

Crossability between *Arachis hypogaea* L. and *Erectoides* species A. sp. 9990 GKP

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Summary Eighteen cultivars representing five botanical types of *Arachis hypogaea* L. were selected as female parents to cross with A. sp. 9990, an *Erectoides* accession with resistance to multiple diseases and pests. All the crosses failed to produce viable seeds mainly due to embryo abortions. Three morphologically different types of embryo abortion were recorded. Marked differences were observed between subspecies, and among botanical types and genotypes in hybrid pod production and embryo development. In terms of pod production per 100 pollinations, subspecies *fastigiata* was superior to subspecies *hypogaea*, and Valencia and Virginia types performed better than Spanish and Peruvian types. Fengqiu Yibazhua and Silihong were among the most acceptable genotypes by showing higher pod productions and delayed embryo abortions. These results suggest that through extensive screenings, particular genotypes with higher crossability or less incompatibility with A. sp. 9990 can be obtained, and the use of such genotypes in hybridization program will certainly facilitate the transfer of desirable genes from A. sp. 9990 into the cultivated peanut.

Key words *Arachis*, peanut, wild species, crossability

Introduction

Peanut is an important industrial crop. Since its seeds are rich in oil and protein, it has been used as food ingredients and a source of edible oil as well. Diseases and pests are major production constraints and have been causing farmers heavy economic losses. For the development of resistant varieties, breeders are paying more and more attentions to the utilization of wild *Arachis* in addition to fully exploiting the cultivated germplasms. *Arachis* is a large genus comprising 40—70 species which are divided into seven sections. *Arachis hypogaea* L., a member of section *Arachis*, is the only cultivated species of great economic importance (Stalker, 1985). Most of the previous studies on wild *Arachis* have been concentrated on members of section *Arachis* (Singh, 1984; Wong, 1986), partly because they are the only accessions compatible with the cultivated species. Section *Erectoides*, however, has recently drawn the interest of researchers with the discovery that some of its accessions are resistant to multiple diseases and/or pests. *Arachis* species 9990 GKP (A. sp. 9990, which has not been validly described and named, the collector's number is still used here) is one such accession. It is immune to rust, highly resistant to early and late leafspots, and thrips (Stalker, 1987; Zhang, 1991). Successful transfer of its multiple resistances into *A. hypogaea* L. may probably lead to a breakthrough in peanut improvement program. It has been found in wheat and other crops that cultivar genotypes may vary significantly in their crossabilities with wild relatives, and researchers have made use of such variations to create interspecific and intergeneric hybrids. In view of these experiences, the present study was designed to: (1) Evaluate a wide range of *A. hypogaea* L. genotypes for their crossabilities with A. sp. 9990, through which genotypes with higher crossability or less incompatibility

may be found out; (2) Examine incompatibility barriers, which may help to determine appropriate ways for gene transfer.

Materials and methods

Seeds of *A. sp.* 9990 was provided by the Institute of Oil Crops, Chinese Academy of Agricultural Sciences. All the 18 cultivars used were from the germplasm bank of Henan Academy of Agricultural Sciences (HAAS). According to the accepted classification, the cultivated peanut is divided into two subspecies, each subspecies is further subdivided into two varieties which correspond to botanical types. Virginia, Peruvian, Spanish and Valencia are the four basic types. Intercrossings among subspecies or varieties allow for the production of intermediate types. Four genotypes were selected from each of the four basic types, and two from the intermediate types (Tab. 1).

