plants with little difference from the receptor was 10. Among the remaining 8 individual plants, the highest number of tillers was 19, the lowest was 12, and the average number of tillers was 15, which increased by 66.7% (Figure 5).

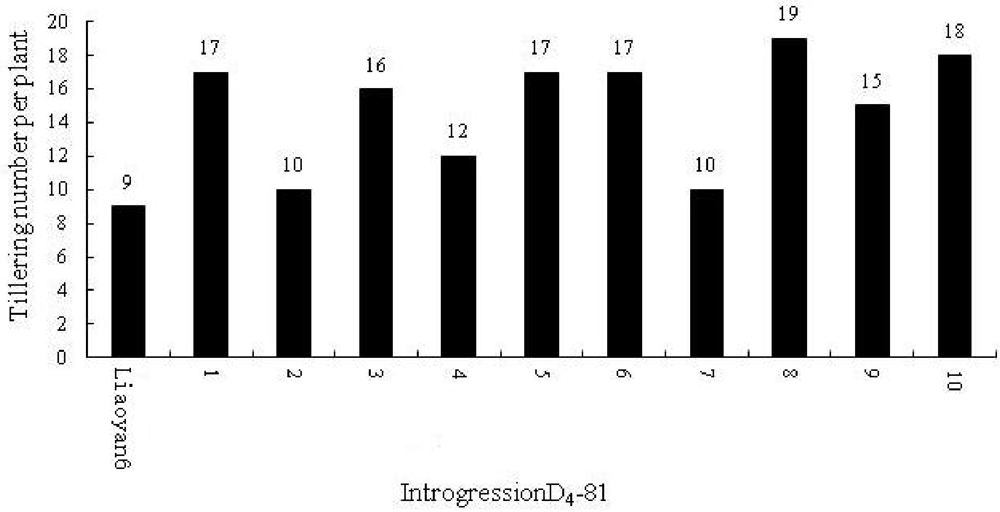

Figure 5 The comparison of tillering number per plant of transferred line D4-81 with its receptor 'Liaoyan6'

2 Discussion

Some studies have pointed out that the homology between rice and sorghum is 16%. By comparing the genome of cereal crops, it has been observed that there are great similarities in the contents and arrangement order of the chromosomal genes in the same family that show collinearity, that is, between different species. Therefore, the successful introduction of sorghum DNA into rice has a certain genetic background support (Zhou et al., 1980, Journal of Genetics and Genomics, 7(2): 119-122; Zheng, 1996, Scientia Agricularia Sinica, 29(6): 2-8; Wang et al., 2007). Among the distant hybridization of rice, the hybridization with sorghum is more thorough and mature. Moreover, sorghum has good plant morphology, thick stems and hard stems, developed roots, fertilizer resistance and toppling resistance, large panicle grain. It belongs to C_4 plants, high photosynthetic efficiency, fast growth rate, smooth operation of photosynthates, good fruiting, full grain; strong adaptability, drought and stain tolerance, fertilizer and poverty tolerance, stable and high yield (Fu et al., 1997). Some good characters of sorghum were transferred to rice through hybridization to produce new rice varieties with high yield and high quality. Sorghum rice and the 'Yuanza 209' and 'Yuanza 235' strains produced with sorghum rice as carriers provided references for the distant hybridization of rice and sorghum (He, 2011, Bulletin of Agricultural Science and Technology, (3): 125-126). In this study, we successfully obtained the D_4 generation rice lines imported with sorghum SSA-1, and identified the agronomic traits of the imported lines. The results showed that the 4th generation rice materials introduced sorghum SSA-1 showed extensive variation. Some of the mutant plants showed the characteristic agronomic traits of sorghum, such as wider leaves, straight blade leaves, deeper leaf color, red glumes and round grain deformation. In addition, the imported line plants showed obvious variation compared with the recipient lines. For example, the earliest imported lines D_4-13 and D_4-27 were 5 days earlier than their receptor 'Liaoyan 28'. The average plant height of imported line D_4-11 was 21.4% higher than that of 'Liaoyan 28', while the plant height of imported line with 'Liaoyan 6' as the receptor was lower than that of the receptor. The panicle length of D_4-66 increased by 2.6% compared with that of the receptor. Among the 27 lines, only D_4-81 showed multiple tillers, and the average tiller number was up to 66.7% higher than that of the receptor. The number of grains per panicle of the imported lines varied more, some were lower than that of the receptor, some were higher than that of the receptor, but the imported lines with 'Liaoyan 6' as the receptor increased more obviously. The significant variation of 1000-grain weight was more prominent in the import lines of 'Liaoyan 6' as the receptor, most of which were higher than the recipient weight.

