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THE INHERITANCE AND EXPRESSION OF STERILITY IN HYBRIDS OF DIHAPLOID AND CULTIVATED DIPLOID POTATOES

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When dihaploids of European *Solanum tuberosum* are used as female parents in crosses with South American cultivated diploid potatoes (Group Phureja/Stenotomum), various kinds and degrees of male sterility are found in the offspring. The effect of using different dihaploid and cultivated diploid parents on shrivelled microspore sterility of F_1 hybrid progenies was studied. Variation in the character was continuous and statistical analyses showed high general combining ability for dihaploid parents but not for cultivated diploids. A significant but non-linear relationship was found between percent of stainable pollen and seed set in crosses with female tester parents, provided that some degree of functional male fertility was present. F_1 clones with pollen of normal appearance but with no functional fertility probably represent a hitherto unclassified cytoplasmic male sterility. The results are discussed from the point of view of methods to be adopted in improving potatoes at the diploid level.

Introduction

Certain of the earlier suggestions for the use as breeding material of dihaploids derived from *Solanum tuberosum* cultivars (cp. CHASE, 1963) have met with difficulties arising from the low fertility of such plants. An initial dihaploid progeny is approximately equivalent to the third generation of selfing at the level of the tetraploid parent (HOUGAS & PELOQUIN, 1958). Inbreeding depression may be partly responsible for sterility, so that fertility should increase when crosses are made between diverse dihaploids (VAN SUCHTELEN, 1966). Dihaploid breeding is subject to additional limitations since relatively few Tuberosum clones provide male-fertile dihaploids and these form a small proportion of the initial progenies (see ROSS et al., 1964). A feasible route for dihaploid utilization was demonstrated by HOUGAS & PELOQUIN (1960) who crossed dihaploids with diploid species. Some of these were forms of primitive cultivated potatoes e.g. *S. ajanhuiri*, *S. goniocalyx*, *S. phureja*, *S. stenotomum*, and the way seemed clear for the breeding of edible potatoes at the diploid level. However, when attempting to apply this method, ROSS et al. (1964) apparently encountered male fertility problems in hybrids with dihaploid Tuberosum seed parents (see also HOUGAS & PELOQUIN, 1961). They advocated the use of male fertile dihaploids as pollen parents only, relying on a wide range of Phureja and Stenotomum seed parents to

introduce genetic variability. Figures were presented amply confirming the fertility of such hybrids.

Rather little has been published on fertility of progenies of which a dihaploid Tuberosum was the seed parent in crosses with edible diploids. DE LA PUENTE & PELOQUIN (1968) stated that only one sixth of such progenies would be fertile, but no supporting data were given for the F_1. HOWARD (1970) studying one cross and its reciprocal, obtained male sterile progeny when the dihaploid was the female but male fertile progeny when it was the male; crossing male-fertile hybrids with other dihaploids showed that the latter would not all produce male sterile offspring. GRUN (1970a) quoted male sterility data for three dihaploid × Phureja progenies and one reciprocal. Male fertility (defined as anther dehiscence and at least 50% stainable pollen) was low or absent when the dihaploids were seed parents. SOSA & HERNANDEZ DE SOSA (1972) gave berry and seed-set data for hydrids of secondary dihaploids (2x Tuberosum × 2x Andigena) ♀ with Phureja ♂ in an investigation of incompatibility. Their results suggested that factors other than S_i alleles were mainly determining fertility which varied greatly from clone to clone. Perhaps the most complete account to date is that of LIBERAL (1966), who gave fertility figures for five F_1 progenies involving two different dihaploids used as females. Both male and female fertilities were lower than those of progenies for dihaploid males, but there was much variation. Continuation of three of the families into the F_2 led to diminution of male and female fertility in two of them.

