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Comparison of Two Methods of Hand-crossing *Vigna unguiculata* (L.) Walp[1]

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Abstract **Five lines of cowpea were crossed in a complete diallel, using 2 methods. Crossing success rate was greatly enhanced by collecting pollen donor flowers on the morning of anthesis, storing them until late afternoon, and then completing the cross on freshly emasculated flowers (method 1) as compared to utilizing pollen donor flowers for crossing immediately upon collection, early to mid-morning on the day of anthesis (method 2). Significant differences in success rate were found among cultivars used as seed parents in method 2, while no such differences were observed in method 1. Significant differences in success rate were found among cultivars used as pollen parents in both methods.**

Hybridization of cowpea involves time-consuming hand-emasculation and cross-fertilization, which often results in abortion of the cross (1, 4, 7, 8). Causes of abortion can include excessive manipulation of the seed parent flower, high temperature during and immediately following emasculation and pollination, low humidity leading to desiccation, inviable pollen, and incompatibility (1, 3, 5, 7, 8).

Optimum time of day for crossing is a major consideration in effectively conducting a cowpea hybridization program. Makie (4) alluded to time of day and possibily high temperature as reasons for failure of cowpea crosses at Davis, Calif., and suggested that all crosses be completed before 0830 hr. Oliver found evening emasculation followed by morning pollination to be ideal (6). Kheradnam and Niknejad (2) recommended morning crossing in Iran, while Sen and Bhowal (9) suggested evening emasculation followed by morning pollination. Rachie et al. (7, 8) stated that crosses can be accomplished either by evening or morning emasculation followed by morning pollination or by evening emasculation and pollination. They also suggested that cowpea pollen viability can be maintained under refrigeration, and that genotypes will perform differently as pollinators. However, no data has been presented to support these statements.

A comparison of 2 methods of hand-crossing cowpeas was conducted at College Station, Texas, in the fall of 1977, in an effort to optimize cowpea hybridization and to document pollen viability following storage and genotype differences. In both methods, 4 cowpea cultivars and 1 breeding line ('Brown Crowder', 'Calico Crowder', 'Knuckle Purple Hull', 'Bush Purple Hull', and TX 460) were grown in a glasshouse under a temperature regime of 18°C night and 28° day. Seed were sown in 4-liter black plastic pots in a soilless medium consisting of 1 sphagnum peatmoss: 1 sand: 1 vermiculite (by volume). The medium was amended with dolomite, gypsum, and inorganic fertilizer to ensure adequate growth. Forty plants of each cultivar were arranged in a 4-replicate randomized complete block design and crossed in a complete diallel.

In method 1, crosses were accomplished by utilizing open flowers of the pollen parent and 1-day pre-anthesis buds of the seed parent. The seed parent was prepared by separating the standard with forceps along the suture and then unfolding back along the wings of the bud. The keel was removed with forceps, exposing the stamens and stigma. Stamens were also removed with forceps. Following each emasculation, the forceps were dipped in 95% alcohol to ensure maintenance of sterile conditions. Flowers from the pollen parent were picked on the morning of anthesis. Pollen, collected in the keel, was squeezed out and transferred directly to the stigmatic surface of the emasculated flower. The wings and standards of the seed parent were then returned to the closed position and secured with a strip of transparent tape to prevent desiccation and contamination. A 2 × 3 cm crossing label, marked with each parent, was attached to the peduncle on which the flower was born (1). All emasculations and pollinations in method 1 were accomplished between 0700 and 1100 hr.

In method 2, crossing was similar to method 1, except that flowers from the pollen parent were picked early (0800 hr) on the morning of anthesis and stored in prelabeled plastic foam cartons at 3 to 4°C and 90–95% relative humidity until late afternoon (1600 hr). Pollen from these flowers was then used to pollinate freshly emasculated pre-anthesis buds, as previously described.

