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CROSS BREEDING EXPERIMENTS : COMPATIBILITY, POLLEN FERTILITY, AND GERMINATION PERCENTAGES OF INTERSPECIFIC HYBRIDS, F1, F2 & F3 IN THE GENUS *Pachyrhizus* Rich. ex DC. (*Fabaceae* ; *Phaseolae*)

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ABSTRACT

A comparative study of the interfertility of the three cultivated species in the genus *Pachyrhizus Rich.* ex DC. (*P. erosus,* incl. wild forms, *P. tuberosus, and P. ahipa*) is presented ; the considerable compatibility is demonstrated based in cytological studies, cross breeding experiments, and examinations of pollen fertility in the parental species and F1 F2 and F3 hybrids. Differences in compatibility between reciprocal crosses are demonstrated, but the pollen fertility of hybrids is only slighty reduced.

Furthermore, seed yields and germination percentages of parent species and hybrids have been examined. The seed yield of the hybrids have been found to be considerably higher than that of the species, and the germination percentages of the hybrid seed have been found not to be reduced, with one exception : *P. tuberosus x P. ahipa.* The possibility of creating new cultivars adapted for specific climatic conditions through interspecific breeding is indicated. Finally, all species and hybrids have been studied cytologically.

RESUME

TAXONOMIE EXPERIMENTALE. EXPERIMENTATION DE CROISEMENTS DE SELECTION : COMPATIBILITE, FERTILITE DU POLLEN, ET POURCENTAGE DE GERMINATION D'HYBRIDES INTERSPECIFIQUES F1, F2 et F3 DANS LE GENRE PACHYRHIZUS RICH.(*Fabaceae*, Phaseoleae)

Une étude comparative de l'interfertilité des trois espèces cultivées du genre *Pachyrhizus* Rich. ex DC (*P. erosus*, espèces sauvages incluses, *P. tuberosum, et P. ahipa*) est présentée. Leur compatibilité considérable est démontrée à partir d'études cytologiques, d'expérimentation de croisements, et d'examen de la fertilité du pollen chez les espèces parentales, les hybrides F1, F2 et F3. Des différences de compatibilité entre espèces parents sont établies, mais la fertilité pollinique des hybrides est seulement très légèrement réduite. De plus, on a examiné le rendement en grain et les taux de germination des espèces parentales et des hybrides. Le rendement en grain des hybrides a été trouvé considérablement supérieur à celui des espèces, et le taux de germination des grains d'hybrides n'a pas été réduit sauf avec *P. tuberosus x P. ahipa*. La possibilité de créer de nouveaux cultivars adaptés à des conditions de climat, spécifiques à travers de sélection interspécifiques est indiquée. Enfin, toutes les espèces et hybrides ont été étudiées cytologiquement.

Note : In this paper the individual serving as the female will always be mentioned before of the pollen donor.

INTRODUCTION

The tuberous legume genus *Pachyrhizus* comprises five species of which the three are to be found in cultivation.

Archaelogical evidence from Mexico and Peru has confirmed that the cultivation of at least two of the species was practiced some 2.000 years ago (Oviedo y Valdès 1535, O'Neale & Whitaker 1947 and Ugent et al. 1986). Thus these cultivated species represent the oldest known leguminous tuber crops found in the new world.

As mentioned by van der Maesen (1972) in his study of the genus *Cicer* L. the opportunity of conducting a biosystematic study of cultivated crops, their wild forms and their wild relatives is rarely possible. The isolation/determination of wild forms of cultivated species is often difficult and even if this obstacle is overcome the material may prove incapable of cross fertilization. However, two of the three cultivated species of *Pachyrhizus* are recorded in their wild form and the two wild species are still to be found in abundance within their distribution area (both of the wild species have been introduced to areas outside their original distribution through botanical gardens, and have subsequently escaped to become naturalized). This makes the genus an excellent object for such studies.

During a four year period the previous study (Sorensen, 1988) of the interspecific relations has been extended through cross breeding experiments, morphological examinations of live material, growth experiments and pollen morphological studies of parent as well as F1-,F2- and F3- hybrids. Estimates of yield performances (tuber yields) have been carried out on plants grown in greenhouses at the Botanical Laboratory, University of Copenhagen and at the Botanical Institute, the Royal Veterinary & Agricultural University, Copenhagen. Further results derived from these studies, e.g. on yield performance, is given in Sorensen (1989b).

The genus has a Neotropical distribution, ranging from Central Mexico through Central America to the central parts of South America, i.e. Bolivia, Paraguay and Southwest Brazil. Of the three cultivated species, *P. erosus* has gained pantropical distribution in post-Columbian times, with the largest area of cultivation today in The Far East, including China. *P. tuberosus* is cultivated sporadically in Brazil, Ecuador and Peru, and has been introduced to a few islands in the Caribbean. It has not been possible to establish the exact time of these introductions, but most likely *P. tuberosus* was introduced to the Caribbean through pre-Columbian immigration of the Amerindian people like a number of other South American tuber crops. The last cultivated species, *P. ahipa*, presumably originates in the Andean regions of Ecuador, Peru and Bolivia. This species has never been introduced outside its recorded distribution area, i.e. areas where cultivation has taken or is taking place, with the exception of some limited cultivation in the northern provinces of Argentina, Jujuy and Salta, where the crop was cultivated by Bolivian farm labourers as recent as in the 1940es (Dr. O. Boelcke, pers. comm.). In contrast to the two previously mentioned cultivated species, *P. ahipa* has never been recorded in the wild state.

As one of the main objectives has been to provide the basic information needed for future breeding of high yielding clones suited for production in third world countries, testing of the material resulting from the cross breeding programme is planned to commence at six tropical agricultural institution in 1989 and continuing to the end of 1991.

Concerning the morphology, distribution, ecology, and taxonomic synonomy of the five *Pachyrhizus* species, see Sorensen (1988). The morphological specificity of the pollen (from cultivated species, their wild forms and wild species) and the pollen morphology of the hybrids is presented in Sorensen (1989a) ; for details on cultivation practices and other information of agronomic interest, see Sorensen (1989b).

MATERIALS AND METHODS

The material used in the present study was collected in the following countries in February - April 1985 : Mexico, Belize, Guatemala, Costa Rica, Colombia, Ecuador, Bolivia, and Argentina. Additional material of both wild and cultivated origin has been supplied by a number of botanical and agricultural institutions worldwide. A list of the total material present in the germplasm collection, stating country of origin and whether wild or cultivated is available from the author.