When a breeding programme was initiated at Pentlandfield using hybrids from dihaploid × cultivated diploid crosses, the number of male-fertile Tuberosum dihaploids was limited, whereas there was a reasonable supply of female fertile clones. As the success of the programme would depend on width of the genetic base as much as on fertility, the decision was taken to include progenies with dihaploid seed parents. This paper presents fertility data, particularly on male fertility, for such hybrids drawn from the breeding scheme, and may help to clarify the extent to which the reciprocal fertility difference, generally explained in terms of dominant major genes and cytoplasmic factors for sensitivity (e.g. GRUN, 1970a, b), is likely to hamper the breeder.

Materials

The primary dihaploids used as parents in this study had the following origins:

(i) from Tuberosum cultivars: HC29 ex "Roswitha;" HC32 and HC56 ex "Majestic;" HC46 ex "Gladstone;" HC161 ex "Maris Piper;" HC162 ex "Pentland Crown;"

(ii) from unnamed breeder's clones: HC35 and HC142 from eelworm resistant clones containing an Andigena genetic contribution in Tuberosum cytoplasm; HC193 ex 6061 b 1 (J. M. Dunnett).

Secondary dihaploids were raised from seed kindly supplied by Dr. H. W. Howard of P.B.I. Cambridge, England and Dr. J. Th. Hermsen of I.V.P., Wageningen, Holland. The Cambridge material, numbered 0117/DH/CB and 0118/DH/CB, had the cultivars "Majestic", "Chippewa", "Maris Piper" and "Ulster Knight" in its ancestry. The Dutch material, numbered SH66-111 and SH66-115, was of more complex origin; including the European cultivars "Grata" and "Maritta" together with dihaploids produced at Wisconsin, U.S.A.

Cultivated diploid parents were elite clones drawn from a scheme for improvement by mass-selection. The series BZ, CL, EL, WF had been selected at Pentlandfield during the period 1967 to 1970. The M series had been selected 1962–65 at the John Innes Institute, Hertford, England. Edible diploid clones had some Group Stenotomum in their ancestry but tended to be of the Phureja type. An unimproved Stenotomum clone, stn 3174, came from Oruro, Bolivia.

Methods of fertility estimation

The only completely reliable estimate of male or female fertility must be the capacity to produce viable seed, but a very large number of test-crosses is sometimes needed to establish the true fertility, making the method uneconomic for large numbers of clones. Various rapid methods for estimating male fertility are commonly used and depend on observing properties of the anthers and pollen. PEREZ-UGALDE et al. (1964) advocated the use of both pollen-shedding and in vitro pollen germination. GRUN and his collaborators have published many data on male sterility, manifested as anther indehiscence due to gene/cytoplasm interactions. Phureja and Stenotomum parents were among those used in their work: see GRUN, AUBERTIN & RADLOW (1962); GRUN & AUBERTIN (1965). However, in the practical breeding situation, it is usual to excise pollen from the anthers. Limiting factors will then lie in the pollen itself. Male fertility is most frequently estimated by recording the percentage of "viable" pollen grains, using an appropriate stain. If a proportion of the grains do not develop to the stage observed in plants known to be highly male fertile, some reduction in male fertility may be expected. In vitro pollen germination should in theory relate more closely to functional fertility than does stainability. In practice little advantage is gained. Figures for germination and stainability in dihaploid × diploid potato hybrids

published by LIBERAL (1966) reveal a close parallel between the two, with germination consistently lower. Since functional male fertility is manifested at very low germination percentages (PEREZ-UGALDE et al., 1964), such data offer little scope for differentiating clones in the lower range. The chief objection to pollen stainability is that measurements often have a low repeatable accuracy. "Within plant" variation can be dealt with by repeated sampling, but difficulties inherent in the types of stain used are more serious. Ideally, only the cytoplasm of pollen grains should be stained, but many commonly used stains, such as aceto-carmine, are also taken up by the walls. The result then depends on the time allowed for staining, the strength of the solution, etc. If a solution of iodine in KI is substituted, more repeatable and clear-cut results ensue (cp. PARKER & BORRILL, 1968). Most potato pollen does not contain starch at the time of liberation, but iodine colours the cytoplasmic contents a dark brownish-yellow, while hardly tinting the wall. Full staining is rapidly reached.

