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Innovation on Distant Hybridization of Saline-tolerant Mud Flat *Spartina* and Rice Germplasm

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Abstract To explore saline-tolerant high yield *Spartina* and rice germplasm resources, innovate upon distant hybridization technology, and develop new saline-tolerant mud flat *Spartina* germplasm, autonomous patent for invention was used to study distant hybridization of *Spartina* and rice in 2005 - 2016. The study adopted tidal flat planting screening, phenotypic screening, molecular marker assisted selection, to find biological evidences of new germplasm, and also applied crossbreeding, conventional breeding, and molecular breeding, to select new saline-tolerant *Spartina* and rice varieties. With 12 years of efforts, the team developed 1498 crossbreeding combinations and established pedigree file and seed bank, tested 307 crossbreeding materials (57 hybrid seeds showed parental genetic components of *Spartina* and Rice); obtained *Spartina*/Rice distant hybridization and seed setting, transplanting, hybridization material economic traits, and molecular biological experimental evidences; Rice ♀ × *Spartina* ♂ hybridization success rate of 1.04 - 1.39%; obtained authorization of patent for invention of *Distant Hybridization Methods for Spartina* and Rice; formulated procedures for high yield cultivation of *Spartina*-Rice, planted 33.33 hm^2 *Spartina*-Rice in coastal saline and alkaline land, cultivated 10 new saline-tolerant *Spartina*-Rice varieties, and the rice yield up to 5.925 - 8.28 t/hm^2. Results indicate that *Spartina*-Rice is saline-tolerant high yield rice germplasm, developed through sexual hybridization, conventional breeding, and molecular breeding technologies, and is optimal crop for saline and alkaline land. Success of *Spartina*-Rice solves the problem of saline-tolerant crop badly for transformation of saline and alkaline land, the problem of feeds for herbivores, as well as problem of environmental pollution resulted from straw burning. Besides, it is of essential scientific significance for resource utilization, increase of agricultural efficiency, grain security, and farmland strategy, so it has broad application prospect.

Key words Mud flat, *Spartina* and rice, Distant Hybridization, New saline-tolerant germplasm, Innovation

1 Introduction

There are 15 varieties of *Spartina* in the world, all of them are perennial C4 herbs and have certain saline and flood tolerance[1]. *Spartina anglica* (cordgrass) and *Spartina alterniflora* (smooth cordgrass) (hereafter referred to as *Spartina*) are varieties with the largest area of distribution in coastal regions of China. *Spartina anglica* was introduced by Nanjing University from Britain in 1963, and the number of chromosomes is 122 and 124. *Spartina anglica* has been included into the Feed Science of China, has strong saline and flood tolerance, and is suitable for mid tide zone. The annual yield of fresh *Spartina anglica* is 15 - 30 t/hm^2, suitable for feeding ruminants[2]. *Spartina alterniflora* was introduced by Nanjing University from the United States of America in 1979, and it has 62 chromosomes. *Spartina alterniflora* also has strong saline and flood tolerance, but the plant is higher than *Spartina anglica* and the grass yield is high, suitable for feeding herbivores, and thus receives wide attention[3].

Graduated from Nanjing Agricultural University in 1975, Chen Qikang has been studying *Spartina*. Under the guidance of Professor Zhong Chongxin from Nanjing University, he introduced *Spartina anglica* in 1975 and *Spartina alterniflora* in 1980, and built 80000 mu tidal flat *Spartina* pasture, and established the first *Spartina* pasture of China. Besides, he is undertaking the Program of Coastal Pasture Experiment of State Science and Technology Commission, and the coastal pasture experiment was identified as the first in China; the *Spartina* can feed 3200 cattle and sheep every year[4-6]. With more than 40 years of study on *Spartina* as feed of livestock and poultry, Chen Qikang found that *Spartina* has excellent properties of saline and flood tolerance, but the yield is low and insufficient to satisfy nutritional demand of cattle and sheep, so he was resolved to explore saline tolerant gene resources of *Spartina*. Rice belongs to graminaceous C3 plant with 2n = 24 chromosomes. Rice varieties are abundant, seeds are plump, the yield is high, and rice chaff and straws can be used as feed for cattle and sheep. According to *Feed Science of China* compiled by Zhang Ziyi, rice contains 8% crude protein, nitrogen-free extract (NFE) content ranks the first in various grains, and the available energy is also the highest, so it is an excellent energy feed[2]. Rice provides excellent germplasm resources for *Spartina* modification. Distant hybridization of rice can introduce favorable genes of paternal plants, generate non-comparable diversity and new varieties, and expand genetic basis of rice[7]. To explore saline toler-

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ant and high yield germplasm resources of *Spartina* and Rice, we carried out distant hybridization study on *Spartina* and Rice in 2005 – 2016.

