Construction of All-male Asparagus Breeding Technology System

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Abstract [**Objectives**] This study was conducted to solve the problems of long breeding cycle and low induction rate of double-haploid plants of asparagus. [**Methods**] Based on the achievements and practice of breeding research, an all-male asparagus breeding technology system was constructed. [**Results**] The technology system includes such six techniques as collection and identification of germplasm resources, tetraploid plant induction, anther culture, artificial hybridization of double-haploid plants, offspring screening and tissue culture and rapid propagation of parents. [**Conclusions**] The construction of this system lays a foundation for breeding new all-male asparagus varieties with excellent quality.

Key words Asparagus; All-male breeding; Germplasm resource; Technology system

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Asparagus is a kind of nutritional and health-care high-grade vegetable deeply loved by consumers, and it has the reputation of "the king of vegetables"[1]. Asparagus is a dioecious plant with low yield of female plants and yield of male plants 20% - 30% higher than that of female plants. Therefore, cultivating all-male varieties has become the main direction of asparagus breeding at home and abroad[2]. Obtaining double-haploid plants is the key in all-male asparagus breeding. At present, the main research is to obtain haploid materials through anther culture of diploid male asparagus plants and then obtain double-haploid plants through chromosome doubling[3]. However, there are some problems in this method, such as very low haploid induction rate[4] and very low haploid plant vitality[5], which leads to the slow pace of all-male breeding. In order to improve the efficiency of all-male asparagus breeding, Institute of Cash Crops, Hebei Academy of Agriculture and Forestry Sciences optimized asparagus breeding process by changing the timing of chromosome doubling, and successfully selected an excellent all-male asparagus variety Jiyu Lvlu 3. Therefore, based on latest achievements of asparagus breeding research, in this study, an all-male asparagus breeding technology system was constructed from the aspects of germplasm collection and identification, tetraploid plant induction, anther culture, artificial hybridization of double-haploid plants and rapid propagation with parental combination, which lays a foundation for asparagus breeding and industrial development.

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Collection and Identification of Germplasm Resources

Germplasm resources are the basis of cross breeding. Asparagus planting resources at home and abroad were widely collected to establish a germplasm resource nursery. Various resources were comprehensively evaluated, including biological characteristics, yield, quality, cold tolerance, drought resistance, disease resistance, and lodging resistance, and the suitability and heritability of each germplasm resource was comprehensively evaluated[6-7]. According to the target characters of new variety breeding, corresponding germplasm resources were selected for further research.

Tetraploid Plant Induction

Germplasm resources with 2 - 3 excellent characters (high quality, disease resistance, high yield, stress resistance) were selected. When the seedling age was 30 - 40 d, the top of the tender stem of each seedling was treated with 0.1% colchicine for 48 h. After 10 d, the chromosome of the stem tip was observed (Table 1), and tetraploid seedlings with 40 chromosomes were selected for further culture.

Table 1 Effects of colchicine concentration on tetraploid induction rate of asparagus plants

No.	Concentration//%	Tetraploid induction rate//%
1	0.05	35.4
2	0.10	59.8
3	0.15	28.9
4	0.20	19.5
5	0.30	15.2

Anther Culture of Tetraploid Plants

After the tetraploid plants bloomed, male plants with strong growth were selected, and buds were taken off, and stored at

2 – 4 ℃ for 4 d in the dark. Anthers (mononuclear side) were stripped for anther culture. The callus induction medium was: 1/2 MS + NAA 2.0 mg/L + 6-BA 1.0 mg/L. The medium for bud differentiation was MS + NAA 0.5 mg/L + 6-BA 1.5 mg/L (Table 2). The rooting medium of regenerated plantlets was: MS + IBA 1.0 mg/L + KT 0.05 mg/L NAA 0.1 mg/L + ancyrmidol 1.5 mg/L (Fig. 1, Table 3). Through chromosome observation, double-haploid plantlets with 20 chromosomes were selected and cultured. The double-haploid plants were distinguished according to sex, and evaluated from biological characters, yield, quality, stress resistance, disease resistance and lodging resistance.

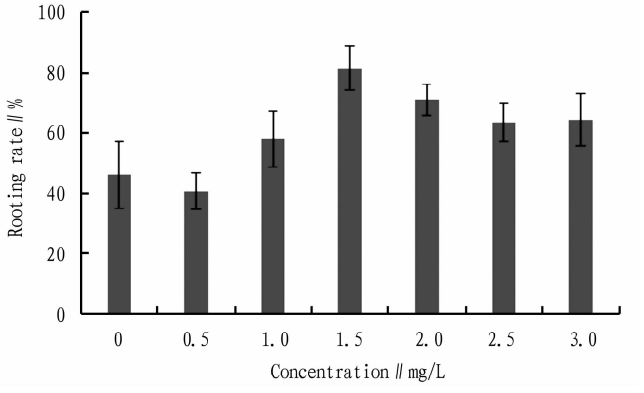

Fig. 1 Effects of ancyrmidol on rooting rate of asparagus

Table 2 Screening of bud differentiation medium

No.	Hormone ratio // mg/L		Bud induction rate // %	Proliferation coefficient
	NAA	6-BA		
1	0.1	0.5	28.5	2.5
2	0.1	1.0	19.7	2.9
3	0.1	1.5	29.5	2.1
4	0.2	0.5	84.0	3.3
5	0.2	1.0	219.5	4.8
6	0.2	1.5	62.5	3.5
7	0.5	0.5	95.3	3.6
8	0.5	1.0	215.6	9.8
9	0.5	1.5	284.5	10.5