Stainability percentages of all clones in this study were based on at least two pollen samples of 200 grains each. If replicates differed by more than 10 percent further samples were taken and a mean calculated.

Seed-set figures are considered to give a reliable estimate of true seed fertility since non-viable seed could be recognized visually and excluded from the counts, provided samples were fresh. Confirmation was obtained by subsequent germination tests.

Crossing was carried out under glass, female parents having been emasculated in the bud stage and protected against stray pollination by "glassine" paper bags. Plants were grown in "John Innes No. 3" compost in 190 mm plastic pots, either on their own roots or grafted on tomato rootstocks.

Results

EFFECT OF PARENTAGE ON POLLEN STERILITY OF PROGENIES

Mean percentages of stainable pollen in 26 F_1 progenies, involving seven different primary dihaploid female parents and eleven different edible diploid male parents, are given in Table 1. Inspection of the means immediately suggests that there may be consistent differences attributable to common seed parents: e.g. between the progenies of HC32 and HC56. Means for the progenies of any given dihaploid have a relatively restricted range of variation: very low and very high means have not occurred within the same common parent group. When the data is re-arranged into groups of progenies according to common edible diploid parent, obvious differences between groups are lost.

TABLE 1
POLLEN STAINABILITY IN PROGENIES OF PRIMARY DIHAPLOID × EDIBLE DIPLOIDS

Progeny No.	Parents ♀	♂	No. plants examined	Mean % stainability	Range
0171/HC-DB	HC32 ×	BZ(1)	15	13.9	0.0–51.0
0272/HC-DB	×	BZ(2)	8	5.1	0.0–41.0
0274/HC-DB	×	BZ(26)	18	38.0	0.0–77.8
0368/HC-DB	×	BZ(65)	14	24.8	0.0–52.5
0369/HC-DB	×	BZ(107)	8	9.8	1.0–27.5
0173/HC-DB	×	EL(2)	8	21.0	0.0–69.5
084/HC-DB	HC35 ×	BZ(1)	22	56.6	11.0–83.5
085/HC-DB	×	BZ(2)	18	36.7	0.5–79.0
0174/HC-DB	×	BZ(25)	35	53.0	5.0–90.5
0290/HC-DB	×	BZ(26)	20	61.7	25.0–89.5
0372/HC-DB	×	BZ(65)	19	54.6	10.0–70.5
0373/HC-DB	×	BZ(115)	20	68.8	50.5–82.0
0176/HC-DB	×	EL(2)	20	53.9	23.5–73.5
0374/HC-DB	×	WF(5)	20	54.3	5.0–79.0
096/HC-DB	HC56 ×	BZ(1)	9	44.5	12.0–73.7
0276/HC-DB	×	BZ(2)	18	41.7	0.0–85.0
0280/HC-DB	×	BZ(58)	15	59.7	10.0–74.0
0279/HC-DB	×	CL(12)	13	51.0	0.0–74.5
0281/HC-DB	×	EL(2)	20	39.1	0.0–77.5
0395/HC-DB	HC63 ×	BZ(2)	20	3.7	0.0–53.5
0396/HC-DB	×	BZ(115)	13	20.3	0.0–61.0
0188/HC-DB	HC161 ×	BZ(1)	13	28.4	0.0–68.0
0380/HC-DB	HC162 ×	BZ(65)	6	9.6	0.0–51.5
0382/HC-DB	×	WF(5)	5	21.5	0.0–82.5
0392/HC-DB	HC193 ×	BZ(25)	20	28.1	0.0–66.5
0393/HC-DB	×	BZ(115)	20	45.9	0.0–73.5

The most convenient method for testing the apparent differences between these progeny groups with common parents is an analysis of the "North Carolina" type (COMSTOCK & ROBINSON, 1952). To make full use of the data, two "North Carolina" I analyses were made, one with dihaploid common parents, the other with diploid common parents. By comparing results from the two analyses, the pattern of inheritance could be inferred. For the purposes of statistical analysis each percentage score was transformed to arcsin (100–x%) degrees. Results are given in Table 2*a*, *b*.