2 Materials and methods

2.1 Parental materials for distant hybridization

2.1.1 Mud flat *Spartina*. (i) In 2005 – 2006, we introduced and cultivated 1720 plants of *Spartina anglica* and 560 plants of *Spartina alterniflora*. The plants of *Spartina anglica* were purchased from Jiangsu Qidong Huanghai Sea Beach Development Co., Ltd (former Qidong mud flat *Spartina* pasture); (ii) in 2014 – 2016, we introduced and cultivated 680 plants of *Spartina alterniflora* from Tongzhou Bay in Nantong of Jiangsu Province by three times. We took them as parental materials of rice hybridization.

2.1.2 Rice varieties. In 2005 – 2016, we introduced and cultivated following rice varieties as parental materials of rice hybridization: 1. Dahua Xiangnuo; 2. Jindao 1007; 3. Wuxiangnuo 8333; 4. Huajing No. 4; 5. Yinxiang 18; 6. Yanjing No. 10; 7. Wuyunjing No. 7; 8. Wujing 15; 9. Chengnuo 218; 10. Zhenxian 866; 11. Ningjing No. 1; 12. Tongyu Jing No. 14; 13. Nanjing 45; 15. Nanjing 47; 16. Nanjing 5055; 17. Shangshi 400; 18. Nipponbare; 19. 7K339; 20. Wujing 13; 21. Yandao No. 9; 22. Nongken 57; 23. Youfujing; 24. Yanjing 98; 25. Yanfeng 47; 26. Zhonghua No. 1; 27. Zhongxiang No. 1; 28. Liuqianxin; 29. Changnongjing No.4; 30. Yangdao No. 6. *Spartina* blooms in the end of July, and becomes exuberant in August to October. To make the florescence coincide with rice, we sowed one batch (4 batches in total) every 15 days from April 20.

2.2 Distant hybridization method of *Spartina* and Rice In 2005 – 2007, we undertook Rice ♀ × *Spartina* ♂ hybridization experiment. We did warm water lanching and ale gametocide treatment of Rice ♀, cut stamen of *Spartina* to pollinate, to explore whether hybridization of Rice and *Spartina*. In 2008 – 2013, on the basis of successful experiment of Rice ♀ × *Spartina* ♂ hybridization, we expanded the scope and quantity of hybridization. In 2014 – 2016, we undertook the study on reciprocal distant hybridization of *Spartina* and Rice. Normal hybridization: Rice ♀ warm water lanching and ale gametocide treatment, cut stamen of *Spartina* to pollinate, and did steam lanching and ale gametocide treatment to pollinate; reverse hybridization: continuously cut stamen to pollinate for *Spartina* ♀.

2.3 Rice ♀ × *Spartina* ♂ distant hybridization method Step 1: taking *Spartina* as male parent and rice as female parent, we carried out genus distant hybridization, obtained the first generation of hybrid, conducted screening test of planting in saline-alkaline land, phenotypic screening, molecular marker assisted selection, and finally obtained *Spartina/Oryza sativa* hybrid seeds containing *Spartina* and Rice. Step 2: using backcross method + molecular marker assisted selection method, we selected new varieties (lines) of *Spartina*/Oryza sativa suitable for growth in mud flat saline-alkaline land. We made a record of crossbreeding, backcross, and molecular marker assisted selection, and annually prepared file of hybrid pedigree, and kept samples of crossbreeding and backcross seeds in seed bank for reference.

2.4 Study methods

2.4.1 Patent for invention. Genus distant hybridization methods of *Spartina* and Rice on mud flat[8]. Patent number: ZL 2007 1 0019635.6.

2.4.2 Three-in-one technology. We combined mud flat planting screening, phenotypic screening, and molecular marker assisted selection methods to breakthrough key technology of saline tolerance biological characteristics.

2.5 RAPD molecular identification of mud flat *Spartina*/ Rice distant hybridization materials RAPD molecular identification of mud flat *Spartina*/ Rice distant hybridization materials was carried out by Professor Qin Pei from Institute of Biological Technology of Nanjing University, Associate Professor Tian Zengyuan from School of Life Sciences, Zhengzhou University, and Associate Professor Chen Shihua from College of Life Sciences of Yantai University. Detection samples: we used D to denote *Spartina anglica*, H to denote *Spartina alterniflora*, R to stand for rice, and RD1-n and RH1-n for hybrid seeds.

