Hybridization of Peanuts in Growth Chambers[1]

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ABSTRACT

Hand crosses in peanuts require much time and generally are done by emasculating the flowers late in the evening or at night, and then pollinating the plant the next morning when pollen is available. A new method, in which a growth chamber is operated on a reversed day-night schedule (12-hour, 29 C day commencing at 4:30 p.m. and 12-hour, 21 C night commencing at 4:30 a.m. CST), allows the emasculations to be made between 8:00 and 10:00 a.m. and the pollinations to follow immediately. This method permits crossing to be done during normal work-day hours and allows up to three crossing cycles and growth generations per year. In 1971 and 1972 tests of this method showed more than 50% success in achieving viable hybrids per pollination.

Additional index words: **Arachis hypogaea,** Emasculation, Genetic markers, Groundnuts, Hybrids, Pollination, Seed dormancy.

Conventional techniques for making hand crosses in peanuts are tedious and time-consuming, and the crosses generally must be made during other than normal work hours. Recently, Norden (1973) reviewed peanut breeding procedures. Norden and Rodriguez (1971) modified some of the conventional crossing procedures of Stokes and Hull (1930) to achieve greater hybridization success. They reported that 70-90% of the pollinations resulted in fertilization with their techniques, whereas those of Stokes and Hull gave only about 50% success. Conventionally, crossing is done in a greenhouse and consists of making emasculations in the evening followed by pollinations the next morning between 7:00 and 10:00 a.m. Norden and Rodriguez placed moistened paper towels above the flowers after the pollinations which probably accounted for much of the success of their method. Earlier, Schultz (1947) used a wetted cheesecloth chamber to increase the humidity in his experiments. Lee, *et al.* (1972) showed that high humidity in plant growth chambers also was beneficial for the developing pods of naturally self-pollinating peanut plants.

To identify the crosses that they had made on a given day and to aid record keeping, Norden and Rodriguez (1971) tagged the flowers and pegs with color-coded strings and wires. This procedure permitted them to harvest the hybrid pods individually (without harvesting the whole plant) a given number of days after the pegs were tagged. They indicated that in their area (Gainesville, Florida) late winter and early spring are conducive to hybridization success in the greenhouse. Hildebrand (1974), working in Rhodesia, controlled bud development of peanuts in a greenhouse by extending the day length 8½ hours (5:00 p.m. to 1:30 a.m.) with artificial lights. He reported that the plants flowered profusely and that buds could be emasculated successfully from 7:30 to 11:30 a.m. following the light treatment.

For several years I have been using a system of crossing peanuts which involves the use of plant growth chambers. The method allows crossing to be done throughout the year and during normal working hours.

Materials and Methods

The peanut cultivars used as parents for the 1971 and 1972 crossing programs are shown in Table 1. Parents with genetic markers were chosen to distinguish the hybrids. For the cultivars used in this study dominant characters were: Virginia growth habit, purple stems, krinkled leaves, narrow leaflet, and coarse stems. Fungicide-treated seeds were planted, one per pot, in September both years. Maternal parents were planted in 33 and 18-cm plastic pots in 1971 and 1972 respectively in a fertilized potting medium consisting of equal parts of sand, soil, perlite, peat, and vermiculite. Pollen parents were planted in 15-cm clay pots in a sandy loam soil and grown in a fiberglass greenhouse at 21 to 29 C. The maternal parents were grown in growth chambers with a 12-hour, 21 C night and 12-hour, 29 C day regime. The day and night schedules began at 4:30 p.m. and 4:30 a.m. CST, respectively. This "reverse" regime results in flower buds which are near optimum for emasculation during the mornings at Stillwater, Oklahoma.

When the maternal plants began blooming, the flowers were removed daily until the plants were producing several flowers per day. Then the crossing cycle was started and continued daily until several pollinations were made on each plant. One plant was used as the maternal parent for each cross combination. The emasculation techniques

[1]Cooperative investigations of the Southern Region, Agricultural Research Service, U.S. Department of Agriculture, and the Oklahoma Agricultural Experiment Station, Oklahoma State University, Stillwater, Oklahoma 74074. Journal Article No. 3088. Oklahoma Agricultural Experiment Station.

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Table 1. Phenotypic Descriptions of Peanut Cultivars used in the Growth Chamber Crossing Trials.