A clear distinction can be made between the effect of common dihaploid and common diploid parents, since there are significant differences between the dihaploid parents but none between the diploids. The analysis has detected significant differences between progenies within dihaploid common parents, although the Mean Square is much smaller than that

TABLE 2
STATISTICAL RESULTS OF N. CAROLINA DESIGN I ANALYSES ON POLLEN STERILITY

Dihaploid common parents

Common parent	Progenies used							
HC32	0171	0173	0272	0274	0368	0369		
HC35	084	085	0174	0176	0290	0372	0373	0374
HC56	096	0276	0279	0280	0281			
HC63	0395	0396						
HC162	0380	0382						
HC193	0392	0393						

Source	d.f.	M.S.	F	p
Parents	5	12943.0832	55.3	0.01
Progenies within parents	19	832.5245	3.5	0.01
Within progenies	379	233.7987		

Diploid common parents

Common parent	Progenies used			
BZ(1)	084	096	0171	0188
BZ(2)	085	0272	0276	0395
BZ(25)	0174	0392		
BZ(26)	0274	0290		
BZ(65)	0368	0372	0380	
BZ(115)	0373	0393	0396	
EL(2)	0173	0176	0281	
WF(5)	0374	0382		

Source	d.f.	M.S.	F	p
Parents	7	2419.4773	1	N.S.
Progenies within parents	15	3816.7017	15.8	0.01
Within progenies	358	240.4984		

between dihaploid parents. In the diploid common parent direction (Table 2*b*), the between progenies within parents Mean Square will have been inflated by g.c.a. effects from the dihaploid parents. That this is not *per se* the reason for lack of significance between diploid common parents can be seen by comparing the actual "between parents" Mean Squares for dihaploids and diploids: the difference is more than five-fold. These results indicate high general combining ability of dihaploid parents but not of diploids and of a lesser degree of specific combining ability in certain parental combinations.

The wide range of variability between plants within progenies should be noted (Table 1). Even in progenies with such low means as those of 0395 and 0380, individual plants occur with 50% stainable pollen.

The intercrossing of diverse dihaploids is known to improve fertility

TABLE 3
POLLEN STAINABILITY IN PROGENIES OF SECONDARY DIHAPLOIDS × EDIBLE DIPLOIDS

Progeny No.	Parents ♀	♂	No. Plants examined	Mean % stainability	Range
0405/CB-DB	0117/DH/CB(22)	× BZ(2)	15	2.0	0.0–10.0
0410/CB-DB	0118/DH/CB(9)	× BZ(25)	20	32.1	0.0–66.5
0411/CB-DB		× BZ(26)	14	54.5	10.0–73.5
0412/CB-DB		× BZ(65)	20	43.5	17.0–65.5
0413/CB-DB		× BZ(107)	20	66.8	55.5–77.0
0414/CB-DB		× BZ(115)	20	72.3	18.0–90.0
0415/CB-DB		× WF(5)	20	61.8	7.0–93.0
0426/ND-DB	SH66-111(1.35)	× BZ(2)	20	20.2	1.0–42.5
0427/ND-DB		× BZ(25)	12	20.8	0.0–42.5
0436/ND-DB	SH66-111(2.32)	× BZ(2)	20	10.0	0.0–38.0
0437/ND-DB		× BZ(25)	20	21.4	0.0–47.0
0442/ND-DB	SH66-115(10)	× BZ(2)	12	19.1	0.0–41.5

in further dihaploid generations. It would be useful to see if secondary dihaploids crossed with edible diploids have more pollen-fertile progenies than do primary dihaploids (see Table 3). Stainability figures are not obviously different from those of Table 1. A "t"-test on mean values from the two tables gave $t = 0.194$, well below significance at 5% (d.f. = 36). The use of secondary dihaploids did not improve pollen fertility.