Until the spring of 1986 only the two species : *P. erosus* and *P. ahipa* were available to the hybridization programm, when material of *P. tuberosus* and of a wild form of *P. erosus* from Guatemala (previously known as P. strigosus Clausen) were added to the programme. This material was then used as soon as the first flowers were available. At present there are only four cultivars of *P. tuberosus* available : one from Haïti, one from Peru and two from Brazil.

Regardless of the origin of the material, i.e. whether from field collections or from germplasm collections, a minimum number of 20 seeds were sown (provided sufficent material was available). The seeds were allowed 60 days to germinate, by which time they would have been infected by fungi had germination not taken place. The germination percentages of hybrid material were calculated when germinating a minimum of half of the seeds resulting from a specific combination.

The flowers functioning as pollen-receptors were emasculated 48 hours prior to anthesis, i.e. prior to the opening of the standard and wing petals. In order to ascertain optimal development of the legume/seeds, flowers, situated in
an intermediary position within the inflorescence, were given preference. Emasculations and artificial pollinations were conducted using flame sterilized instruments. All flowers and buds in the inflorescence more than 5 mm long were removed in order to eliminate unwanted pollination from adjacent flowers. The whole inflorescence was then covered by a paper bag and a celluloid bag (reducing desiccation of the emasculated flower). When using *P. erosus* as the pollen receptor only a single flower per inflorescence, situated on a short shoot in the middle of the inflorescence, was emasculated. Fertilization was carried out 24 hours after emasculation. The celluloid bag was removed after 52 hours when observations of the ovule indicated that the artificial hybridization had been successful. The paper bag was left in place until the time when the young legume reached a length of approx. 2.5 cm. Failure of pollination led to abscission following 24-48 hours. Abscission would rarely occur at a later stage. Reciprocal crosses were conducted

simultaneously. Emasculation of the *P. tuberosus* material had to take place at least 52 hours prior to anthesis as the pollen sacs would open 36-48 hours before opening of the standard and wing petals. Furthermore, the flowers of *P. tuberosus* tended to be more brittle due their succulent nature, thus profuse bleeding often occurred. The inflorescences of the wild form of *P. erosus* had only few flowers per inflorescence, therefore the initial ten plants did not suffice should all combination be possible (only ten plants had been germinated, as the amount of seed material available was always limited). Fortunately, this material was markedly sensitive to short day treatment, and an additional 5 plants were manipulated during the spring of 1987 in order to provide the flowers needed

Unfortunately, it has not been possible to include the material of wild forms of *P. erosus* from Costa Rica, the material of P. panamensis and the material of *P. ferrugineus* in the hybridization programme. Although the Costa Rican material and the material of *P. panamensis* have had fertile shoots, initiated through short day treatments, they abscised when the young inflorescences reached 3 mm in length. The *P. ferrugineus* material has not responded to short day treatment, and no fertile shoots have been observed.

Pollen samples for the calculation of fertility were taken from live material only. The samples obtained from flowers at anthesis were stained in cotton blue, and examined in a Wild M5 ligth microscope ; the method used was according to Gustafsson (1973). A minimum of two pollen samples per plant were taken from different inflorescences, counting 400 pollen grains per sample.

When germinating the seeds for the cross breeding programm, the study of chromosome numbers in somatic cells of the parent material were carried out. Subsequently, when germinating the resulting hybrid seeds, the study of their somatic chromosome numbers were added. Seedlings taken one week after germination were placed in a dark room at 20°C for 12 hours, then placed under a grouth lamp for one hour Root tips were pretreated in 2mM 8-hydroxyquinoline for 2-3 hours at 18°C, fixed in a cold acetic-ethanol (1:3) solution, changed after one hour, and stored in a deep-freezer until squashing. Hydrolyzed in 1N HCL, stained in Feulgen, and squashed in acetic-acid (Östergren & Heneen 1962).

The examinations of meiotic cells were conducted on both parents, as well as on hybrid material. Flower buds, approx. 1.5 mm long, were collected at 15 min. intervals during the first hour following sunrise. The pollen sacs were isolated and pretreated in the same acetic-ethanol (1:3) solution as the somatic cells in order to fixate the chromosomal divisions, subsequently hydrolyzed in 1N HCL. Two different methods of staining the material were

employed : 1. stained in Feulgen and squashed in a 45% acetic acid solution ; 2. squashed directly in aceto-orcein.

RESULTS

The origin, germination percentages, and pollen fertilities of the material used in this study, are given in App. 1.

1. Cytology of parent, F1 and F2 material

The results are presented in Fig. 1.a. & b. All the material studied, parent as well as hybrid, had 2n=22. This is in agreement with all reports on the chromosome base number of the genus (Lackey 1981). The two wild species (*P. ferrugineus and P. panamensis*) are examined here for the first time.

The study of chromosome numbers in meiotic cells of interspecific hybrids was attempted ; however, although some 200 flowers buds were examined, no meiotic divisions were observed, i.e. the material was either too young or too old. This study was abandoned because it was thought too time consuming to validate the results. In addition as no phenotypic abnormalities have been observed in the hybrids and as the pollen fertility of the hybrids does not indicate the presence of non-homologous chromosomes (the presence of non-homeologous chromosome cannot be excluded), this methods of analysis has not been considered to merit further contemplation.

2. Pollen fertility

The results of the examinations of pollen fertility in parent species as well as in interspecific hybrids were as follows :

9 cultivars of *P. erosus* were examined, 5 Mexican, 2 Guatemalan, 1 Brazilian and one Indonesian. An average of 9 samples were studied per accession. The 82 pollen samples showed a mean percentage stained grains of 96±5%. The fertility of the individual accessions are presented in App. 1.

Flowering of the wild forms of *P. erosus*, of which 8 accessions are represented, have proved difficult to induce. Only 2 accessions, one from Guatemala (the «*P. strigosus*» Clausen-type) and one from the botanical Garden at Reduit, Mauritius, have had well developed flowers so far. The remaining 6 accessions from Costa Rican populations did develop young fertile shoots, but is not included in the pollen fertility, as mentioned, because abscission occurred prior to completion of their development.

Material of 3 cultivars of *P. ahipa* has been examined. Unfortunately, the origin of two of the accessions could not be determined, one from the Devonian Botanical Garden, University of Alberta, Canada, had a morphological appearance very similar to the cultivar obtained from Tarija, Bolivia, i.e. with violet blue flowers and dull black seeds. The remaining cultivar of unknown origin is slightly taller, with white flowers and with black and white mottled seeds, obtained from the Jardin Botanique National, Meise, Belgium. A total number of 60 samples yielded an overall fertility percentage of 98±1%.