Pollen tube channel method has become one of the effective technical means of transgenic at present. Especially from the perspective of breeding, it has lower cost, stronger operability and is more suitable for traditional field breeding. Direct introduction of foreign DNA is one of the contents of plant molecular breeding. It is used to directly introduce the total DNA or target genes of the donor genetic material with target traits into the plant as the recipient, create a large number of variation materials, and obtain the offspring with target traits through screening, so as to achieve the purpose of improving varieties. Through the introduction of foreign total DNA, a large number of variations can be produced. Although many valuable variants can be screened out, the screening cost is high, the generation cycle is long, and the purpose of variation is not strong. In this study, the sorghum apomomous germline SSA-1 was introduced into the rice varieties 'Liaoyan 28' and 'Liaoyan 6', and the progeny caused a large number of trait separation, resulting in many phenotypes that rice did not have. The selected imported lines were homozygous only after four consecutive self-crossing, so as to obtain stable homozygous progeny of the D_4 generation material consistent with the target trait. It took a relatively long time for the results to become clear. In recent years, with the rapid development of molecular breeding science and technology, directional transgenic technology is becoming more mature. The selection of DNA is more purposeful and targeted, and the DNA is closer to the target gene. The success rate should be higher, and the breeding generation can be greatly shortened, so that the target varieties can be bred faster and more accurately.

3 Materials and Methods

3.1 Extraction of total DNA from sorghum apomic Germline SSA-1

After germination, the donor sorghum germline SSA-1 seeds were incubated in a 28℃ incubator for 10 days. 5 g of seedling leaves were cut and ground in a mortar with liquid nitrogen. DNA was extracted by trypsin method. The extracted DNA A260/A230=2.3 and A260/A280=1.9 were determined by Beckman DV-50 spectrophotometer, which met the requirements of foreign DNA import test.

3.2 Introduction of total DNA from sorghum apomic Germline SSA-1

A DNA solution was prepared with 0.1 ssc (soluble solids content) solution. At the heading stage of the rice receptor, the pollinated and unflowering grains were removed, and the flowering grains on the same day were cut with scissors. Then, the pistils of the rice varieties ‘Liaoyan 28’ and ‘Liaoyan 6’ were coated with a brush dipped in an appropriate amount of DNA saturated solution, and the pistils were bagged immediately to prevent the overcrossing of exogenous pollen. The harvested seeds were planted in the experimental field in the next year, and the traits were observed and the lines with obvious variation were selected for self-crossing. In the test, D was used as the representative, the imported lines were self-crossed for four times, and finally the stable material D_4 generation was obtained. A total of 27 D_4 generation lines were identified (Table 2).

Table 2 Receptors and the transferred lines

Receptor	Transferred line
Liaoyan28	D_4-7、D_4-11、D_4-13、D_4-17、D_4-21、D_4-22、D_4 -24、D_4-25 D_4-27、D_4-28、D_4-29、 D_4-33、D_4-37、D_4-39、D_4-47
Liaoyan6	D_4-52、D_4-57、D_4-59、D_4-62、D_4-66、D_4-71、D_4-73、D_4-76 D_4-81、 D_4-83、D_4-85、D_4-87

3.3 Design of field experiment

The experiment was carried out in Wang Guo Experimental Base (37°46′N, 112°34′E), Institute of Crop Science, Shanxi Academy of Agricultural Sciences in 2019, and the sowing time was April 21, 2019. In this experiment, ‘Liaoyan 28’ was used as Control 1 and ‘Liaoyan’ was used as Control 2. The tests were arranged in sequence. Each plant was inserted in 3 rows, each plot was 3 m long and 1 m wide, and the row spacing of planted plants was 15 cm×24 cm.

During the period from germination to harvest to seed, the agronomic characters of the planted materials, such as tiller number, heading time, plant height, grain number per panicle, grain weight per panicle, leaf color, grain shape and 1000-grain weight, were observed and recorded. The obtained data were calculated and statistically analyzed by Excel and DPS 6.50 software.

Authors’ contributions

LGX was the experimental designer and the executor of this research. He also completed the data analysis and the writing of the first draft of the paper. WGY was the proposer and the person in charge of the project. He participated in guiding the experimental design, data analysis and paper writing. YXH and MQ participated in the experiment execution and field investigation. WGP completed the experimental data sorting. All authors read and approved the final manuscript.

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References

Chen J., Luo M., Li S.N., Tao M., Ye X.L., Duan W., Zhang C., Qin Q.B., Xiao J., and Liu S.J., 2018, A comparative study of distant hybridization in plants and animals, Science China (Life Sciences), 61(3): 285-309
https://doi.org/10.1007/s11427-017-9094-2
PMid:28861869

Fu J., Xu Q.G., and Zhou W.X., 1997, On breeding rice through wide hybridization between rice (*Oryza sativa*) and sorghum (*Sorghum bicolor*), Hunan Nongye Daxue Xuebao (Journal of Hunan Agricultural University), 23(6): 509-514

Fu J., Xu Q.G., Luo H., and Hong Y.H., 1992, Effect of introduced exogenous DNA on rice breeding, Hunan Nongxueyuan Xuebao (Journal of Hunan Agricultural College), 18(1): 10-17

Huang X., Chen W.S., Zhang H.M., and Zhang L., 2015, Application and prospect of biotechnology in research and development for medicinal plant, Zhongcaoyao (Chinese Traditional and Herbal Drugs), 46(16): 2343-2354