FUNCTIONAL FERTILITY

Data on berry-set and seed-set in 24 F_1 clones, involving six different dihaploid and seven different edible diploid parents, are presented in Table 4, alongside pollen stainability figures. For each common dihaploid parent, clones are listed in descending order of pollen stainability. Only crosses between hybrids and tester parents of proven female fertility are included in the table, which abstracts data accumulated during a breeding programme; hence the variation in number of "test" pollinations per clone. The F_1 clones had been selected for yield and tuber size but not for male fertility. A major effect of the dihaploid parent is again discernible: only HC29 and HC35 of the dihaploids listed have given male fertile offspring. Low levels of normally developed pollen could sufficiently explain the sterility of certain clones (e.g. 097(17)) but it would be difficult to identify a particular stainability "threshold" for fertility. The finding of male fertility in M476(1), for instance, was probably due to the large number of test pollinations made. One cannot assert, therefore, that M472(27) and M476(20) are completely male sterile, though at best their

fertility must be very low. On the other hand, in the 090 clones (6), (7), (8) high levels of stainable, apparently normal, pollen are not accompanied by functional fertility, despite adequate numbers of test pollinations. From seven to nine different female tester parents were used with each clone, so *S* allele incompatibility cannot be held responsible.

Therefore, at least two factors appear to affect the expression of male fertility in this material. First, low levels of normal pollen depress fertility but its extinction is hard to demonstrate owing to the large number of test crosses needed. Second, apparently normal pollen may be unable to effect fertilization due to failures at, or after, germination. This could well explain why attempts to demonstrate a quantitative relationship between pollen stainability and functional male fertility usually fail. However, success might be attained by using data only from plants in which some degree of fertility had already been established (see Table 4).

TABLE 4

POLLEN STAINABILITY AND FUNCTIONAL MALE FERTILITY IN DIHAPLOID × DIPLOID HYBRIDS

Clone No.		Parents ♀ × ♂	Stainability %	No. flowers pollin.	No. berries	Seeds per berry	Seeds per pollin.
M472	(10)	HC29 × M468(2)	70.5	6	1	31	5.2
	(27)		12.0	3	1	—	—
M476	(20)	HC29 × M468(6)	50.0	33	—	—	—
	(10)		43.0	107	1	16	0.15
	(1)		15.5	193	3	17	0.26
0171/HC-DB	(8)	HC32 × BZ(1)	51.0	56	—	—	—
	(3)		39.0	3	—	—	—
0173/HC-DB	(13)	HC32 × EL(2)	69.5	9	—	—	—
084/HC-DB	(28)	HC35 × BZ(1)	81.0	5	3	57	34.2
	(4)		71.0	129	38	106	31.2
	(11)		61.5	106	10	66	6.2
	(42)		56.5	40	1	21	0.5
	(12)		44.0	100	5	87	4.4
085/HC-DB	(10)	HC35 × BZ(2)	71.0	13	1	36	2.8
	(33)		64.5	14	6	13	5.6
	(25)		63.0	12	3	67	16.8
	(16)		58.0	11	5	14	6.4
0176/HC-DB	(29)	HC35 × EL(2)	66.0	37	7	73	13.8
M475	(15)	HC46 × M468(5)	36.5	66	—	—	—
097/HC-DV	(17)	HC63 × stn 3174	9.5	18	—	—	—
	(46)		5.0	9	—	—	—
090/HC-DB	(6)	HC142 × M468(6)	85.0	61	—	—	—
	(8)		79.0	117	—	—	—
	(7)		66.5	144	—	—	—

