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A comparison between emasculated and non-emasculated artificial hybridisation methods with *Phaseolus vulgaris* L.

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Summary

Complete emasculation and non-emasculation methods of artificial hybridisation were compared. Higher pod set was obtained with non-emascualtion, although frequency of self-pollination was higher. The number of hybrid plants per pollination was slightly higher with non-emasculation, although differences in operator proficiency using complete emasculation was a compounding factor. Cultivar effects may differentially influence the success rates for the two hybridisation methods.

INTRODUCTION

Information on the effectiveness of different hybridisation methods was required when planning the new navy bean breeding programme. Two methods described by Bliss (1980), complete emasculation and non-emasculation were used. Pollination in each method is achieved by inserting the detached pollinated stigma from male flowers onto the female stigma. Hybrids should be more easily obtained with non-emasculation because of the relative ease of the method, which should reduce floral damage and subsequent abortion.

Successful pollination rates of 70 to 90% pod set, with 4 to 6 seeds per pod, have been obtained by 'hooking' pollinated stigmas onto female stigmas protruded through the keel (Buishand 1956; Bliss 1980). In the present study an incision was made in the non-emasculated female keel through which the pollinated stigma was inserted.

MATERIALS AND METHODS

Advanced navy bean lines and other beans chosen from the Queensland Department of Primary Industries germplasm collection as parents (Redden *et al.* 1985) were serially sown in deep beds of soil in a glasshouse at Hermitage Research Station during May and June in 1983 and 1984. The glasshouse was heated at night to maintain temperatures above 15°C.

Artificial hybridisation was conducted by two operators from late July to early September. In 1983 both operators used the complete emasculation methods whereas in 1984 Operator B also used the non-emasculation method in an attempt to increase the recovery of hybrids. Peduncles of the female flowers were tagged to indicate emasculation method, parents, date and operator for all manual pollinations. All tags were recovered including those from aborted flowers and each hand pollinated pod was harvested and threshed separately.

Plots of each pod progeny were planted in October 1984. The occurrence of self-pollinated plants was recorded at flowering and harvest using the characters flower colour, seed colour and seed type to identify hybrid and selfed progeny. All female parents of non-emasculated pollinations had white flowers and seeds which are recessive characters (Prakken 1970) and male parents had coloured flowers and seeds. Selfed progenies had white seed and flowers whilst hybrid progenies had coloured flowers and seeds.

RESULTS AND DISCUSSION

The level of abortion was substantially reduced with the non-emasculated technique (50%) compared with emasculation (78%) (Table 1). Number of seeds per pod, however, was similar for each method. The number of hybrid plants obtained per pollination was significantly higher for non-emasculation as too was the proportion of self-pollination.The germination of hybrid seed appeared to be similar for each method.

Table 1. Summary of hybridisations attempted in *Phaseolus vulgaris* 1984

	Emasculated	Non-emasculated	χ^2_1
No. of pollinations	340	123	26.23***¶
No. of pods set	74	62	131.98***¶
Total seed set	307	272	
Average seeds pod†	4.1	4.4	
F_1 hybrid plants	143	76	8.73**††
Selfed plants	3‡	51§	
No. of hybrid plants/ total pollinations	0.43	0.62	

†Standard deviations of 1.8 and 1.7 respectively for emasculated and non-emasculated.

‡Includes 1 wholly selfed pod + 1 partly selfed pod.

§Includes 12 wholly selfed pods + 8 partly selfed pods.

¶χ^2_1 calculated on mean expectation per total pollinations.

*** = significant at 0.1% level.

††χ^2_1 calculated on mean expectation per total seed set.

** = significant at 1% level.

Operator proficiency is obviously important, since Operator A was better than Operator B using the emasculated method in both years. Operator B had substantially higher hybridisation success with non-emasculation but the case for adopting the method is less clear for Operator A. The percentage pod set without emasculation was lower than reported by Bliss (1980).This may have been due to cutting the female keel rather than to protrude the female stigma to allow 'hooking' with the pollinated stigma.

	Emasculated		Non-emasculated
	Operator A	Operator B	Operator B
1983 % pod set	29.8	15.4	
1984 % pod set	25.3	9.3	50.4
1984 % selfed	2.1 (A + B)		40.2

(1983 A:B χ^2_1 = 3.97 using Yates correction for continuity (all χ^2 pair comparisons, Little and Hills 1972) is significant at the 5% level.

1984 A:B χ^2_1 = 3.5 significant at the 5% level.

Operator A 1983:1984 χ^2_1 = 0.227, not significant.

Operator B 1983:1984 χ^2_1 = 1.05, not significant.

Emasculated − A + B × years χ^2_3 = 13.02 significant at the 1% level.

1984 Operator B emasculated:non-emasculated χ^2_1 = 26.93 significant at the 0.1% level.

Selfs − emasculated:non-emasculated χ^2_1 = 32.54 significant at the 0.1% level.

All available weather data from the Bureau of Meteorology's weather observation station no. 041044 based on the Hermitage Research Station was checked for an association with pod set, but none was found. Many factors might be expected to influence abortion such as moisture regime in relation to temperature, position in the glasshouse, age of plants, etc. The possible existence of different cultivar reactions to hybridisation methods

is suggested by Table 2 but this would need to be addressed in a better controlled environment than the glasshouses used.

Table 2. Pod set of the most frequently used parents for two hybridisation techniques. Proportion and percentage of pods set per total pollination

	Parent	Emasculation	Non-emasculated	χ^2_1 using Yates' correction for continuation
Female	Kerman	1/9 =11.1%	6/7 =85.7%	27.98***
	Actolac	10/47=21.3%	8/12 =66.7%†	22.4***
	Revenue	18/60=30.0%	7/12 =58.3%†	8.44**
	Sel^n 46	6/32 =18.7%	4/12 =33.3%	3.56#
	Campbell 11	2/19 =10.5%	17/27=63.0%†	36.08***
	Campbell 16	2/19 =10.5%	8/17 =47.1%	22.0***
	Campbell 18	5/33 =15.2%	15/21=71.4%†	35.18***
		$\chi_2$6=15.6**	$\chi_2$6=32.76***	
Male	Kerman	0/11 =0%	11/22=50%	5.29*
	Bac 57	2/7 =28.6%	11/22=50%	5.29*
	Antioquia 23	3/10 =30%	5/18 =27.8%†	0.02n.s
	PI 207263	1/5 =20%	10/21=47.6%†	10.46***
	CPI 95894	1/7 =14.3%	7/10 =70%†	35.49***
	CPI 95875	4/16 =25%	5/6 =83.3%†	30.32***
	GO 3974	2/11 =18.2%	0/5 =0%	16.25***
	Puebla 152	0/12 =0%	4/8 =50%	48.02***
		χ^2_7=66.96***	χ^2_6=94.6***	

†2 or more pods with selfed seed.

‡Level of significance 10%, 5%, 1% and 0.1% indicated respectively by #, *, **, and ***.

The advantages of the non-emasculation method would appear to be; a higher percentage of hybrids per pollination, less time spent per pollination and a relatively favourable response of some cultivars with this method compared with emasculation.

The disadvantages of non-emasculation are; a higher percentage of selfed seed, and parents must have different agronomic characteristics for positive detection of self-pollinations.

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