The only accession of *P. tuberosus* to be included in the cross breeding experiments, a cultivar from Haïti, was obtained in July, 1986. This cultivar had a fertility percentage of 97±1, based on 28 samples ; one pollen sample from this cultivar had a few grains non-reduced (2n) pollen. The remaining three accessions from Brazil (2) and Peru (1) have yet to be included in the hybridization experiments.

In the interspecific F1-hybrids of *P. erosus* x *P. ahipa* (both parents cultivars), an overall fertility of 84±4%, based on 134 samples. 9 different crosses involving 5 different accessions yielded very equal fertilities ; see Tab. 1.

The overall fertilities of the F2, F3, and F4 generations were respectively 92±3%, 78±0%, and 77±0%.

In the reciprocal cross *P. erosus* x *P. ahipa*, both parents cultivars, the calculation of the total fertility, based on 12 samples, is 88±% (see Tab. 1).

Artificial hybrids resulting from the combination of *P. erosus* x *P. tuberosus* (both parents cultivars) and the reciprocal combination, *P. tuberosus* x *P. erosus*, yielded the pollen fertilities presented below, based on 10 samples.

In the combination : *P. ahipa* x *P. tuberosus*, both parents cultivars, the overall percentage stained pollen grains was 94±3%, calculated on the basis of 25 samples.

There are no figures available for the reciprocal combination as the seed material resulting from the artificial pollination was incapable of germinating. See Tab. 3.

The following three combinations representing the F2 generation have flowered : *P. tuberosus* x (*P. erosus* x *P. ahipa*), *P. tuberosus* x (*P. ahipa* x *P. erosus*) and (*P. erosus* x *P. ahipa*)*x (*P. erosus* x *P. ahipa*). The first two combinations were produced through artificial pollination and the third

Table 1 : Pollen fertility in <u>P. erosus x P. ahipa</u> and the reciprocal combination

Hybrid combinaison	Accession number	Percentage stained pollen								
		Parent			F1			F2		
		No. ind	No. X	%±SD Xmean	No. ind	No. X	%±SD Xmean	No. ind	No. X	%±SD Xmean
P. erosus x	ECO32/285	3	18	98 ±1	25	119	85 ±4	6	11	92 ±3
P. ahipa	AC102/485	7	31	100 ±0	-	-	-	-	-	-
					F3			F4		
					No. ind	No. X	%±SD Xmean	No. ind	No. X	%±SD Xmean
					1	2	78 ±0	1	2	77 ±0
					-	-	-	-	-	-
		Parent			F1			F2		
		No. ind	No. X	%±SD Xmean	No. ind	No. X	%±SD Xmean	No. ind	No. X	%±SD Xmean
P. erosus x	EC512/385	2	8	96 ±2	2	9	80 ±3	-	-	-
P. ahipa	AC102/485	7	31	100 ±0	-	-	-	-	-	-
P. erosus x	EC116/186	2	6	88 ±3	3	6	84 ±6	1	2	91 ±1
P. ahipa	AC102/485	3	10	100 ±0	-	-	-	-	-	-
P. erosus x	EC503/882	2	6	99 2	3	6	84 ±5	-	-	-
P. ahipa	AC102/485	3	10	100 ±1	-	-	-	-	-	-
P. ahipa x	AC102/485	7	31	100 ±0	2	10	88 ±4	-	-	-
P. erosus	EC508/583	2	6	87 ±2	-	-	-	-	-	-
P. ahipa x	AC102/485	3	10	100 =0	1	2	89 ±1	-	-	-
P. erosus	EC114/1285	3	10		-	-	-	-	-	-

derived from self pollination. The first F2 combination yielded a fertility percentage of 75±3%, based on four samples. The fertility of the second combination was calculated to 88±6% on the basis of six samples, results representing the F3 of this combination (or rather the true F2 i.e. selfed) yielded an overall pollen fertility of 84±5, based on four samples, see Tab. 4. The final combination showed the highest fertility of the F2 material, i.e. 92±3%, on the basis of four samples, the F3 and F4 yielding 78±0% and 77±0% respectively, Tab. 1.

Overall fertility percentages of the parental species and the hybrid combinations are given in Tab. 5.

3. Compatibility («cross-ability») of parent and F1 material

The occurence of autonomous agamospermy has never been recorded within the genus, but in order to rule out the possibility of this phenomenon a number of flowers from the parental species were emasculated and isolated. All emasculated flowers abscised within 48 hours following emasculation.

A total of 172 artificial pollinations were attempted, including parent as well as hybrid material; of these 71 (41%) were successful, i.e. resulted in mature seeds, and 6 (3,5%) aborted before complete development.

The only combinations where no registrable ovary growth occurred were when *P. erosus* cultivars had been artificially pollinated with *P. erosus* wild form pollen and the reciprocal combination. All other combination induced varying degrees of avariall growth, although differences in the willingness to accept interspecific pollen by *P. tuberosus* appeared to be somewhat varying.

The results are presented in Fig.2.

4. Germination percentages

Because the parent material, germinated for use in cross pollination and growth experiments, was obtained both from direct collection in the field as well as a range of agricultural and botanical institutions, the germination percentages observed were extremely heterogeneous, i.e. some accessions had been stored for a number of years, thus reducing the germination capability of the material. In order to evaluate the actual germination percentages of the parent material it was therefore decided to include the material from field collections only ; only material included in the cross breeding experiments are included in Tab. 6

Table 2 : Pollen fertility in <u>P. erosus</u> x <u>P. tuberosus</u> and the reciprocal combination

Hybrid combinaison	Accession number	Percentage stained pollen								
		Parent			F1			F2		
		No. ind	No. X	%±SD Xmean	No. ind	No. X	%±SD Xmean	No. ind	No. X	%±SD Xmean
P. erosus x	EC503/882	5	20	99 ±2	2	8	91 ±1	-	-	-
tuberosus	TC118/186	7	28	97 ±1	-	-	-	-	-	-
P. erosus x	EC116/186	2	6	88 ±3	1	2	85 ±3	-	-	-
tuberosus	TC118/186	7	28	97 ±1	-	-	-	-	-	-
tuberosus x	TC118/186	7	28	97 ±1	1	2	93 ±3	-	-	-
P. erosus	EW522/386	3	11	99 ±1	-	-	-	-	-	-
tuberosus x	TC118/186	7	28	97 ±1	1	2	88 ±2	-	-	-
P. erosus	EC032/285	3	18	98 ±1	-	-	-	-	-	-