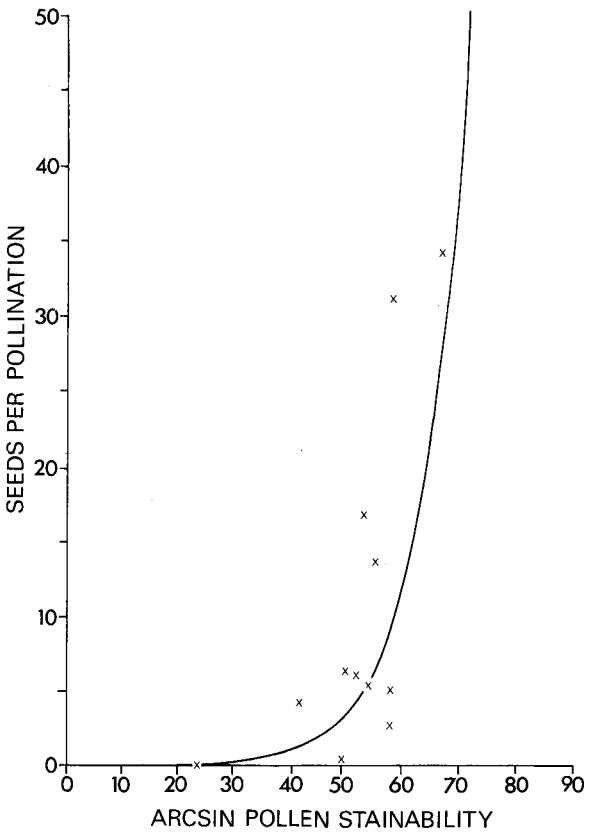

Fig. 1

"Seeds per pollination" was selected as the best measure of fertility because it bears a direct relation to the effort involved in obtaining a successful cross. A plot of stainability against "seeds per pollination" (Fig. 1) suggests that, while a relationship is apparent, it is not a simple linear one. As stainability increased, there was a disproportionate increase in seeds produced. When the data were rectified, taking $\log_e$ values for "seeds per pollination" data, a significant linear regression was obtained: $y = -4.938 + 0.126x$ ($a = 4.938 \pm 1.815$, $b = 0.1257 \pm 0.0355$). An exponential relationship frequently indicates an additional influence of concentration in the independent variable factor. Several authors have noted an enhancement of pollen germination and subsequent tube growth due to "crowding" of the grains (e.g. ARIYASU, 1959). While clones with a low stainability often produce smaller amounts of pollen, in these artificial crosses enough pollen was normally collected and applied to

cover the stigmatic surface. Even then, if only a few grains were viable they would be diluted and separated by physiologically inert microspores. Thus it seems likely that the "crowding" effect is responsible for the observed relationship and significantly contributes to the low functional fertility of male parents with poor pollen development. A curve, calculated from the rectified linear regression (such that $y = 0.0072e^{0.126x}$) has been fitted to the points of Fig. 1, and could be used as a basis for the selection of a threshold value for stainability in a male parent proposed for crossing. The bulk of the improvement in fertility lies above 50 percent, which provides limited justification for the choice by GRUN (1970a) of that level as indicating full male fertility. Clearly, clone M476(1) must lie near the lower limit for practical functional fertility. The curve shows also that, in the stainability range up to 81.0 percent, "saturation" of potentially functional ovules was not approached.

As male sterility in dihaploid ♀ x diploid ♂ hybrids is believed by some workers to be of the cytoplasmic type, it is interesting to examine whether the female fertility of the hybrids is, in fact, higher than the male. Table 5 gives functional fertility data for 16 F_1 clones, involving six different dihaploid parents. Pollen stainabilities are included for comparison. Testing was done only with male parents of proven fertility. It will be seen that there are no cases of consistent lack of fertility associated with