Cultivar	Okla. P-No[1]	P.I. No.	Botanical type	Characteristics
Apaxuc	2398	268661	Spanish	Short, compact, early maturity
Aureus	1284	--	Spanish	"Goldenleaf" mutant
F-416	1452	--	Virginia	Runner-type
Guanajuato-2	326	280688	Virginia	Purple stems, late maturity
Krinkle II	291	--	Spanish	Wine seed, "krinkled" leaves
Mani Pintar II	935	268837	Virginia	Red and white mottled seeds
Narrowleaflet	1286	--	Spanish	Dwarf, very small leaflets
Spanhoma	112	--	Spanish	Typical improved Spanish
Peru	936	262129	Spanish	Large leaves, coarse, Valencia

[1]P-numbers assigned by the Oklahoma Agricultural Experiment Station.

are similar to those used by Norden and Rodriguez 1971), except that the flower petals were not removed in my procedure. Between 8:00 and 9:30 a.m., plants were removed from the chamber, placed on a nearby table with adequate overhead lighting, emasculated, and immediately pollinated with pollen from flowers taken from greenhouse plants. Only one pollen genotype was applied to flowers of each maternal parent, thereby eliminating the necessity of tagging flowers and pegs. After pollination, the plants were returned to the chamber and the floor was sprinkled with water to provide additional humidity. The plants were watered in the late afternoon to avoid possible disturbance of the pollinated flowers.

After the crossing cycle was completed flowers were removed daily between 8:00 and 9:30 a.m. for at least 3 weeks to prevent natural selfing of these later flowers. Plants were grown in the growth chambers for a least 60 days after the last pollination before being harvested. At harvest, the plants were removed from the potting medium and the pods were removed by hand, washed, and air dried in small cloth bags hung in the greenhouse for 2-3 weeks. Pods were shelled by hand and the seeds were stored at room temperature until they were planted in the field in early June each year. During the growing season, the resulting plants were counted and the number of hybrids were recorded.

Results and Discussion

Results of the crossing attempts in 1971 are shown in Table 2. Interruption of the dark period during crossing did not appear to influence flowering response in general but it will be noted that the numbers of pollinations differed among plants because of variations in flowering of the individual plants. Reasons for the flowering variation are not known. The ratios of hybrids achieved to pollinations accomplished also varied (0.38 to 0.89). The day and night temperature regimes used in this study were based on observations of favorable growth and flowering responses in growth chambers in other peanut experiments

Table 2. Results of Peanut Crossing Trials in Growth Chamber (33-cm pots), 1971.

Cross No.	Parents ♀	Parents ♂	Pollinations	Period of Pollinations (Days)	Seed Harvested	Germ. (%)	Hybrids (%)	Hybrids / Pollinations
71-2	Aureus	Guanajuato-2	61	12	28	89	92	.38
71-3	Aureus	Mani Pintar II	28	10	18	100	94	.61
71-5	Spanhoma	Mani Pintar II	36	13	36	89	100	.89
71-6	Spanhoma	P-936	27	17	21	81	100	.63
71-7	Mani Pintar II	P-936	35	17	30	87	65	.49
71-9	Tifspan	Mani Pintar II	51	9	36	97	97	.67
		Mean	40	13	28	91	93	.59

(unpublished). General health and vigor of all plants was good. Apparently, genotypes differ in their ability to function effectively in fertilization either as males or females. Schultz (1947) attributed some of the differences in crossability to flower accessibility and suggested flower injury as a probable factor, but he also indicated some of the differences might be genetic. Because my trials were not replicated, good analyses cannot be made in regard to these possibilities.

Results for 1972 are shown in Table 3. The mean pollinations attempted per plant were fewer than in 1971 (29 versus 40) and more days (16 versus 13) were required to achieve the pollinations. These differences are probably due, in part, to the slightly less vigorous plants grown in the smaller pots in 1972. The larger pots apparently provided a more optimum rooting and pegging environment and the plants looked healthier. The smaller pots were used mainly because they were more easily handled and required less chamber space.