Table 3 : Pollen fertility in <u>P. ahipa</u> x <u>P. tuberosus</u> and the reciprocal combination

Hybrid combinaison	Accession number	Percentage stained pollen								
		Parent			F1			F2		
		No. ind	No. X	%±SD Xmean	No. ind	No. X	%±SD Xmean	No. ind	No. X	%±SD Xmean
P. ahipa x	AC102/485	7	31	100 ±0	3	10	95 ±3	-	-	-
tuberosus	TC118/186	7	28	97 ±1	-	-	-	-	-	-
P. ahipa x	AC524/387	3	10	100 ±1	1	2	92 ±1	-	-	-
tuberosus	TC118/186	7	28	97 ±1	-	-	-	-	-	-
tuberosus x	TC118/186	7	28	91 ±1	-	-	-	-	-	-
P. ahipa	AC102/485	7	31	100 ±0	-	-	-	-	-	-

Table 4 : Pollen fertility in <u>P. tuberosus x (P. erosus x P. ahipa) and in P. tuberosus x</u> <u>(P. ahipa x P. erosus)</u>

Hybrid combinaison	Accession number	Percentage stained pollen								
		Parent			F1			F2		
		No. ind	No. X	%±SD Xmean	No. ind	No. X	%±SD Xmean	No. ind	No. X	%±SD Xmean
P.tuberosus x	TC118/186	7	28	97 ±1	-	-	-	2	4	76 ±3
(P. erosus x	ECO32/285	3	18	98 ±1	2	6	85 ±2	-	-	-
P. ahipa)	AC102/485	7	31	100 ±0	-	-	-	-	-	-
P.tuberosus x	TC118/385	7	28	97 ±1	-	-	-	3	8	84 ±6
(P. ahipa	AC102/485	7	31	100 ±0	2	8	88 ±4	-	-	-
P. erosus)	EC508/583	2	6	87 ±2	-	-	-	-	-	-

		F3		
		No. ind	No. X	%±SD Xmean
		1	2	88 ±0
		-	-	-
		-	-	-

The period from sowing till germination was recorded, and as would be expected, due to more uniform storage, the cultivars germinated more uniformly, i.e. within a few days. In contrast the wild species and wild forms of cultivated species would germinate over a period of as many as 20 days.

Another factor limiting germination is the varying tolerance towards the moisture level in the medium, i.e. non-fertilized peat was used for germinating the material. Especially the seeds of *P. ahipa* and *P. ferrugineus* would succumb to fungal attacks if insufficient drainage was provided. Hence, the seeds of these two species were germinated in cleaned quarts sand. This method increased the germination percentage significantly.

Hybrid F1 and F2 seed exhibited germination percentages very similar to the percentages of parent material originating from field collections with one exception only : *P. tuberosus* x *P. ahipa*. This observation is remarkable considering the interspecific nature of the material, i.e. most interspefic material originating from artificial pollination exhibit a significant decrease in the germination percent, especially F1 material (Hoover et al. 1985, Baden 1987) ; see Tab. 7.

Although normal in appearance, the hybrid seed from the combination of *P. tuberosus* x *P. ahipa*, was, as mentioned, incapable of germinating. However, because the embryos were well developed the possibility of artificial embryo rescue is currently being contemplated.

5. Legume (pod)/seed yield of hybrid, F1 and F2 material

The average seed yield per pod per artificial pollination in the hybrid combinations and in combinations involving F1 material are shown in Fig. 3. The maximal number of seeds recorded in a single pod is 11, i.e. in a Mexican cultivar of *P. erosus* , but this number has not been observed in any of the pods resulting from artificial pollination.

The pod/seed yield observed in the hybrid, F1 and F2 material as a result of unassisted self-pollination was similar or higher than that observed in the parental species, with the exception of some plants belonging to *P. ahipa* . Hence, the observation that hybrid material involving *P. ahipa* had remarkably high seed/pod yields cannot surprise.

The seed yield per hybrid combination, F1 individuals, resulting from unassisted selfing are presented in Tab. 8.

Table 5 : Overall pollen fertilities of species and hybrid combinations

Species/ Hybrid combinaison	% stained pollen			
	F1 %±SD Xmean	F1 %±SD Xmean	F1 %±SD Xmean	F1 %±SD Xmean
P. erosus	96 ±5	-	-	-
P. ahipa	98 ±1	-	-	-
P. tuberosus	97 ±1	-	-	-
P. erosus x P. ahipa	84 ±4	92 ±3	78 ±0	77 ±0
P. ahipa x P. erosus	88 ±2	-	-	-
P. erosus x P.tuberosus	90 ±3	-	-	-
P.tuberosus x P. erosus	91 ±4	-	-	-
P. ahipa x P. tuberosus	94 ±3	-	-	-
P.tuberosus x P. ahipa	-	-	-	-

Table 6 : Germination percentages in parental species
Stored for minimum of one growth season prior to germination

Accession Number	Origin/Locality	No. seeds	No. seeds germinated	Germ.
ECO32/285*	Yucatant, Mex.	45	8	18
EC114/1285	Parà, Brazil	14	12	86
EC116/186	Jutiapa, Guat.	10	10	100
EC503/882	Gto, Mex.	15	8	53
EC508/583*	-, Mex.	20	12	60
EC512/385*	-, Mex.	10	9	90
EW522/386*	Reduit, Maurit.	17	16	94
Total	erosus	131	75	57
AC102/485	Tarija, Bolivia	165	69	42
TC118/186	N. Esté, Haïti	18	18	100

Table 7 : Germination percentages in F1 - and F2 - hybrids

Cross number(s)	Combinaison accession numbers	Species	F1/F2	No. seeds sown	No. seeds germ.	Germ. %
14	EC032/285 x EC508/583	erosus erosus	F1	3	3	100
10, 20, 21, 23, 24, 40, 50	EC032/282 x AC102/485	erosus ahipa	F1	43	39	91
27, 28	EC512/385 x AC102/485	erosus ahipa	F1	16	5	31
50	EC116/186 x AC102/485	erosus ahipa	F1	4	3	75
Total	erosus x ahipa		F1	63	47	75
31	AC102/485 x EC508/583	ahipa erosus	F1	3	2	67
38	AC102/485 x EC032/285	ahipa erosus	F1	3	0	0
64	AC102/485 x EC114/1285	ahipa erosus	F1	7	3	43
Total	ahipa x erosus		F1	13	5	38
43	EC503/882 x TC118/186	erosus tuberosus	F1	9	7	78
52	EC116/186 x TC118/186	erosus tuberosus	F1	3	1	33
55, 56	EC114/1285 x TC118/186	erosus tuberosus	F1	6	5	83
Total	erosus x tuberosus		F1	18	13	72
68	TC118/186 x EW522/386	tuberosus erosus	F1	5	5	100
86	TC118/186 x EW115/1285	tuberosus	F1	4	2	50
89	TC118/186 x EC032/285	tuberosus erosus	F1	3	3	100
Total	tuberosus x erosus		F1	12	10	83