3 Results and analyses

3.1 Experiment of *Spartina* and Rice hybridization and mud flat planting in 2005 – 2008 In 2005, we obtained 149 seeds from hybridization of Peiai 64S/04141(7K339 ♀) R and D (♂). In 2006, we obtained 224 ears of F_1 generation selfed seeds, 11842 grains of selfed seeds, 1000-grain weight 18.38 g; F_1(♀) ×D(♂) backcross 17 ears, obtained 24 grains of BC_1F_1 seeds, the seed setting rate of backcross 2.84%; 6 varieties of R(♀)× D(♂) hybrid 23 ears, 63 grains of seeds, and the seed setting rate of crossbreeding 4.2%[9,10]. We introduced and cultivated 560 plants of *Spartina alterniflora* in 2005, and carried out *Spartina alterniflora* and Rice hybridization experiment in 2006. Results indicate that R(♀) 7 varieties (groups) × H (♂) hybridization 39 ears obtained 115 grains of hybrid seeds, with seed setting rate of 5.4%; $F_1$32, $F_1$34(♀) × H (♂) hybridization 12 ears obtained 18 grains of hybrid seeds, with seed setting rate of 2%. In December 2006, the Institute of Biological Technology of Nanjing University measured samples of hybrid seeds, *Spartina anglica* and Rice hybrid varieties: $RDF_1$32 and $RDF_1$34; *Spartina alterniflora* and Rice hybrid varieties: $RHF_1$37 and $RHF_1$39, RAPD reaction indicates that compared with rice parental materials, the strap of hybrid generation had distinct difference, indicating that distant hybridization leads to recombination of rice genes[11,12].

To increase quantity of new germplasm and expand scope of germplasm resources of *Spartina* and Rice hybridization, we hybridized R(♀)×D(♂) (RD) for 110 groups in 2007, the seed setting rate up to 20.3%, we hybridized R(♀)×H(♂) (RH) for 50 groups in 2007, the seed setting rate up to 23.1%. In the same year, we rented 1.33 hm^2 mud flat in Xingken of Qidong City and carried out saline tolerance adaptation experiment for

Spartina and Rice hybrid seeds. Under the conditions of soil salt content 7.83‰, pH 8.20, and irrigated water 10.37‰, 1000-grain weight of *Spartina* and Rice hybrid F2 was 18g, seed setting rate was 52.8%, and single plant seed weight was 19.97 g. In 2007, under the conditions of soil salt content 5.28‰ and pH 7.9, 1000-grain weight of *Spartina* and Rice hybrid F2 reached 22g, seed setting rate reached 51.1%, and single plant seed weight was 19.1g. 1000-grain weight of rice × maize × *Spartina* hybrid seeds was 22.8g, seed setting rate reached 69.9%, and single plant seed weight up to 41.4g; in experiment of Institute of Agricultural Sciences of the Area along Yangtze of Jiangsu Province, 1000-grain weight of *Spartina anglica* and Rice F2 hybrid seeds reached 22.4g, seed setting rate was 58.2%, and single plant seed weight was 33.7%[13,14]. In 2008, saline tolerance adaptation experiment was carried out for *Spartina anglica* / Rice hybrid seed No. 34 and No. 32, and 3.72 t/hm^2 hybrid seeds were obtained. In 2005 – 2008, we hybridized Rice (♀) × *Spartina* (♂) 358 groups.

3.2 Innovation on *Spartina* and Rice hybridization and new saline tolerant *Spartina* / Rice hybrid varieties in 2009 – 2012

Based on successful *Spartina* and Rice distant hybridization and trial planting in mud flat saline-alkaline land in 2005 – 2008, we carried out innovation on *Spartina* and Rice hybridization and new saline tolerant *Spartina* / Rice hybrid varieties in 2009 – 2012. We hybridized Rice (♀) × *Spartina* (♂) 669 groups in 2009 – 2012. Hybridization results: 421 groups of R(♀) × D(♂) (RD) were hybridized, and 248 groups of R(♀) × H(♂) (RH) were hybridized. A total of 289 samples of *Spartina*/Rice hybrid seeds were detected in 2009 – 2012. Among them, 48 samples had genetic elements of parental *Spartina* and *Oryza sativa*, and we screened new saline *Spartina* and *Oryza sativa* hybrid varieties. In 2009, through mud flat screening combined with molecular marker assisted selection, in 32 groups, 20 groups obtained saline tolerant rice higher than 3.0 t/hm^2 with the average rice yield 4.89 t/hm^2 (the minimum yield was 3.11 t/hm^2 and the maximum yield was 8.94 t/hm^2). We selected 6 new saline-tolerant *Spartina*/Rice hybrid varieties with yield of 6.0 – 7.5 t/hm^2. We found breeding materials of saline tolerant sweet stalk japonica rice germplasm resources, soluble sugar content of straw was above 5.0%, which was more than 65% higher than the control group. In 60 samples of hybrid seeds, we selected 18 hybrid groups. Through RAPD analysis, 9 groups had common strap with parental *Spartina anglica* and had vacancy of parental Rice materials, indicating that 9 groups of distant hybrid seeds had genetic elements of parental *Spartina anglica*[15,16].