TABLE 5

POLLEN STAINABILITY AND FUNCTIONAL FEMALE FERTILITY IN DIHAPLOID × DIPLOID HYBRIDS

Clone No.		Parents ♀ ♂	Pollen stainability %	No. flowers pollin.	No. berries	Seeds per berry	Seeds per pollin.
M472	(35)	HC29 × M468(2)	84.0	6	1	31	5.2
	(29)		39.0	3	1	—	—
M476	(20)	HC29 × M468(6)	50.0	50	3	68	4.1
	(10)		43.0	69	10	95	13.8
	(1)		15.5	87	7	60	4.8
0171/HC-DB	(8)	HC32 × BZ(1)	51.0	19	4	116	24.4
	(3)		39.0	19	2	38	4.0
084/HC-DB	(11)	HC35 × BZ(1)	61.5	2	2	131	131.0
M475	(15)	HC46 × M468(5)	36.5	47	9	127	24.3
	(13)		Nil	26	2	98	7.5
097/HC-DV	(9)	HC63 × stn 3174	29.0	59	8	95	12.9
	(17)		9.5	54	5	78	7.2
	(46)		5.0	84	1	—	—
090/HC-DB	(6)	HC142 × M468(6)	85.0	59	8	71	9.6
	(8)		79.0	95	24	40	10.1
	(7)		66.5	92	2	29	0.63

particular dihaploid parents. Compared with the 13 male fertile clones of Table 4, figures for "seeds per berry" are significantly higher ($t = 2.38$; $p < 0.05$) although "seeds per pollination" do not differ significantly ($t = 0.90$). The contrast between male sterility and female fertility in the 090 clones is noteworthy, but does not correspond with the types of cytoplasmic male sterility previously described (GRUN, 1970a: GRUN & AUBERTIN, 1966), where impaired pollen was visually recognizable. It should be noted that even on the female side values for "seed per berry" are somewhat lower than would be expected for edible diploids under similar (glasshouse) conditions. The one clear instance of female sterility (in 097(46)) was associated with a low percentage of stainable pollen, suggesting a common basis in abnormal meiosis, but this is not invariably the case since the female fertile M475(13) produced no normal pollen.

Discussion

Data presented in this paper demonstrate the occurrence of varying degrees of male sterility in F_1 hybrids from dihaploid female parents, confirming the reports of ROSS, PELOQUIN & HOUGAS (1964), LIBERAL (1966) and GRUN (1970a). The importance of the phenomenon from the point of view of genetic utilization of such hybrids depends therefore on its extent, severity and inheritance. The model proposed by GRUN (1970*a* and *b*) invokes sensitive cytoplasmic factors present in dihaploid Tuberosum which with dominant nuclear genes, produce various types of sterility, derived from group Phureja and Stenotomum sources (among others). Of three types listed by GRUN as showing high expressivity in the F_1 generation, namely indehiscence, "sporads" and shrivelled microspores, it is the shrivelled microspore sterility which is most important from a practical point of view. Indehiscence is automatically overcome by artificial pollen extraction during crossing. "Sporad" sterility has been observed at Pentlandfield, but only in occasional progeny from certain crosses with secondary dihaploids. The absence of a reasonable proportion of normally developed pollen is, however, a serious obstacle to crossing.

It would hardly be expected that potato hybrids should show a complete and invariable cytoplasmic male sterility since even the earliest reports of this phenomenon mention differences in its expression, and the search in crop plants for sterilities which are reliable against varying environmental conditions and genetic backgrounds has often been prolonged and arduous (DUVICK, 1959). The genetical component of such variation

could be explained in several ways;

(i) not all dihaploids possess equally sensitive cytoplasms;
(ii) not all edible diploid clones carry appropriate dominant sterility genes;
(iii) either or both types of parent contain other genes which modify the effect, partly or completely restoring fertility.