The hybridization experiments were conducted in the solarium at HAAS from July through Sept. of 1991 and 1992. Eighteen crosses were made with cultivars as female parents, and *A. sp.* 9990 as pollen donor. All plants were grown in clay pots. In each cultivar, four plants were cross-pollinated and two plants self-pollinated as control. The well-accepted emasculation and pollination methods were followed. Pegs were counted 7–10 days after pollination, and plants harvested 30–40 days after the cease of pollinations. Percentages of pegs (pegs produced per 100 pollinations), pods (pods produced per 100 pollinations), effective pegs (pods formed per 100 pegs), and normal pods (well developed pods per 100 pods) were calculated. At harvest, pods were dissected and ovules measured and cultured on MS media supplemented with various growth regulators. 3–5 weeks after culture initiation, ovules were dissected and embryos were examined. During the analysis of data expressed as percentage, inverse sine transformation was performed.

Results

Crossabilities between A. hypogaea L. cultivars and *A. sp.* 9990

Among all the 18 crosses, percentages of pegs, pods, effective pegs, and normal pods varied significantly. When cultivars were selfed, genotypic variations were also observed in the characters mentioned above (Tab. 1). Correlations between corresponding characters in selfs and crosses were analysed. The correlation coefficients for percentages of pegs, pods, effective pegs, and normal pods were 0.195, 0.143, 0.378, and 0.205, respectively, none of them was significant, indicating that variation among crosses do manifest the differences of crossability of the cultivars.

Statistical analysis showed that subspecies *fastigiata* maintained a significantly higher pod percentage than that of subspecies *hypogaea* when crossed with *A. sp.* 9990. Among the four basic botanical types, peg and pod productions in Valencia and Virginia types were significantly higher as compared with that in Peruvian type.

For all the characters, each cross was paired with its corresponding self. Analysis of the paired data revealed that percentages of pegs and pods were not affected in cross pollinations, but percentages of effective pegs and normal pods were significantly reduced. The growth of hybrid ovules and embryos was seriously retarded, and no viable seeds were obtained in all the crosses, indicating the existence of strong incompatibility barriers between *A. hypogaea* L. and *A. sp.* 9990.

Table 1. Results of cross-and self-pollination

Cultivar genotypes		Cultivars \times A. sp. 9990					Cultivars selfing				
Subsp.	Type	No. of polli- nation	Peg per- cent- tage	Pod per- cent- tage	Effec- tive peg %	Nor- mal pod %	No. of polli- nation	Peg per- cent- tage	Pod per- cent- tage	Effec- tive peg %	Normal pod %
Subspecies	Virginia type										
<i>hypogaea</i>	1. Kaifeng Dahuasheng	124	24. 2defg *	15. 3cd *	63. 3	10. 5	77	32. 5	31. 2	96. 0	
	2. Fengqiu Yibazhua	123	42. 3 bcd	28. 5 abc	67. 3	71. 4	57	24. 6	24. 6	100. 0	
	3. Gushi Bazihuasheng	155	41. 9 bcd	27. 1 abc	64. 6	42. 8	43	44. 2	41. 9	94. 7	
	4. Xihua Xiaozibai	138	50. 0 abc	34. 8 abc	69. 6	54. 2	52	57. 7	57. 7	100. 0	
	Peruvian type										
	1. Yongcheng Make	158	19. 0 fg	15. 2 cd	80. 0	87. 5	45	26. 7	26. 7	100. 0	83. 3
	2. Lankao SansiLi	145	29. 0 cdef	24. 8 abc	85. 7	83. 3	76	14. 5	14. 5	100. 0	90. 9
	3. Luojiang Jiwo	139	20. 9 efg	15. 1 cd	72. 4	47. 6	77	23. 4	19. 5	83. 3	53. 3
	4. Hainan Make	121	9. 1 g	4. 1 d	45. 4	20. 0	83	30. 1	24. 1	80. 0	75. 0
Subspecies	Spanish type										
<i>fastigiata</i>	1. Fuqing	129	42. 6 bcd	25. 6 abc	60. 0	66. 7	58	27. 6	20. 7	75. 0	75. 0
	2. Guangliu Zhenzhudou	129	25. 6 defg	18. 6 bcd	72. 7	79. 2	70	42. 9	31. 4	73. 3	72. 7
	3. Fuhuasheng	157	31. 9 bcdef	24. 8 abc	78. 0	64. 1	73	34. 3	30. 1	88. 0	77. 3
	4. Baisha 1016	181	27. 6 cdef	21. 0 bc	76. 0	55. 3	65	43. 1	41. 5	96. 4	59. 3
	Valencia type										
	1. Silihong	128	50. 8 ab	37. 5 ab	73. 8	66. 7	67	29. 9	26. 9	90. 0	94. 4
	2. Funing Duoli	139	36. 7 bcde	30. 2 abc	82. 4	42. 9	75	24. 0	24. 0	100. 0	94. 4
	3. UF 71513	122	65. 6 a	45. 9 a	70. 0	69. 6	47	29. 8	25. 5	85. 7	75. 0
	4. NCAC 17090	80	40. 0 bcdef	32. 5 abc	81. 3	69. 2	55	29. 1	21. 8	75. 0	50. 0
	Intermediate type										
	1. Yuhua 3	124	34. 7 bcdef	24. 2 abc	69. 8	50. 0	59	39. 0	35. 6	91. 5	95. 2
	2. Yuhua 4	91	29. 7 cdef	26. 4 abc	88. 9	95. 8	74	27. 0	27. 0	100. 0	95. 0