3.2.1 Results of *Oryza sativa* ♀ × *Spartina anglica* ♂ hybridization. In 2009 – 2011, we obtained experimental evidence of seed setting and transplantation of *Spartina anglica* and Rice distant hybridization, and economic traits of hybrid materials; through RAPD analysis, 22 samples of hybrid materials had common straps with parental *Spartina anglica* but no parental Rice, indicating that 22 samples of distant hybridization materials had genetic elements of parental *Spartina anglica*[17]. In 2009, we hybridized 30 groups of seeds, cut hull of 1737 grains, obtained 409 grains of seeds, and seed setting rate reached 22.1%. In 2010, we sowed 30 groups of hybrid seeds, transplanted 8 groups, the germination rate reached 26.67%; we sowed 409 grains of seeds, transplanted 18 grains, and the germination and transplantation rate was 4.4%. Analysis: in 2009, we cut hull of 1737 grains; in 2010, we transplanted 18 grains, the actual hybridization success rate was 1.04%. In 2010, we hybridized 3 groups of seeds, cut hull of 126 grains, obtained 31 grains of seeds, and seed setting rate reached 27.1%. In 2011, we sowed 3 groups of hybrid seeds, one group germinated, the germination rate reached 33.33%; we sowed 31 grains of seeds, transplanted one grain, and the germination and transplantation rate was 3.2%. Analysis: In 2010, we cut hull of 126 grains; in 2011, we transplanted one grain, the actual hybridization success rate was 0.79%. Experimental results of these two years: success rate of Rice ♀ × *Spartina anglica* ♂ hybridization was 0.79 – 1.04%.

3.2.2 Results of *Spartina Oryza sativa* ♀ × *Spartina anglica* ♂ backcross. In 2009, we hybridized 11 groups of seeds, cut hull of 769 grains, obtained 176 grains of seeds, and seed setting rate reached 21.64%; in 2010, we sowed 11 groups of hybrid seeds, transplanted 7 groups, the germination rate reached 63.64%; we sowed 176 grains of seeds, transplanted 15 grains, and the germination and transplantation rate was 8.52%. Analysis: In 2009, we cut hull of 769 grains; in 2011, we transplanted 15 grain, the actual hybridization success rate was 1.95%. In 2010, we hybridized 13 groups of seeds, cut hull of 679 grains, obtained 75 grains of seeds, and seed setting rate reached 9.52%. In 2011, we sowed 13 groups of hybrid seeds, transplanted 4 groups, the germination rate reached 30.77%; we sowed 75 grains of seeds, transplanted 12 grains, and the germination and transplantation rate was 16%. Analysis: in 2010, we cut hull of 679 grains; in 2011, we transplanted 12 grains, the actual hybridization success rate was 1.77%. Experimental results of these two years: success rate of *Spartina Oryza sativa* ♀ × *Spartina anglica* ♂ backcross was 1.77 – 1.95%.

3.2.3 Results of *Spartina Oryza sativa* ♀ × *Oryza sativa* 7K339 ♂ backcross. In 2009, we hybridized 130 groups of seeds, cut hull of 8630 grains, obtained 2509 grains of seeds, and seed setting rate reached 26.89%. In 2010, we sowed 130 groups of hybrid seeds, 45 group germinated, the germination rate reached 34.62%; we sowed 2509 grains of seeds, transplanted 177 grain, and the germination and transplantation rate was 7.05%. Analysis: In 2009, we cut hull of 8630 grains; in 2010, we transplanted 177 grain, the actual hybridization success rate was 2.05%. In 2010, we hybridized 43 groups of seeds, cut hull of 2600 grains, obtained 1134 grains of seeds, and seed setting rate reached 35.27%. In 2011, we sowed 43 groups of hybrid seeds, 16 group germinated, the germination rate reached 37.21%; we sowed 1134 grains of seeds, transplanted 49 grain, and the germination and transplantation rate was 4.32%. Analysis: In 2010, we cut hull of 2600 grains; in 2011, we transplanted 49 grain, the actual hybridization success rate was 1.88%. Experimental results of these two years: success rate of *Spartina Oryza sativa* ♀ × *Spartina anglica* 7K339 ♂ backcross was 1.88 – 2.05%.

3.2.4 Results of Rice ♀ × *Spartina alterniflora* ♂ hybridization. In 2009 – 2011, we undertook study of *Oryza sativa* and *Spartina alterniflora* distant hybridization, applied *Oryza sativa* and *Spartina alterniflora* genus distant hybridization methods, and combined mud flat planting screening, backcross phenotypic screening, cell-based screening, and molecular marker assisted selection technologies, to find new saline tolerant rice varieties suitable for planting in mud flat and using as feed. In 2009 – 2011, the success rate of actual distant hybridization was 1.39%. We randomly selected 6 samples of 7C14 and Zhongxiang No. 1 rice female parent and *Spartina alterniflora* (H) male parent distant hybrid seeds to make RAPD molecular analysis, found that 10K205-7C14 × H, 8K157-7C14 × H, 9H5 Zhongxiang No. 1 × H had common strap with parental *Spartina alterniflora*, but no parental Rice, showing that the above distant hybrid seeds had genetic elements of parental *Spartina alterniflora*. Besides, we selected 12 samples of 7K339 rice female parent and *Spartina alterniflora* (H) male parent distant hybrid seeds and carried out RAPD analysis, results indicated that 10K215, 8K48, 9H9, 9H8, and 9H28 had common strap with parental *Spartina alterniflora* but no parental rice, showing that the above distant hybrid seeds had genetic elements of parental *Spartina alterniflora*, while there was different degree of variation in other hybrid seeds, parental *Spartina alterniflora*, and parental rice[18, 19].