Table 3 Screening of rooting medium

No.	Hormone ratio // mg/L				Rooting rate %
	NAA	IBA	KT	Ancyrmidol	
1	0.1	0.5	0.05	1.5	0.0
2	0.1	0.5	0.10	1.5	0.0
3	0.1	1.0	0.05	1.5	35.4
4	0.1	1.0	0.10	1.5	25.8
5	0.1	1.5	0.05	1.5	5.0
6	0.1	1.5	0.10	1.5	6.0
7	0.2	0.5	0.05	1.5	0.0
8	0.2	0.5	0.10	1.5	0.0
9	0.2	1.0	0.05	1.5	34.8
10	0.2	1.0	0.10	1.5	24.2
11	0.2	1.5	0.05	1.5	32.4
12	0.2	1.5	0.10	1.5	20.6

Artificial Hybridization Pollination

Artificial pollination is the core link of asparagus hybrid breeding. Through artificial pollination, hybrid offspring varieties that meet the breeding objectives are obtained, and then new varieties are screened out. Female plants and male plants that met the target traits and had 2 – 3 excellent traits such as yield, quality, stress resistance and disease resistance were selected from the double-haploid plantlets respectively, and artificial pollination was carried out according to the principle of complementary traits. Female plants need to be isolated before and after pollination.

Screening of Hybrid Offspring

The evaluation and screening workload of the F_1 generation is huge, so early selection is very important. The hybrid combinations were planted in a suitable ecological area, and their future yield potential was evaluated according to the growth at seedling stage and in the second year after planting, and the indexes such as stress resistance, disease resistance, plant height, stem diameter, tender stem number, scale bud wrapping and branchiness were determined. Through the comprehensive evaluation of phenotypic variation analysis, correlation analysis, cluster analysis and principal component analysis, excellent lines were obtained, and then regional tests and production demonstration were carried out to finally obtain excellent new asparagus varieties.

Tissue Culture and Rapid Propagation of Parents

The plants with strong growth of male and female parents were selected respectively, and new strong tender stems with a length of about 15 – 25 cm were selected as explants. The explants were washed with tap water for 5 min, soaked in 75% ethanol for 30 s under aseptic conditions and then in 2% sodium hypochlorite for 5 min, and washed with sterile water for 3 – 5 times. The sterilized tender stems were cut into stem segments with 1 – 2 axillary buds each, which were inoculated on the axillary bud induction medium. The culture conditions were as follows: temperature 27 ℃, relative humidity 70% – 80%, illumination time 12 h/d and illumination intensity 2 500 lux. The tender stems over 5 cm induced from the axillary buds of each stem segment were cut off, and the stem tips were cut off. Then, the obtained materials were inoculated on the proliferation medium, and sub-cultured for 1 – 2 cycles until 3 – 8 clustered stems grew in each leaf axil. Next, the stem segments were cut into 2 – 3 cm and inoculated on the rooting medium. When 2 – 3 stout fleshy roots grew out, they could be transplanted.

Conclusions

The all-male hybrid breeding technology system of asparagus constructed in this study consists of six techniques, among which germplasm resources are the material basis of hybrid breeding, the creation of double-haploid materials in line with target traits is the core of the technology, and artificial pollination and early evaluation of the F_1 generation are the key technique. The three complement

each other. Because asparagus is dioecious, the tissue culture and propagation of parents is also the key technique of hybrid seed production which ensures the breeding of hybrids. In a word, asparagus industry needs new all-male varieties with high quality, high yield and good disease resistance, and systematic cross breeding is expected to realize this ideal.

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components of enzyme protection system are SOD and POD. The coordination of SOD and POD can keep active oxygen free radicals in plants in a stable state, so that wheat can carry out normal circular metabolism. On this basis, in the early stage of this study, the longest root length and seedling length of Zhoumai 28 seedlings were used as survey indicators for measuring the effects of salt stress on the characters of Zhoumai 28. The activities of SOD and POD in Zhoumai 28 were determined in the middle stage, and basic physical and chemical properties of POD and SOD in wheat were studied by bioinformatics analysis in the later stage. The results showed that the SOD activity of Zhoumai 28 decreased with the concentration of NaCl increasing, indicating that the balance of produced normal and scavenged superoxide radicals in wheat cells was damaged under salt stress, so the SOD activity decreased, and the superoxide radicals that could not be effectively eliminated caused harm to the growth of the body[13]. The activity of POD was consistent with SOD. The MDA content increased significantly, which led to a significant impact on seedling growth, which was reflected in a significant decline in root length and seedling height, resulting in salt damage, and finally a decline in yield[14]. It shows that membrane lipid peroxidation often occurs when plants are in adversity or organ aging[15]. There were differences between SOD (ABN50913.1) and POD (XP_044344719.1) of Zhoumain in terms of signal peptide, structure and transmembrane domain.

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