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Table 7 : Germination percentages in F1 - and F2 - hybrids

Cross number	Combinaison accession numbers	Species	F1/F2	No. seeds sown	No. seeds germ.	Germ. %
47, 48, 64B, 65	Ac102/485 x TC118/186	ahipa tuberosus	F1	12	12	100
60, 70	TC118/186 x AC102/485	tuberosus ahipa	F1	10	0	0
23*, 24*	EC032/282 x AC102/485	erosus ahipa	F2	5	5	100
84	EW522/386 x (EC032/285) x (AC102/485)	erosus erosus ahipa	F2	5	4	80
87, 88	TC118/186 x AC102/485 EC508/583	tuberosus ahipa erosus	F2	8	7	88
91	TC118/186 x (EC032/285) x AC102/485	tuberosus erosus ahipa	F2	1	1	100

6. Morphology and vigour of hybrid, F1, and F2 material

Less than 1% of the material resulting from articial pollination, i.e. including hybrid, F1 and F2 generations, aborted following germination. The few individuals that did abort cannot be demonstrated to have perished due to lethal genetic causes, as the deaths were observed in material of different genetic origin. A too high moisture content of the medium may have had a detrimental effect on the development of the young seedling ; i.e. the hybrid nature of the material does not affect germination capability.

A number of parental characters were found to be dominant when studying the morphology of the hybrid, F1 and F2 material. E.g. only two hybrid individuals F1 resulting from artificial pollinations involving *P. ahipa* exhibited a semi-erect habit, in the remaining material (F1) involving this species the twining habit of *P. erosus* or *P. tuberosus* dominated. However, in hybrids belonging to the F2-generation the complete range of growth habit, i.e. from very low erect and bushy types to coarse twining types are found. All combinations, in which *P. ahipa* had been used either as pollen receptor or as donator, possesses wing petals curling outward following anthesis ; this character is exclusively found in *P. ahipa*. Furthermore, all hybrid material involving this species showed varying degrees of reductions in structure of the inflorescence, especially reductions in the number of short shoots and number of flowers per short shoot. This observation indicates that some of the genetic factors, responsible for the structural complexity of the inflorescence within this genus, are indeed specific, i.e. although a considerable variation in the structure of the inflorescence can be observed within a single species, the existence of dominant genes on the interspecific level has been demonstrated, see also discussion on inflorescence types in papilionoid legumes by Tucker (1987).

The colour of the seed coat is a traditional character used when studying hybridization in legumes. The variation of this character on the species level has previously been studied by Clausen (1945) when reviewing the genus. Clausen's use of this character in his proposed taxonomy, is regrettably not entirely correct. In the description of *P. tuberosus* Clausen stated that the seeds of this species are red, black or black and white mottled. This has recently been confirmed but, black and white mottled seeds are in addition found in *P. ahipa*. The hybrid seeds resulting from an artificial interspecific hybridization will have the seed colour of the acting female where the species *P. erosus* and *P. ahipa* are concerned. In combinations using *P. tuberosus* as the acting female, i.e. the orange red seeded cultivar from Haïti, the hybrid seeds will not possess the orange red colour, but when *P. ahipa* is used as pollen donor the seeds will be black, or if *P. erosus* acts as the pollen donor the seeds will be greenish mauve. However, in all seed

Table 8 : seed yields in F1-hybrids resulting from unassisted selfing

* figures in brackets are lowest / highest seed yield per plant

Combinaisons Species	Duration harv. days	No. of plants in test	Mean no. seeds harv./plant*
erosus x ahipa	30 210	20	(9-) 77 (-212)
ahipa x erosus	30	1	58
erosus x tuberosus	30	2	(3-) 6 (-8)
ahipa x tuberosus	60 90	8	(47-) 78 (-139)
tuberosus x ahipa x erosus	30 600	3	(3-) 14 (-28)
tuberosus x erosus x ahipa	30	2	(68-) 93 (-117)

material resulting from selfing of hybrids, the seed colour has been intermediary between the seed colour of the parental species. The seeds resulting from selfing F1 material have maintained this intermediary colour.

Seed shap will be identical to that of the pollen receptor, if only the seeds resulting from the initial pollination is considered. Individuals of the F1 generation
where *P. erosus* has been involved (a species with flat, square to rounded seeds) and any of the two other species will have considerably thicker seeds, although not necessarily kidney shaped.

Also the morphology of the pod has been used as a species delimitating character. Like the seed colour the morphology of the pod in the F1-generation following an artificial pollination, will possess characters identical to those of the parent pollen receptor. This means that when studying the legume of a fruiting plant the hybrid nature of the individual will only become apparent in F2 material.

Viviparity was observed in *P. ahipa*, up to 90% of the seeds in one pod would occasionally be germinated ; the phenomenon was also recorded in combinations between *P. ahipa* and *P. tuberosus*. No other material, species or hybrids, have shown signs of viviparity.

Finally, all hybrids involving *P. ahipa* have shown to be non-sensitive to day-length variations, i.e. flowering of these hybrids occurs approx. 1 1/2 months following germination regardless of day-length.

DISCUSSION

The chromosome number, n=11, shared by all members of the genus is in concordance with the base number of the majority of the genera of the subtribe *Diocleinae* ; according to Goldblatt (1981) four of the seven genera of this subtribe have n=11. Some authors place *Pachyrhizus* in the subtribe Phaseolinae, e.g. Ingham (1979), based on a possible closer chemical relationship with the genus *Neorautanenia* Schinz (see note). This hypotesis cannot be rejected on differences in cytology as *Neorautanenia* likewise has n=11 ; Goldblatt (1981) states that ten of the fourteen genera of the subtribe Phaseolinae include species with n=11.

In the genus self-pollination is the rule, a fact confirmed through the isolation

Note :
Ingham (1979) incorrectly cited Lackey 1977) for placing the genus *Neorautanenia* in the subtribe Glycinae; Lackey (1977) includes *Neorautanenia* in the subtribe : *Phaseolinae*

of both entire inflorescences and single flowers. The pollen sacs will release the pollen unto the stigma prior to the opening of the standard and wing petals. The method used here for estimating the percentage live pollen has limitations : the stained pollen, although morphologically well developed, i.e. normal sized, non-shrivelled, not disfigured and non-vacuolized, may not necessarily be capable of fertilizing an ovule. However, this method gives a fair estimate of the percentage live pollen, and the term «pollen fertility» is used here for the percentage stained pollen.