3.3 Breeding of new saline tolerant *Spartina* / Rice hybrid varieties in 2013 – 2016

3.3.1 Study on innovation methods for reciprocal distant hybridization of *Spartina alterniflora* and Rice. In 2014 – 2016, we undertook the study on suitable methods for reciprocal distant hybridization of *Spartina* and Rice. Normal hybridization: Rice ♀ warm water lanching and ale gametocide treatment, cut stamen of *Spartina* to pollinate, and did steam lanching and ale gametocide treatment to pollinate; reverse hybridization: cut stamen of *Spartina alterniflora* ♀ to cut hull to pollinate, and warm water lanching and ale gametocide treatment. Results indicate that Rice ♀ hot steam ale gametocide treatment pollination method could increase the seed setting rate by 34.09%, increase the average ear seed setting by 121.21%, the germination rate by 60.07%, work efficiency by 6 – 7 times; *Spartina alterniflora* ♀ continuous ale gametocide treatment pollination could increase the seed setting rate by 3.14 times, the average ear seed setting by 4.21 times, germination rate by 68.74%, and work efficiency by 7 – 8 times. Practice proved that Rice ♀ hot steam ale gametocide treatment pollination method is suitable for Rice ♀ × *Spartina alterniflora* ♂, while *Spartina alterniflora* ♀ continuous ale gametocide treatment pollination method is suitable for *Spartina alterniflora* ♀ × Rice ♂[20].

3.3.2 Study on innovation methods for reciprocal distant hybridization of *Spartina anglica* and Rice. In 2014 – 2016, we undertook the study on suitable methods for reciprocal distant hybridization of *Spartina anglica* and Rice. The study method is the same as that for reciprocal distant hybridization of *Spartina alterniflora* and Rice. Results indicate that Rice ♀ hot steam ale gametocide treatment pollination method could increase the seed setting rate by 103.23%, increase the average ear seed setting by 65.58%, the germination rate by 88.67%, work efficiency by 5 – 6 times; *Spartina anglica* ♀ continuous ale gametocide treatment pollination could increase the seed setting rate by 1.67 times, the average ear seed setting by 2.83 times, germination rate by 41.79%, and work efficiency by 6 – 7 times. Practice proved that Rice ♀ hot steam ale gametocide treatment pollination method is suitable for Rice ♀ × *Spartina anglica* ♂, while *Spartina anglica* ♀ continuous ale gametocide treatment pollination method is suitable for *Spartina anglica* ♀ × Rice ♂. In 2013 – 2016, we hybridized 471 groups of *Spartina* and Rice.

3.3.3 Study on high yield cultivation technologies for mud flat saline tolerant *Spartina* Rice. In view of characteristics of saline-alkaline mud flat, through implementing measures such as water and soil resource utilization, farming modification, supporting river system construction, seed selection and treatment, water direct sowing, raising rice seedlings on seedbed, field transplantation, weeding, fertilizer and water management, prevention and control of plant diseases and insect pests, the yield of saline tolerant *Spartina* Rice can reach 5.925 – 8.28 t/hm^2. Then, we summarized high yield cultivation technologies for mud flat saline tolerant *Spartina* Rice, formulated technical procedures, to provide basis for scientific cultivation of mud flat saline tolerant *Spartina* Rice[21,22].

3.3.4 Breeding of new saline tolerant *Spartina* and Rice lines in 2013 – 2016. On the basis of innovation of *Spartina* and Rice germplasm in 2005 – 2013, we carried out saline tolerant germination experiment for 223 groups of *Spartina* and Rice hybrid seeds. Under the conditions of the salt content of 15‰ and pH 7.0 water environment, 81 groups had germination rate above 50%, accounting for 36.32%; at salt content of 20‰ and pH 7.0 water environment, 17 groups had germination rate higher than 50%, accounting for 7.62%. In 2014 – 2016, we selected 20 groups of excellent hybrid seeds, and combined mud flat planting screening, phenotypic screening, and molecular marker assisted selection methods to breakthrough key technology of saline tolerance biological characteristics. Using hybrid breeding, conventional breeding, and molecular breeding methods, we cultivated 10 new lines of saline tolerant *Spartina* and Rice suitable for planting in mud flat, and the rice yield reached 5.925 – 8.28 t/hm^2. Among them, 2 lines had yield up to 7.68 – 8.28 t/hm^2. In addition, we established 2 hm^2 new line cultivation and seed breeding base, made a trial planting of new lines of saline tolerant *Spartina* and Rice in 20 hm^2 mud flat, carried out cultivation demonstration for new line high yield varieties, and formulated technical procedure for high yield cultivation of saline tolerant *Spartina* and Rice.