Germination percentage of the seeds planted in the field was lower in 1972 than in 1971 (77 versus 91). Part of the differences might be due to seed dormancy, particularly in crosses involving Guanajuato-2 and F-416 which we know impart some dormancy in certain crosses. We did not use any dormancy-breaking procedures as suggested by Toole, *et al.* (1964). In spite of differences in germination in the two years, the percentages of hybrids achieved and the hybrids/pollinations ratios of the two years were similar. However, considerable selfing took place occasionally in both years (see 71-7, 72-4, 72-9, 72-11). These unwanted selfs detract from the successfulness of the methods used. These apparent selfs are difficult to eliminate from a crossing program but they are not of much importance when they can be distinguished readily from the hybrids by genetic markers. Earlier published works on peanut breeding generally have not provided sufficient information on the numbers of selfs to allow suitable comparisons to be made. The apparent selfs in these experiments may have been due to one of several causes: failure to remove the flowers (either before the crossing cycle was started or after it was ended) early enough to preclude selfing; overlooking occasional flowers during the crossing or flower pulling cycles (flower buds are sometimes hidden in the leaf axiles, or covered by the soil at the plant base); or the use of plants for pollen sources that were heterozygous for the appropriate dominant genetic markers. This last possibility is not remote when field seed sources are used in the crosses. Natural crossing estimates in the field have varied between 0.73% and 2.56% in Georgia (Leuck and Hammons, 1969). The possibility of apomixis cannot be overlooked, although it is yet to be reported in peanuts.

Because no tags or strings are used with this method for identifying crosses, considerable time is saved. Tagging is inconvenient and unnecessary except for very critical crossing experiments. However, tagging may reduce the number of harvested selfs. The number of days required to complete each phase of the crossing cycle is approximately as follows: planting to flowering, 25-35; crossing, 10-14; flower removal, 21; last pollination to harvest, 60-80; curing, 7-14 days. The crossing cycle should be completed as quickly as possible once it is started so that the subsequent harvest will yield pods of fairly uniform maturity. If the flowers are removed daily from the mater-

Table 3 Results of Peanut Crossing Trials in Growth Chamber (18-cm pots), 1972.

Cross No.	Parents ♀	Parents ♂	Pollinations	Period of Pollinations (Days)	Seed Harvested	Germ. (%)	Hybrids (%)	Hybrids / Pollinations
72-1	P-936	Narrowleaflet	25	20	20	60	100	.48
72-2	P-936	Guanajuato-2	17	17	15	53	100	.47
72-3	Mani Pintar II	Narrowleaflet	26	22	13	62	100	.31
72-4	Mani Pintar II	Guanajuato-2	19	20	18	44	63	.26
72-5	Spanhoma	Krinkle II	27	8	31	81	84	.78
72-9	P-936	Krinkle II	26	12	21	86	61	.42
72-11	Mani Pintar II	Krinkle II	25	15	24	92	59	.52
72-13	Apaxuc	Narrowleaflet	32	15	23	100	96	.69
72-14	Apaxuc	F-416	43	16	34	68	100	.54
72-15	Apaxuc	P-936	48	14	25	100	96	.50
		Mean	29	16	22	77	85	.51

nal parents for at least 3 weeks after the last pollination, the subsequent late-formed selfs will be sufficiently immature to be distinguished from the putative hybrid pods. By removing all remnants of flower parts (remaining from the previous pollinations) from the plants after the pollinations are stopped, new flowers are more easily detected, especially those near the plant crown. Because of space restrictions, I use Spanish cultivars as maternal parents when possible. These plants are small, provide most of their pods near the plant base, and hybrids are usually easy to detect. With these cultivars, the 18-cm pots are generally sufficient. However when using the Virginia botanical type or wild species for maternal parents large pots are essential.

By using these procedures, one can complete three crossing cycles per year. If seed dormancy is a factor, ethephon can be used to speed germination (Bailey and Bear, 1973). Because growth chamber space is generally limited, it is advantageous to start seedlings in pots in the greenhouse and transfer these to growth chambers in about 20 days. Such plants require only 3-4 days to adjust their flowering and pollen shedding habits to the new environment. Likewise, after the crossing and flower removal cycles have been completed, the plants can be transferred back to the greenhouse for the seed maturation period.

The procedures described here for making hand crosses in peanuts allow the work to be accomplished during regular hours and even more important, they help to distribute the crossing work load more uniformly throughout the year. Elimination of flower and peg tagging speeds the procedure considerably, but all subsequent flowers must be removed daily to eliminate unwanted selfs.

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