P. erosus : the somewhat lower overall pollen fertility of this species (96±5%) can partly be ascribed to the heterogenous nature of the material and possibly partly to the fact that all accessions used were cultivars. The cultivation practice of this crop is based on raising plants from seeds collected the previous season, thus maintaining material with lower fertility which would have succumbed in nature.

All pollen samples from the three accessions of *P. ahipa* had high pollen fertility percentages (overall 98±1%). Two of the three accessions of this species have been included in the cross breeding experiments presented here (the hybrids originating from combinations involving the last accession have yet to flower).

The pollen fertility (97±1%) of *P. tuberosus* being intermediary between the two other species is based on three accession only. This species has the highest abscission rate recorded in the genus.

The interspecific F1-hybrids of *P. erosus* x *P. ahipa* and the reciprocal combination do not exhibit any significant differences in pollen fertility.

The pollen fertilities of artificial hybrids resulting from combinations between *P. erosus* x *P. tuberosus* (i.e. including the reciprocal combination) as recorded here exhibit no significant differences.

Pollen fertility in the combination *P. ahipa* x *P. tuberosus* though slightly lower than the fertility of both parental species.

The pollen fertility of the three F2 combinations studied, viz. *P. tuberosus* x (*P. erosus* x *P. ahipa*), *P. tuberosus* x (*P. ahipa* x *P. erosus*) and (*P. erosus* x *P. ahipa*) x (*P. erosus* x *P. ahipa*), compares well with the F1 fertilities. However, the fertilities recorded in the F3 and F4 hybrids tend to be somewhat lower. Further recordings of the fertilities of subsequent generations, resulting from unassisted selfing, are needed to demonstrate whether these hybrids will retain this level of fertility.

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In summary, the estimates of pollen fertility in the interspecific crosses presented here do not indicate the existence of any cross breeding barriers. However, the experiments will be continued, in order to further substantiate the postulated non existence of cross breeding barriers, i.e. in order to include all species within the genus.

Germination percentages of parental material (species) : A number of the accessions obtained from germplasm collections had been treated with various fungicides, this treatment appeared in some instances to reduce germination considerably. The germination percentages in the two combination involving *P. erosus* and *P. ahipa* indicate that some germination inhibiting factor may be present when *P. ahipa* acts as the pollen receptor. The remarkable difference in germination percentage, however, does not agree with the similarity in pollen fertility of the hybrids. Likewise, when comparing the germination percentage of *P. erosus* x *P. tuberosus* combinations with the pollen fertility of these combinations, there appears to be no basis for linking observations on pollen fertilities of interspecific hybrids and the germination capability of hybrid seeds. This conclusion is further substanciated when the combinations of *P. ahipa* x *P. tuberosus* are examined. Even though seeds will be produced in both combinations, only the seeds (as mentioned previously) from hybrids in which *P. ahipa* were used as pollen receptor will germinate. Furthermore, as stated, the pollen fertility of these hybrids do not have any significant reduction in pollen fertility. Germination percentages of the F2 material
indicate that germination will not become reduced as a result of possible segregation. As only a limited number of seeds belonging to this generation has been germinated, (the hybrid seeds will be germinated under natural growth conditions as part of the running project) any conclusions based on this material must be regarded as premature. However, the material resulting from selfing of the *P. erosus* x *P. ahipa* combination did exhibit a remarkably high germination capability.

The examinations of intraspecific compatibility have demonstrated the existence of barriers between cultivated material of *P. erosus* and a wild form of this species. This may seem paradoxical, and it may even be argued that the material determined as a wild form of *P. erosus* , because of the barrier towards the cultivars of the same species, could serve to validate Clausen's (1945) species : P. strigosus. However, the existence of such a barrier between a wildform and an intraspecific cultivar may be due to the nature/ origin of the material forming the basis for the cultivar. A review of this phenomenon is given in de Nettancourt (1977). Because of the apparent capability of interspecific cross breeding with cultivars of the two south American species, the conclusion transpiring from this result (until further

experiments involving wild forms have been conducted) must be that infraspecific hybridization barriers are present. The wild forms may thus be regarded as sibling species according to Grant (1981), though whether this occurrence constitutes a general character within the genus must await the addition of material of wild forms, not only belonging to *P. erosus*, but also of wild material of the two remaining cultivated species. Another explanation of the apparent existence of infraspecific hybridization barriers may be that such barriers are supressed in the process of selection for factors governing uniformity and yield performance. Finally, breeding experiments involving the species P. ferrugineus and P. panamensis will provide very important information on the existence of hybridization barriers between the species in the genus.

The viviparous behaviour of *P. ahipa* cultivars and *P. ahipa* x *P. tuberosus* combinations has yet to be examined in detail. Whether this observation constitutes a characteristic confined to some cultivars (of *P. ahipa*) or whether the phenomenon occurs as a result of cultivation under greenhouse conditions cannot be determined as yet. However, as the character appears dominant in *P. ahipa* x *P. tuberosus* combinations, this may indicate a closer resemblance between these two species than between *P. ahipa* and *P. erosus* ; viviparity has neither been recorded in *P. ahipa* x *P. erosus* combinations nor in *P. erosus.* The phenomenon must be regarded as a character to be found exclusively in cultivars, as such a character, if occurring in wild forms (or wild species), would be eliminated after one or very few generations, i.e. viviparous seeds will only complete a growth cycle if the pods are harvested and/or the germinated seeds are deposited in a growth medium instaneously.