4 Conclusions and discussions

4.1 Conclusions Using autonomous patent for invention, we studied distant hybridization of *Spartina* and Rice. We adopted tidal flat planting screening, phenotypic screening, molecular marker assisted selection, to find biological evidences of new germplasm, and also applied crossbreeding, conventional breeding, and molecular breeding, to select new saline-tolerant *Spartina* and Rice varieties. With 12 years of efforts, we developed 1498 crossbreeding combinations and established pedigree file and seed bank, tested 307 crossbreeding materials (57 hybrid seeds showed parental genetic components of *Spartina* and Rice); obtained *Spartina* /Rice distant hybridization and seed setting, transplanting, hybridization material economic traits, and molecular biological experimental evidences; Rice ♀ × *Spartina* ♂ hybridization success rate of 1.04 – 1.39%; obtained authorization of patent for invention of *Distant Hybridization Methods for Spartina* and Rice; formulated procedures for high yield cultivation of *Spartina*-Rice, planted 33.33 hm^2 *Spartina*-Rice in coastal saline and alkaline land, cultivated 10 new saline-tolerant *Spartina*-Rice varieties, and the rice yield up to 5.925 – 8.28 t/hm^2. *Spartina*-Rice is saline-tolerant high yield rice germplasm, developed through sexual hybridization, conventional breeding, and molecular breeding technologies, and is optimal crop for saline and alkaline land. Success of *Spartina*-Rice solves the problem of saline-tolerant crop badly for transformation of saline and alkaline land, the problem of feeds for herbivores, as well as problem of environmental pollution resulted from straw burning. Besides, it is of essential scientific significance for resource utilization, increase of agricultural efficiency, grain security, and farmland strategy, so it has broad application prospect.

4.2 Discussions According to statistics of the United Nations Educational, Scientific and Cultural Organization (UNESCO), the area of saline-alkaline land in the world is up to 950 million hm^2; in China, the saline-alkaline land is up to 37 million hm^2, accounting for 1/28 of the world saline-alkaline land, equivalent to 1/4 existing farmland of China. Using traditional and modern genetic engineering method to raise saline tolerance and cultivate saline-tolerant varieties is a most effective approach for development and use of saline-alkaline land[23]. Distant hybridization, as a breeding means, is mainly applied for introducing useful genes of different genus and varieties, to improve existing varieties. Distant hybrid rice, specially hybrid rice with genes of different genus, may have powerful advantages beyond our imagination[24]. Under the conditions of constant increase in population and grain demand and gradual decline in farmland area and fresh water resources, coastal development and saline-alkaline land transformation projects badly need solving problems such as saline tolerance of crops and high efficient use of straws. Exploration and innovation of favorable genes of distant varieties of crops will become an essential approach for modification of crop varieties. *Spartina*-Rice is saline-tolerant high yield rice germplasm, developed through sexual hybridization, conventional breeding, and molecular breeding technologies, and is optimal crop for saline and alkaline land. Success of *Spartina*-Rice solves the problem of saline-tolerant crop badly for transformation of saline and alkaline land, the problem of feeds for herbivores, as well as problem of environmental pollution resulted from straw burning. Besides, it is of essential scientific significance for resource utilization, increase of agricultural efficiency, grain security, and farmland strategy, so it has broad application prospect.

References

[1] GUAN DM. On the ecological system and management of *Spartina alterniflorea* salt-marshes in Chinesecoastal wetland [M]. Beijing: China Ocean Press, 2009. 12: 1 – 15. (in Chinese).

[2] ZHANG ZY. Chinese feed science [M]. Beijing: China Agriculture Press, 2000, 10: 350 – 351, 607. (in Chinese).

[3] QIN P, JING MD, ZHANG ZR, *et al*. The experiment of seed salt-tolerance of three ecotypes of *Spartina alterniflora* [J]. Journal of Nanjing University, 1985(5): 237 – 244. (in Chinese).

[4] ZHONG CX, CHEN QK. Chinese first common cordgrass sheep yard [C]. Nanjing: Nanjing University Press, 1993. 1: 359 – 366. (in Chinese).

[5] CHEN QK. The experiment of sheep breeding using *Spartina anglica* in the seabeach [J]. Animal Husbandry & Veterinary Medicine, 1986, 18(4): 170 – 171. (in Chinese).

[6] CHEN QK, MEI ZP. Observation on the effect of beef cattle in coastal *Spartina* rice field [J]. Animal Husbandry & Veterinary Medicine, 1987, 19(3): 107 – 109. (in Chinese).