* There is no significant differences ($\alpha=0.05$) among combinations marked by at least one common letter.

The development of hybrid ovules and embryos

In the crosses, the growth of ovules was generally slow. About 20—30 days after pollination, ovules reached a length of 1.5—2.5mm (Tab. 2), only accounting for 20—30% of that of the normal seed, then they stopped developing. So there was no correlation between ovule size and its age (Fig. 1). Ovules of all crosses were dissected, but only those crosses with a larger number of ovules are tabulated here. The development of embryos was extremely abnormal. In more than 70% of the ovules dissected, no embryos or only a bit trace of degenerated embryos could be found. Even in those cases where embryo degenerations occurred later, the embryo differentiation was also very poor. Most embryos were found to be amorphous or globular. Variations among crosses and even among individual embryos in the same cross were observed. In crosses involving Silihong and Fengqiu Yibazhua as female parents, embryo development was much better than other crosses.

Table 2. Ovule length and embryo status in *A. hypogaea* L. × *A. sp.* 9990

Cultivar × <i>A. sp.</i> 9990	No. of ovules measured	Percent of ovules with length (mm)			No. of ovules dissected	Percent of ovules of different status				
		>2.5	2.5–1.5	<1.5		Glo-bular	Tor-pedo	Heart-shaped	Coty-ledon	Degene-rated
Kaifeng Dahuasheng ×	14	7.1	21.5	71.4	14	14.3	0.0	0.0	0.0	85.7
Fengqiu Yibazhua ×	47	55.3	31.9	12.8	47	19.1	0.0	4.3	2.1	74.5
Gushi Bazihuasheng ×	50	2.0	22.0	76.0	50	20.0	2.0	0.0	0.0	78.0
Xihua Xiaozibai ×	66	0.0	22.7	77.3	66	27.3	0.0	0.0	0.0	72.7
Yongcheng Make ×	63	7.9	73.1	19.0						
Lankao Shansili ×	73	17.8	74.0	8.2						
Luojiang Jiwo ×	36	8.3	63.9	27.8						
Hainan Make ×	6	0.0	83.3	16.7						
Fuqing ×	37	2.7	73.0	24.3						
Guangliu Zhenzhudou ×	35	0.0	91.4	8.6						
Fuhuasheng ×	58	15.5	84.5	0.0						
Baisha 1016 ×	66	7.5	80.4	12.1						
Silihong ×	133	15.0	70.0	15.0	104	23.1	3.8	1.0	0.0	72.1
Funing Duoli ×	91	0.0	42.9	57.1	85	9.4	0.0	0.0	0.0	90.6
UF 71513 ×	110	13.6	67.3	19.1	95	2.1	0.0	0.0	0.0	97.9
NCAC 17090 ×	66	4.5	28.8	66.7	66	0.0	0.0	0.0	0.0	100
Yuhua 3 ×	44	15.9	70.5	13.6						
Yuhua 4 ×	44	45.5	45.4	9.1						