In conclusion, the experiments conducted so far have clearly demonstrated that possibilities of interspecific hybridization between the cultivated species exist, thus providing opportunities for future breeding programmes aimed at creating new cultivars for specific conditions.
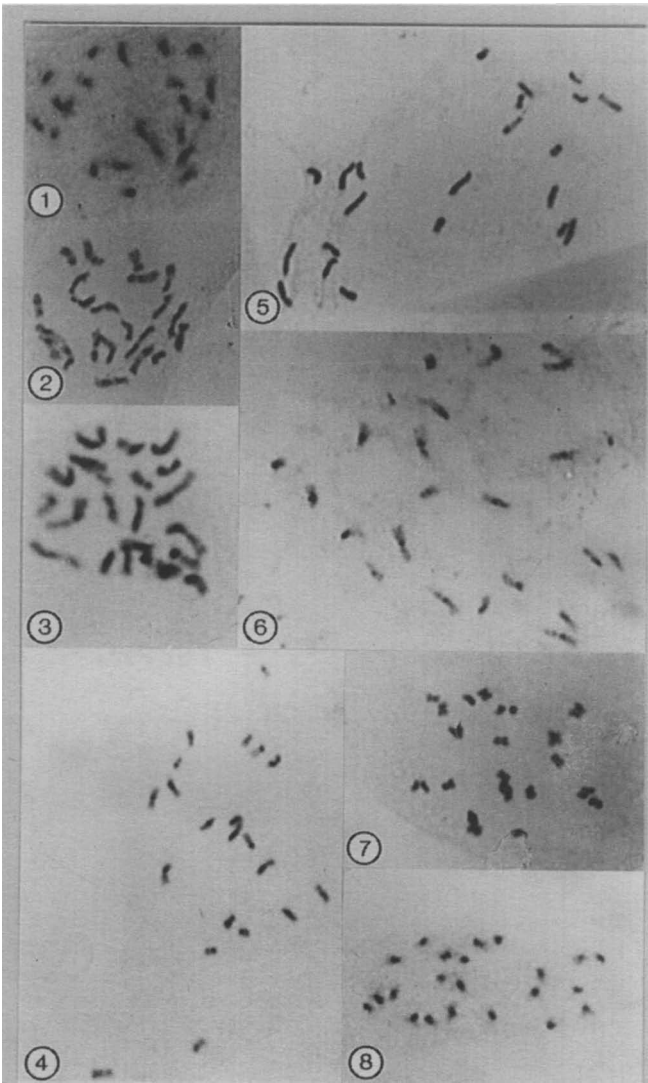
Fig. 1.a.

Cytological observations of mitotic cells from root tips of species : 1. *P. erosus,*
cultivar acc. no. EC107/1086 ; 2. *P. erosus,* wild form acc. no. EW048/385
; 3. *P. erosus,* wild form acc. no. EW115/1285 ; 4. *P. ahipa,* cultivar acc. no.
AC102/485 ; 5. P. panamensis, acc. no. PW055/385 ; 6. P. ferrugineus, acc.
no. FW044/385 ; 7. *P. tuberosus,* cultivar acc. no. TC118/186 ; 8. *P. tuberosus,*
cultivar acc. no. TC526/287.
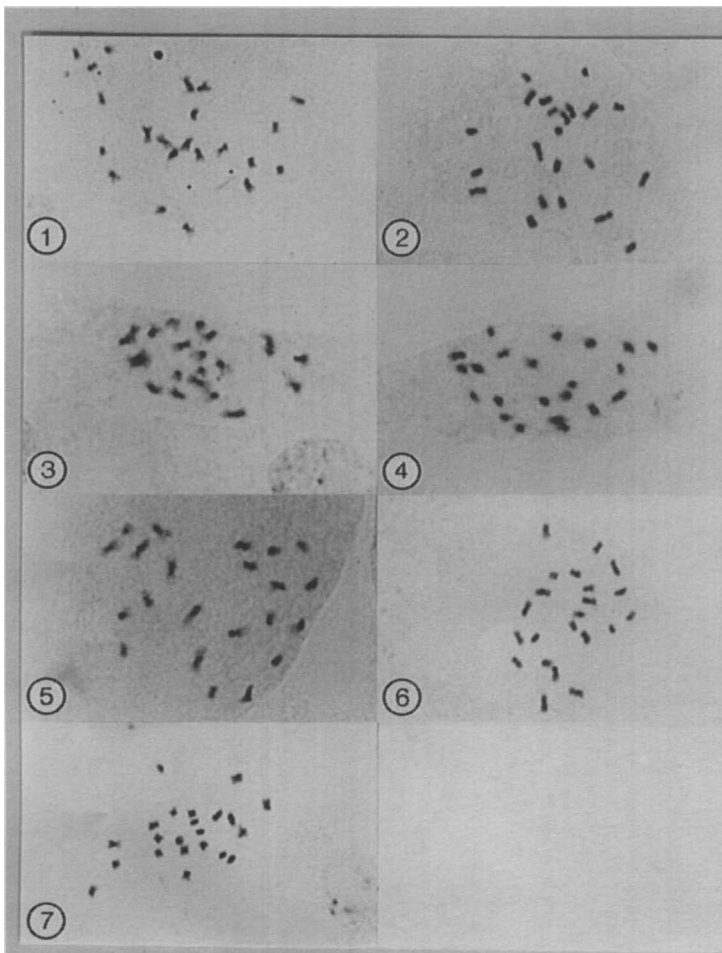All illustrations in this figure are x 2,800.

Fig. 1.b.

Cytological observations of mitotic cells from root tips of F1- and F2-hybrids
: 1. *P. erosus* cultivar acc. no. EC032/285 x *P. ahipa* cultivar acc. no. AC102/
485 ; 2. *P. ahipa* cultivar acc. no. AC102/485 x *P. erosus* cultivar acc. no.
EC114/1285 ; 3. *P. erosus* cultivar acc. no. EC503/882 x *P. tuberosus*
cultivar acc. no. TC118/186 ; 4. *P. tuberosus* cultivar acc. no. TC118/186
x *P. erosus* wild form acc. no. EW8522/386 ; 5. *P. ahipa* cultivar acc. no.
AC102/485 x *P. tuberosus* cultivar acc. no. TC118/186 ; 6. *P. erosus* wild
form acc. no. EW522/386 x (*P. erosus* cultivar acc. no. EC032/285 x *P. ahipa*
cultivar acc. no. AC102/485) ; 7. *P. tuberosus* cultivar acc. no. TC118/186
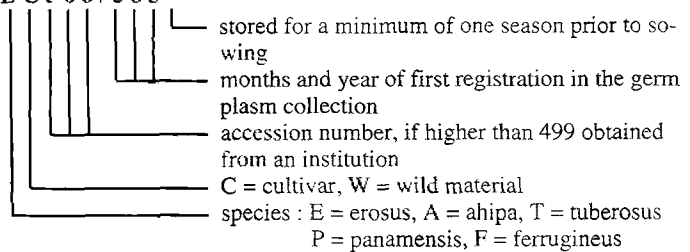x (*P. ahipa* cultivar acc. no. AC102/485 x *P. erosus* cultivar acc. no. EC508/
583).
All illustrations in this figure are x 2,400.