DE LA PUENTE & PELOQUIN (1968), referring to indehiscence and functional sterility, reported differences between Tuberosum and Andigena cytoplasms but not differences within Tuberosum. HOWARD (1970) reported cytoplasmic differences within Tuberosum for pollen sterility. GRUN (1970a) suggested explanations (ii) and (iii) above to explain variation in shrivelled microspore sterility. Since the statistical results of the present study show that most of the general combining ability is attributable to the dihaploid female parents, it is unlikely that any "restorer" genes were present on the cultivated diploid side. Of course, as the variation was all of the continuous type, no progeny showed clear Mendelian segregation and a distinction between the effect of sterility gene dosage and of restorer genes would be operationally meaningless. Evidence that dihaploids from U.S. cultivars show similar characteristics as female parents can be found by referring to data in LIBERAL (1966): five crosses of Phureja and Stenotomum clones involved the two dihaploids, US-W3 and US-W4. Pollen stainabilities and functional male fertilities quoted for the two US-W4 F_1 combinations are much lower, though US-W4 is itself male-fertile (see PELOQUIN & HOUGAS, 1960). Although LIBERAL interpreted these results in terms of the Phureja contribution, they tend to add further weight to the view that it is the selection of appropriate Tuberosum dihaploids which determines success.

Plant to plant variation was such that, in almost all progenies, clones with quite high levels of normal pollen could have been selected (Table 1). The use as parents of secondary dihaploids (which, in culture here, have generally shown higher levels of male fertility than primary dihaploids) did not raise the level of stainable pollen in F_1 hybrids (Table 3). This tends to bear out the importance of the cytoplasmic factors in determining fertility, as does the relative insignificance of choice of edible diploid pollen parent. Data on functional male fertility obtained from the more detailed investigation of individual clones (Table 4) followed the same general pattern as the pollen sterility results. This raises the question as to what relationship may exist between the two types of data. The chief difference lies in the existence of a class of F_1 plants with medium to high pollen fertility which are functionally male sterile. Such plants showed usual F_1 levels of female fertility and had common dihaploid parentage, strongly

suggesting another cytoplasmic male sterility. This cannot, however, be equated with any of the five types listed by GRUN (1970b), although it would be analogous to his [Fm^S]/FM system involving Tuberosum and *S. vernei*. The existence of male sterile plants with stainable pollen will obviously interfere with attempts to investigate the relation between amount of stainable pollen and male potency. Nevertheless, by using only data from F_1 clones which had shown some degree of male fertility, however small, it proved possible to obtain a significant, non-linear relationship between stainability and functional fertility. The non-linearity could be explained by the known effect of pollen density on germination and growth. At high stainability levels, changes of a few percent would produce a marked change in the success of crosses; at low levels a large percentage change would be needed and success in crossing would appear uniformly low. A threshold value of stainability, for predicting the suitability of clones as male parents, could be selected by balancing the number of crosses needed against the desirability of the result, and might lie between 10% and 25%. It would be unrealistic, however, to choose some specific stainability as indicating a fertility-sterility threshold (cf. GRUN, 1970*a*, Table 2; also FINEMAN, 1947).

There was an indication that, even in functionally male fertile clones, the average level of male fertility was slightly lower than the female: this might be expected if cytoplasmic male sterility factors were present. It is worth noting that evidence of possible cytoplasmic differentiation within Tuberosum has long been available: SALAMAN & LESLEY (1922) found striking reciprocal differences in progenies of cv. "Edgecote Purple" and cv. "Edzell Blue", in pollen quality, in male fertility, and in amount of flowering.

In conclusion, it may be said that the male sterility in F_1 hybrids from dihaploid Tuberosum seed parents is notable for its variability so that it constitutes a hindrance rather than a barrier to crossing work. The choice of dihaploid parent clones is all important in obtaining a desired level of male fertility in the offspring. In view of the report of DE LA PUENTE & PELOQUIN (1968), regarding the absence of sensitive cytoplasms in group Andigena, the advent of Neo-Tuberosum clones with agronomic characteristics comparable to those of conventional Tuberosum cultivars (SIMMONDS, 1970/71) should considerably simplify dihaploid breeding.

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