[7] Chinese Academy of Agricultural Sciences. Chinese rice science [M]. Beijing: China Agriculture Press, 1986, 8: 317, 327 – 328. (in Chinese).

[8] Distant hybridization breeding method between distant hybridization of rice and seabeach spartina [P]. 2007 1 0019635.6. (in Chinese).

[9] CHEN QK, SHA WF, GU YJ, *et al*. A preliminary study on distant hybridization of rice and seabeach spartina [J]. Journal of Jinling Institute of Technology, 2007, 23(1): 60 – 65. (in Chinese).

[10] CHEN QK, SHA WF, GU YJ, *et al*. Study on distant hybridization of rice and seabeach *spartina* [J]. Journal of Nanjing University (Natural Sciences), 2007, 43(5): 245 – 251. (in Chinese).

[11] CHEN QK, SHA WF, GU YJ, *et al*. A preliminary study on *Spartina alterniflora* and distant hybridization of rice [J]. Livestock and Poultry Industry, 2007(2): 24 – 25. (in Chinese).

[12] CHEN QK, SHA WF, GU YJ, *et al*. Study on *Spartina alterniflora* and distant hybridization of rice [J]. Journal of Nanjing University (Natural Sciences), 2007, 43(5): 309 – 313. (in Chinese).

[13] CHEN QK, SHA WF, GU YJ, *et al*. Excavation and innovation germplasm resources by distant hybridization of rice and seabeach spartina [J]. Chinese Agricultural Science Bulletin, 2007, 23(S1): 10 – 13. (in Chinese).

[14] CHEN QK, SHA WF, GU YJ, *et al*. Excavation and innovation germplasm resources by distant hybridization of rice and seabeach spartina [J]. Southwest China Journal of Agricultural Sciences, 2009, 22(4): 877 – 883. (in Chinese).

[15] CHEN QK, TIAN ZY, SHA WF, *et al*. Excavation and innovation germplasm resources by distant hybridization of rice and seabeach *spartina* [J]. Jiangsu Agricultural Sciences, 2010(5): 311 – 315. (in Chinese).

[16] CHEN QK. Study on the new germplasm mechanism of salt-tolerant japonica rice [J]. Hybrid Rice, 2010, 25(S1): 532 – 536. (in Chinese).

(To page 94)

mg/g to 1.477 mg/g, and it is highest for Yuanhuang and Jincunqiu, followed by Xinshiji, Cuilu and Xishui. With E'li 2, Xinshiji and Xizilu as pollination varieties, Qingxiang pear has the best internal quality, followed by Yuanhuang, Cuiguan, Cuilu and Xishui.

Table 3 Effect of different pollination varieties on internal quality of Qingxiang pear

Pollination varieties	Single fruit weight g	Fruit shape index	Fruit core size flesh size	Hardness Pa/cm^2	Soluble solids %	Soluble sugar %	Titratable acid %	Vc content mg/g
Yuanhuang	305.99 ±45.38^{ab}	0.945 ±0.036^{bc}	0.224^{ab}	4.97^{bcd}	10.6^{bc}	4.753^{b}	0.290^{bcd}	1.477^{a}
Cuiguan	324.13 ±70.91^{ab}	0.941 ±0.068^{bc}	0.211^{b}	4.972^{bcd}	10.4^{c}	5.379^{ab}	0.313^{bcd}	0.706^{bcd}
E'li 2	319.45 ±78.45^{ab}	0.931 ±0.064^{bc}	0.22^{ab}	5.03^{abcd}	10.5^{bc}	6.264^{ab}	0.335^{bcd}	0.706^{bcd}
Xuehua	305.62 ±35.59^{ab}	0.918 ±0.084^{c}	0.237^{ab}	4.858^{bcd}	10.35^{c}	4.869^{ab}	0.379^{bcd}	0.562^{d}
Xinshiji	326.32 ±85.45^{ab}	0.966 ±0.047^{bc}	0.246^{bcd}	5.538^{bcd}	11.35^{ab}	5.689^{ab}	0.379^{bcd}	0.915^{b}
Cuilu	359.87 ±71.39^{bcd}	1.039 ±0.093^{bcd}	0.207^{b}	4.96^{bcd}	10.7^{bc}	6.04^{ab}	0.357^{bcd}	0.899^{b}
Xizilu	353.34 ±52.90^{bcd}	0.995 ±0.052^{ab}	0.229^{ab}	4.919^{bcd}	11.5^{bcd}	6.507^{bcd}	0.290^{bcd}	0.642^{bcd}
Huanghua	331.66 ±33.66^{ab}	0.976 ±0.065^{bc}	0.23^{ab}	5.275^{ab}	10.55^{bc}	6.002^{ab}	0.335^{bcd}	0.770^{bcd}
Xishui	354.79 ±50.75^{bcd}	0.942 ±0.047^{bc}	0.218^{ab}	4.638^{cd}	10.7^{bc}	4.729^{b}	0.313^{bcd}	0.867^{bc}
Jincunqiu	281.66 ±56.71^{b}	0.921 ±0.092^{c}	0.227^{ab}	4.515^{d}	10.7^{bc}	6.457^{bcd}	0.335^{bcd}	1.380^{a}
CK	304.30 ±51.25^{ab}	0.959 ±0.047^{bc}	0.212^{ab}	5.193^{abc}	9.65^{d}	5.229^{ab}	0.335^{bcd}	0.802^{bcd}

Note: SPSS is used for variance analysis of the data in table, and different letters indicate significant difference at the 5% level.