The situations of embryo abortion observed in the present study could be roughly divided into three types based on the morphology of ovule and embryo development. First, earlier embryo abortion. In this case, when embryo degeneration occurred, ovules could expand further before they completely stopped developing. So a cavity was usually found in the embryo sac. The cross Luojiang Jiwo × *A. sp.* 9990 belonged to this type. Second, synchronous degeneration of ovules and embryos. The embryo development was in harmony with ovules. At degeneration, ovules were usually plump, and the structure of embryo sac was clear. Silihong × *A. sp.* 9990 and Fengqiu Yibazhua × *A. sp.* 9990 represented this type. Third, excessive growth of the integument. The ovules usually grew rapidly, simultaneously, the inner layer of the integument expanded inward and finally filled up the embryo sac. The developing embryos were suppressed to collapse, as in the case of UF 71513 × *A. sp.* 9990.

Discussion

Differences of A. hypogaea L. cultivars in their crossabilities with *A. sp.* 9990

It is probably the first time that the crossabilities between five botanical types of *A. hypogaea* L. and *A. sp.* 9990 were evaluated on a large scale. Although all the 18 cultivars are

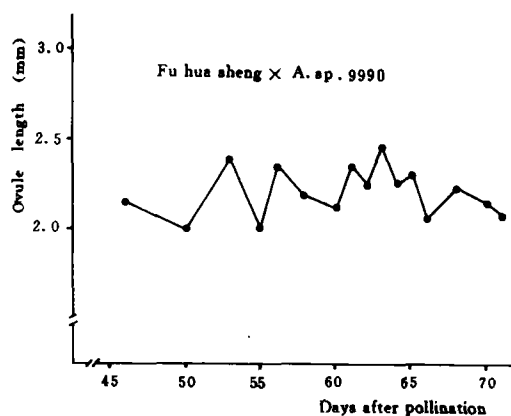


Fig. 1. Development of hybrid ovules

incompatible with *A. sp.* 9990, significant differences among subspecies, botanical types and genotypes do exist. These results indicate that through extensive screening, the genotypes having higher crossability or less incompatibility with *A. sp.* 9990 could probably be obtained. The use of such genotypes in hybridization program will certainly reduce the difficulties of desirable gene transfer. Among the 18 cultivars, Silihong and Fengqiu Yibazhua were the most acceptable female parents by showing higher pod production, later embryo abortion, and higher level of embryo differentiation that occurred sporadically. Hybrid ovules and embryos from these two cultivars also gave a better response to media during *in vitro* culture, from which calli have been obtained. The differentiation of calli is still under-way.

Incompatibility barriers between A. hypogaea L. and A. sp. 9990

The process from pollination to seed formation is quite complex in seed plants. It is much more complicated in the genus *Arachis* where seed formation has two phases. In the aerial phase, dominated by geotropic elongation of the gynophore (peg), the preembryos formed after first few divisions cease to grow, and the development is resumed only after the peg has entered the subterranean phase (Smith, 1956). The peg formation should be an important sign of successful fertilization. In the present investigation, peg setting in all crosses was fairly normal, indicating that prefertilization incompatibility barriers between *A. hypogaea* L. and *A. sp.* 9990 do not exist. The most serious incompatibility barrier is early embryo abortion as evidenced by pod and ovule dissections. In the three types of embryo abortion, the excessive growth of the integument was similar to that reported by Johanson (1956). The reports of the other two types have not been seen yet. Further studies on the exact time when embryo abortion takes place, and physiological and biochemical mechanisms behind the abortion are apparently needed.

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