Figure 2 : Percentage successful crosses per total number of crosses; germination percentages of F_1 and F_2 hybrids; Mean number of seeds per successful cross
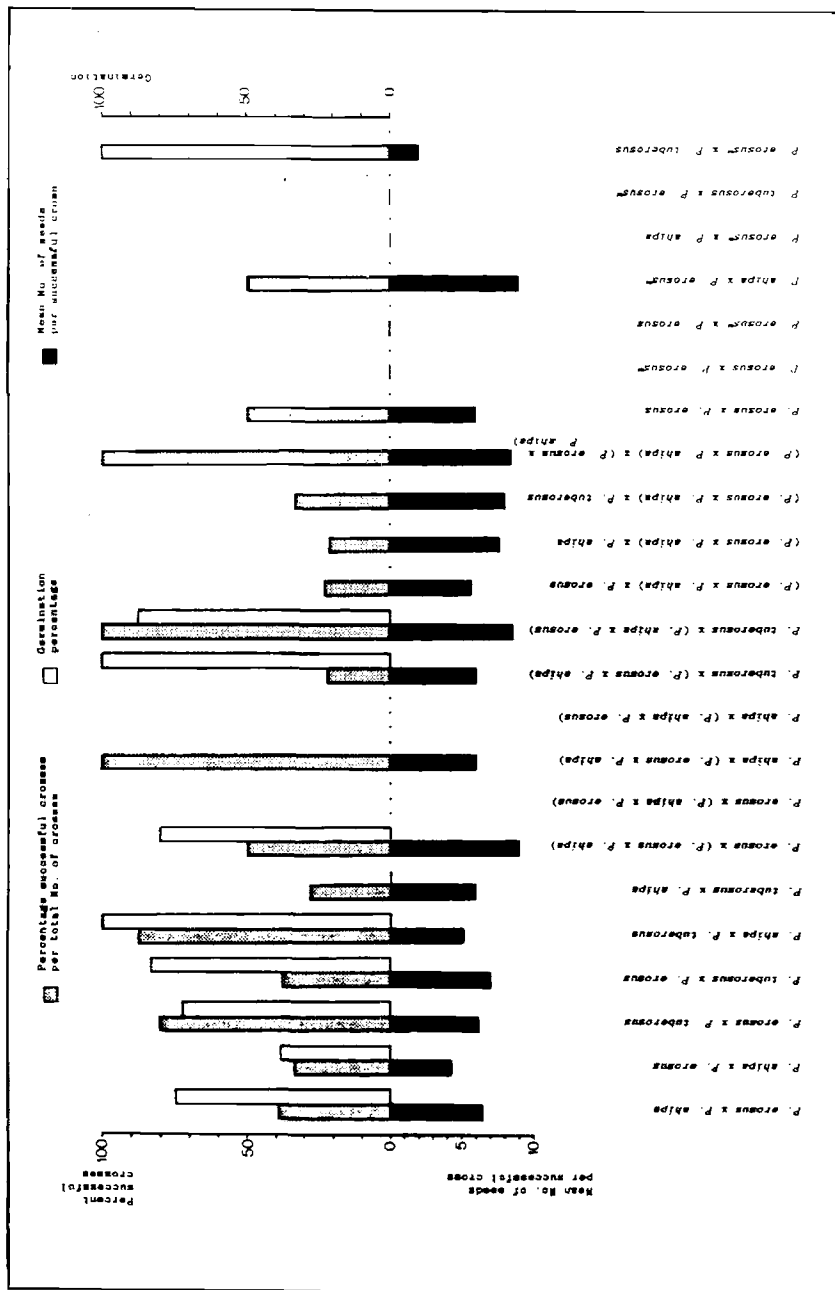
Appendix 1 : Accessions included in the cross breeding experiments

Accession number code : e.g. E C 5 0 8 / 5 8 3 *

stored for a minimum of one season prior to sowing

months and year of first registration in the germ plasm collection

accession number, if higher than 499 obtained from an institution

C = cultivar, W = wild material

species : E = erosus, A = ahipa, T = tuberosus
P = panamensis, F = ferrugineus

Pollen fertility (percentage stainable pollen grains) is based on the two highest observation. * indicates that the material has a reduced pollen fertility due to infection with the "sinkama mosaic virus".

tuber weights are calculated on a 250 days growth period.

<u>Species : P. erosus</u>
Accession number : ECO32/285*
           Origin : Kantunil, Edo. Yucatan, Mexico
           Status : Cultivated
      Germination : 18%
   Pollen fertility : 84 ±14%
    Tuber weight : 161 ±148 g

Accession number : EC114/1285
           Origin : Feijo Maneo, Para, Brazil
           Status : Cultivated
      Germination : 86%
   Pollen fertility : 98 ±2%
    Tuber weight : 249 g

Accession number : EC116/186
           Origin : Chimaltenango, Mun. Quesada, Depto. Jutiapa, Guatemala
           Status : Cultivated
      Germination : 100 %
   Pollen fertility : 80 ±10%
    Tuber weight : 218 ±55 g

Accession number : EC503/882
           Origin : C.A.E.B., Celaya, Edo. Guanajuato, Mexico
           Status : Cultivated
      Germination : 53%
   Pollen fertility : 68 ±30% %
    Tuber weight : 191 ±57 g

Appendix 1 : cont.

Species : P. erosus

Accession number : Ec508/583
> Origin : Mexico, Botanical garden, university of Copenhagen, Denmark. No. 7123
> Status : Cultivated
> Germination : 60 %
> Pollen fertility : 80 ±12%
> Tuber weight : -

Accession number : EC512/385*
> Origin : Mexico ? , C.I.A.T., Cali, Colombia. No. s.n.
> Status : Cultivated
> Germination : 90 %
> Pollen fertility : -
> Tuber weight : -

Accession number : EW522/386
> Origin : Central America ?, school o Agriculture, University of Mauritius, Reduit, Mauritius. No. CM1/84
> Status : Wild
> Germination : 100 %
> Pollen fertility : 94 ±5%
> Tuber weight : 159 ±49 g

Accession number : TC118/186
> Origin : Parc National Historique Citadelle Sans Souci, Ramier, Dept. Nord Este, Haïti
> Status : Cultivated
> Germination : 100%
> Pollen fertility : 89 ±7%
> Tuber weight : 84 ±48 g

Species : P. ahipa
Accession number : AC102/485* and AC102/1185 (2nd generation)
> Origin : Tarija, Prov. Tarija, Bolivia
> Status : Cultivated
> Germination : 42%
> Pollen fertility : 95 ±10%
> Tuber weight : 101 ±62 g

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