A comparison of results using the 2 methods is presented in Table 1. Within crossing method 1, significant differences were found among pollen parents used with a specific female. For example, 'Bush Purple Hull' was most compatible with pollen parent TX 460 and least compatible with 'Knuckle Purple Hull'. When 'Brown Crowder' was the seed parent, 'Bush Purple Hull' was the most compatible, while 'Knuckle Purple Hull' was the least compatible pollen parent. Similar results were found when 'Calico Crowder' and 'Knuckle Purple Hull' were used as seed parents. In all situations, 'Bush Purple Hull' was the most compatible pollen parent. In method 2, significant differences were also found with pollen parents crossed to a specific female. However, these differences were generally less pronounced than in method 1, ranging from no significant differences for pollen parents crossed to 'Bush Purple Hill'

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to considerable variation for pollen parents crossed to TX 460. Within crossing method 1, significant differences were also found among cultivars used as seed parents. 'Knuckle Purple Hull' yielded the greatest seed set (35%), which was significantly greater than other seed parents. With method 2, no significant differences were found among seed parents, although 'Calico Crowder' and 'Bush Purple Hull' tended toward a greater percentage of seed set. Reciprocal differences were also observed. For example, within method 1, 'Bush Purple Hull' x TX 460 yielded a success rate of 44%, while the reciprocal was 51% successful. Reciprocal effects were also observed in method 2.

Method 2 was significantly better than method 1 for every cross. For example, 'Brown Crowder' in method 1 yielded an overall seed set of 24%, while in method 2 the mean was 56%. Similarly, seed set was 24% vs. 56% for TX 460 and 26% vs. 59% for 'Calico Crowder'.

Our results illustrate that differences in artifical cross-pollination success rate exist among cowpea genotypes when used as pollen and/or seed parents and indicate the superiority of method 2 (late afternoon emasculation and pollination) for crossing under conditions exemplified by warm (>25°C.) and dry (<50% relative humidity) conditions (southwestern United States). The differences in success rate between the 2 methods could be attributed to the maintenance of excellent viability in the stored pollen and cooler, more humid conditions during and immediately following late afternoon vs. morning pollination. The late afternoon conditions offer a more suitable environment for manipulation of the flower of the female parent and growth and development of pollen on the stigmatic surface, as severity of the late morning and early afternoon heat and lack of humidity is avoided. It also may be possible that the pre-anthesis stigmatic surface is more receptive to pollen in the late afternoon vs. early morning.

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Table 1. Pollination success rates among and between parents utilizing 2 methods of bud pollination.[z]

Parent		Method 1			Method 2		
Seed	Pollen	No. pollinations	No. set	Success (%)	No. pollinations	No. set	Success (%)
Bush Purple Hull	Brown Crowder	86	19	22a[y]	93	59	63a
	Calico Crowder	96	12	12a	70	36	51a
	TX 460	81	36	44b	86	52	60a
	Knuckle Purple Hull	64	9	12a	72	41	57a
	Total	327	76		321	18	
	Mean	82	19	23a[x] a[w]	80	47	59a b
Brown Crowder	Bush Purple Hull	50	17	34c	78	51	65b
	Calico Crowder	86	21	24b	63	38	60b
	TX 460	38	9	24b	84	48	57b
	Knuckle Purple Hull	58	6	10a	60	26	43a
	Total	238	58		257	146	
	Mean	59	14	24a a	64	36	56a b
TX 460	Bush Purple Hull	43	22	51d	81	58	72b
	Calico Crowder	68	19	28c	70	38	54a
	Brown Crowder	76	8	10a	42	19	45a
	Knuckle Purple Hull	51	9	18b	64	31	48a
	Total	238	58		257	146	
	Mean	59	14	24a a	64	36	56a b
Calico Crowder	Bush Purple Hull	60	23	38b	71	42	59b
	TX 460	81	16	20a	60	39	65b
	Brown Crowder	90	22	24a	78	40	51a
	Knuckle Purple Hull	42	13	31a	52	31	60b
	Total	273	74		261	152	
	Mean	68	18	26a a	65	38	59a b
Knuckle Purple Hull	Bush Purple Hull	59	26	44b	69	46	67b
	Calico Crowder	36	13	36a	42	26	62b
	TX 460	70	21	27a	42	26	62b
	Brown Crowder	41	11	30a	97	46	47a
	Total	206	71		286	149	
	Mean	51	18	35b a	71	39	52a b

[z]Method 1 utilized pollen from flowers collected in the early morning, with bud emasculation and pollination following immediately, while method 2 used pollen from flowers collected in early morning and stored until late-afternoon emasculation and pollination.

[y]Means percent success rate of the 4 replicates separated in columns within each parent for each crossing method by Duncan's multiple range test, 5% level.

[x]Means separated in columns for each crossing method by Duncan's multiple range test, 5% level.

[w]Means separated in rows between methods by Duncan's multiple range test, 5% level.

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