Kong Q., Feng Z., Liu L., Kong Y.G., and Zhang Y., 2005, Application and development of foreign DNA introduction via the pollen-tube pathway, Fenzi Zhiwu Yuzhong (Molecular Plant Breeding), 3(1): 113-116

Li G.X., Wang G.Y., Yu X.H., Mei Q., and Qiao Y.X., 2008, The character of stabilizing materials by injecting sorghum DNA into rice and its isozyme analysis, Shanxi Nongye Kexue (Journal of Shanxi Agricultural Sciences), 36(1): 28-29

Liu G.F., Chen M.J., Li M., Lü H.Y., Ge Y.Q., Wei X., and Yang W.C., 2018, Advance and innovation of rice breeding, Zhiwu Yichuan Ziyuan Xuebao (Journal of Plant Genetic Resources), 19(3): 416-429

Luo X.L., Xiao J.L., Zhang A.H., Wang Z.A., Xu Q., Ding X., and Liu C.L., 2016, Research progress and prospect of cotton artificial mutant, Zhongguo Mianhua (China Cotton), 43(8): 5-9

Meng Z.H., Liu X.J., Wang Y.J., Meng Q.X., Li C.G., Liu Y.W., and Zhang J.L., 2006, The research and development about exogenous DNA introduced into paddy rice by pollen-tube pathway, Zhongguo Nongxue Tongbao (Chinese Agricultural Science Bulletin), 22(6): 52-56

Peng S.B., Tang Q.Y., and Zou Y.B., 2009, Current status and challenges of rice production inchina, Plant Production Science, 12(1): 3-8
https://doi.org/10.1626/pps.12.3

Ping J.A., Zhang F.Y., Lü X., Du Z.H., Guo J.W., and Wang J.Z., 2009, Research progress of apomixis technology in sorghum, Shanxi Nongye Kexue (Journal of Shanxi Agricultural Sciences), 36(1): 81-84

Sun X.P., Liu Z.S., Li S.H., Yang Q.W., and Zhang W.X., 2009, Genetic variation of pollen-tube transferred lines of cultivated rice with *Oryza rufipogon* DNA, Zhiwu Yichuan Ziyuan Xuebao (Journal of Plant Genetic Resources), 10(2): 267-271

Wang F., Zhang Q.P., Chen J.X., Zhou Z.H., Li B., He W., Zhang H., and Li R.L., 2014, Transformation of *Bt-Cry5Aa* in cotton and identification of its insect-resistant, Zhiwu Yichuan Ziyuan Xuebao (Journal of Plant Genetic Resources), 15(4): 877-881

Wang H.G., Zhang J.M., Liu S.B., and Kong F.J., 1999, The ways and characteristics of improving modern plant varieties and its development tendency, Shandong Nongye Daxue Xuebao (Journal of Shandong Agricultural University), 30(4): 471-480

Wang L., Chen J.T., and Zhang Z.X., 2007, Strategies and progress on cereal comparative genomics, Yichuan (Hereditas), 29(9): 1055-1060
https://doi.org/10.1360/yc-007-1055
PMid:17855253

Wang Z.C., Liao M.S., Muherepiya A., Xu J.H., Li C.P., and Zhang F.C., 2011, Study on pollen-tube pathway mediated cotton (*Gossypium hirsutum*) transformation with salt-tolerant gene from *Halostachys caspica*, Fenzi Zhiwu Yuzhong (Molecular Plant Breeding), 9(2): 180-184

Wu B., Hu W., and Xing Y.Z., 2018, The history and prospect of rice genetic breeding in China, Yichuan (Hereditas), 40(10): 841-857

Xiao J.Z., and Deng J.P., 2006, Pollen tube pathway transgenic technique and application in rice molecular breeding, Zhongguo Nongxue Tongbao (Chinese Agricultural Science Bulletin), 22(2): 87-90

Yang J.S., Ge K.L., Wang Y.H., Wang B., and Tan C.C., 1993, Highly efficient transfer and stable integration of foreign DNA into partially digested rice cells using a pulsed electrophoretic drive, Transgenic Res., 2(5): 245-251
https://doi.org/10.1007/BF01968837

Zhang X.W., Duan X.J., Tang Y.Q., Xiao R.P., Liu Q.M., and Li J.Y., 2019, Rice germplasm 'Yu819B' with barnyard grass: photosynthetic characteristics analysis, Zhongguo Nongxue Tongbao (Chinese Agricultural Science Bulletin), 35(27): 8-11

Zhao B.R., Huang J.L., Liu C.L., Fu X.Q., Li H.S., and Yuan L.P., 1998, A study on the transportation way of exogenous DNA *in vivo* and a female sterile variant of rice, Hunan Nongye Daxue Xuebao (Journal of Hunan Agricultural University), 24(6): 17-22

Zhao B.R., Jia J.H., Yan H.H., Li C.Y., Zhan Q.C., Wang B., Zhou K.L., and Yuan L.P., 2000, RAPD analysis of new rice strains developed through the method of panicle-stalk-injecting DNA from wild relative, Zuowu Xuebao (Acta Agronomica Sinica), 26(4): 424-430