4 Conclusions

Almost all pear varieties are self-incompatible, and there are great differences in compatibility due to variety difference[1]. This test also demonstrates that 10 pear varieties are all compatible with Qingxiang pear, and the fruit-setting rate under artificial pollination is significantly higher than under natural pollination. With Xinshiji as pollination variety, the value of some indicators (single fruit weight, hardness, soluble solids content, soluble sugar content, Vc content) is high, the fruit is sweet and fragrant, and there are few stone cells in the fruit. With Xizilu as pollination variety, the fruit appearance quality is high, the single fruit weight is large and soluble solids and soluble sugar content is high, but the ratio of fruit core size to flesh size and titratable acid content are low. The fruit is sweet and fragrant, and there are few stone cells in the fruit. With Cuiguan as pollination variety, the fruit appearance quality is good, and the value of some indicators (single fruit weight, hardness, soluble solids content, soluble sugar content) is high. With Yuanhuang as pollination variety, the fruit is fine, crisp and sweet, and there are few stone cells in the fruit; the intrinsic quality is similar to the control, but Vc content is high. With Cuilu, Xishui as pollination varieties, the single fruit weight is large and the soluble solids and Vc content is high. Therefore, through comprehensive comparison of different pollination varieties of Qingxiang pear, it is found that Xinshiji, Xizilu, Cuiguan and Yuanhuang can be chosen as the best pollination varieties, followed by Cuilu and Xishui.

References

[1] ZHANG SL, XU YL, CHEN DX, *et al*. Selection of pollination varieties of pear and the cause of nonbearing after pollination[J]. South China Fruits, 2002, 31(6):52 –54. (in Chinese).

[2] GUAN JF. Study on the quality of fruit[M]. Shijiazhaung: Hebei Science and Technology Publishing House, 2001:412 –414. (in Chinese).

[3] LONG SZ, HE YQ. The corelation between titratable acid and vitamin C test in litchi[J]. Guangxi Agricultural Sciences, 2002 (4) :188 –189. (in Chinese).

[4] LI J. Study on molybdenum blue method of L-VC test by spectrometry [J]. Food Science, 2000,21(8):42 –43. (in Chinese).

[5] ZHOU XZ. The technique of artificial pollination of Cuiguan pear[J]. Zhejiang Ganju, 2005, 22(3): 40 –41. (in Chinese).

(From page 91)

[17] CHEN QK, TIAN ZY, SHA WF, *et al*. Excavation and innovation germplasm resources by distant hybridization of rice and seabeach *spartina* [J]. Jiangsu Agricultural Sciences,2012,40(1):65 –69. (in Chinese).

[18] CHEN QK, TIAN ZY, SHA WF, *et al*. Exploration and innovation of distant hybridization germplasm of *Oryza sativa* x *Spartina alterniflora* in Tideland [J]. Journal of Anhui Agricultural Sciences,2011,39(36): 22251 –22253. (in Chinese).

[19] CHEN QK, TIAN XY, SHA WF, *et al*. Exploration and innovation of distant hybridization germplasm of *Oryza Sativa* and *Spartina alterniflora* [J]. Agricultural Science & Technology,2012,13(1):131 –133,172.

[20] CHEN QK, CHEN B, LU HL, *et al*. An innovative strategy for reciprocal distant hybridization between *Spartina alterniflora* and rice [J]. Agricultural Science & Technology,2015,16(12):2604 –2611.

[21] CHEN QK, CHEN B, LU HL, *et al*. High-yield cultivation technique of salt-tolerant *Spartina* and rice in coastal mudflat [J]. Agricultural Science & Technology,2016,17(8):1852 –1855,1925.

[22] CHEN QK, CHEN B, LU HL, *et al*. High-yield cultivation technology of salt-tolerant *spatrina* rice in coastal mud flat [J]. Journal of Anhui Agricultural Sciences,2016,44(18):18 –20. (in Chinese).

[23] ZHAO KF, FAN H. On halophyte and its physiology adaption to saline-alkali habitat [M]. Beijing: Science Press,2005, 9:1 –31. (in Chinese).

[24] YUAN LP. Key technology of super hybrid rice with 800 kg per unit area [M]. Beijing: China Three Gorges Publishing House,2006, 1:11 –12. (in Chinese).