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INBREEDING IN CULTIVATED DIPLOID POTATOES

A thesis submitted to the Graduate School of
the University of Wisconsin in partial fulfillment
of the requirements for the degree of Doctor of
Philosophy.

by

Hielke De Jong

Degree to be awarded

January 19—

June 19—

August 19⁷¹—

To Professors:

Rowe
Bingham
Peloquin

This thesis having been approved in respect
to form and mechanical execution is referred to
you for judgment upon its substantial merit.

Robert M. Beck
Dean

Approved as satisfying in substance the
doctoral thesis requirement of the University of
Wisconsin.

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Date of Examination, Aug. 10 1971

INBREEDING IN CULTIVATED
DIPLOID POTATOES

BY

HIELKE DE JONG

A thesis submitted in partial fulfillment of the
requirements for the degree of

DOCTOR OF PHILOSOPHY
(Plant Breeding and Plant Genetics)

at the
UNIVERSITY OF WISCONSIN

1971

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ACKNOWLEDGMENTS

The author wishes to express his gratitude to Dr. P.R. Rowe for providing the plant material and financial support and for his enthusiastic guidance and encouragement of these investigations. I am also grateful to Dr. E.T. Bingham and to Dr. S.J. Peloquin for a critical reading of this manuscript.

I am indebted to the staff of the Interregional Potato Introduction Station at Sturgeon Bay, Mr. R.W. Ross, Mr. H.J. Schmidt and Mrs. R.F. Smejkal for their assistance in growing the plant material. In Madison, similar assistance was received from Mr. R.W. Ruhde, for which I am grateful. The assistance of several seasonal student helpers, especially Mr. P.W. Simon, is acknowledged.

Dr. J.H. Von Elbe, Department of Food Science, kindly loaned me several laboratory instruments. Laboratory space at the Experiment Station at Sturgeon Bay was provided by Dr. C.F. Koval, Department of Entomology.

I also wish to thank my co-workers, Mr. E.R. Everhart, Mr. R. Kessel, Dr. Heilyoung K. Lee and Dr. B. Maris for many stimulating discussions and evaluating ideas which are relevant to this thesis. The assistance of my wife, Siegelinde, for typing the manuscript is deeply appreciated. Her patience and understanding as well as that of our children, Walter, Werner and Margaret deserve my thanks. It is my hope that in the future I will be able to devote more time to my responsibility as husband and father.

I am grateful to the faculty, staff and students of this University and to the people of Wisconsin who have provided an environment conducive to a search for truth.

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INTRODUCTION

The successful exploitation of heterosis as a result of hybridization between inbred lines in maize has led to many similar investigations with other crops. The cultivated potato of Europe and North America, Solanum tuberosum L. Group Tuberosum ($2n = 4x = 48$) is generally considered a tetrasomic polyploid by most investigators. Although several workers have reported inbreeding studies in cultivated tetraploid potatoes, similar investigations in cultivated diploid potatoes have not been reported probably because the diploids are mostly self-incompatible. Research in this area is now possible as a result of studies of haploids of the cultivated tetraploid potato. Crosses between the cultivated diploid Groups Phureja and Stenotomum ($2n = 2x = 24$) and male-fertile Group Tuberosum haploids produce vigorous, highly fertile progeny. Most of these 24-chromosome hybrids are self-incompatible, but crosses involving haploids from an inbred Minnesota seedling, Minn. 20-20-34 (primarily US-W 4), were found to be self-compatible (Cipar, 1964; Smiley, 1963).

Genetic studies in potatoes have been hampered by a scarcity of suitable well defined marker genes. This can in part be attributed to the fact that the early genetic work was done exclusively with the tetrasomic Group Tuberosum. Thus progress in describing new markers has been very slow. The self-compatible, highly heterozygous 24-chromosome hybrids that are now available form the genetic base for much more efficient studies of inbreeding and potato genetics.

An investigation with a genetic base of diploid hybrids involving cultivated diploids and Group Tuberosum haploids, has several advantages over similar studies on the tetraploid level :

- 1) Since both the tetraploids and the self-compatible diploids may have several recessive genes not frequently expressed, these hybrids should be an extremely fertile source of unexplored genetic variability (Hougas and Peloquin, 1962).
- 2) The rate of progress towards homozygosity following selfing is much faster with diploids than with autotetraploids. The number of generations needed to reduce heterozygosity by 50 % by self-pollinating is 1 in diploids and 3.8 in autotetraploids (Bartlett and Haldane, 1934).
- 3) Stocks homozygous for marker genes of potential value for future genetic investigations can be obtained much faster with diploids.
- 4) The segregation ratios obtained for progenies from selfed seed would be much easier to analyze with a disomic inheritance pattern.

Self-compatible diploid hybrids were utilized therefore to evaluate the effects of inbreeding and to characterize some of the qualitative genetic variation present in these hybrids. The specific objectives of this study were :

- 1) To measure the inbreeding depression for several quantitatively inherited characters.
- 2) To search for marker genes which can be used in future genetic studies.
- 3) To develop clones and inbred lines with known genotypes for such marker genes.

LITERATURE REVIEW

A. Inbreeding in tetraploid potatoes

Several inbreeding studies have been reported in tetraploid potatoes and have been reviewed by Abdalla (1970), Engel (1957), Feistritzer (1952), Howard (1960) and Rudorf (1958). Inbreeding by selfing tetraploid potatoes has been proposed as a method of genetic analysis and for the development of superior lines for future breeding work (Feistritzer, 1952; Gowen, 1956; Krantz, 1946; Rieman et al. 1956). Some old American potato varieties such as Early Rose are reported to be derived by selfing Rough Purple Chili (Fruwirth, 1935; Salaman, 1926).

Krantz (1946) reported high yielding selections from the crosses of variety x inbred but none from the inbred x inbred crosses. Guern (1940) was also unable to produce higher yields than the parental varieties from inbred x inbred crosses. To determine whether heterosis is playing an important role in superior varieties, Engel (1957) calculated the coefficients of inbreeding and relationship for several commercial varieties and their parents. He concluded that the presence of heterosis in the common potato was still an unproven hypothesis.

Crossing inbred lines to obtain heterosis has also been proposed for tetraploid potatoes (Rudorf and Ross, 1952). However, in a theoretical model, considering two alleles per locus, Dudley (1964) has shown that the system of using inbred lines to produce highly heterozygous single crosses

cannot be used with autotetraploid crops. In this model, maximum heterozygosity would be realized in single crosses between non-inbred plants rather than in crosses between their selfed progenies unless effective selection occurs during selfing.

In another model, Busbice and Wilsie (1966) suggested that allelic interactions at trigenic and tetragenic loci are important factors contributing to hybrid vigor in autotetraploids. With this model, inbreeding would result in a very rapid loss of second and third order interactions, but crosses between two homozygous inbred lines could at best result in progeny in the duplex condition. The observations that several autotetraploid crops have an inbreeding depression which is more nearly like that expected of a diploid than of a tetraploid (Busbice and Wilsie, 1966; Dewey, 1966; Kalton *et al.*, 1952; Lundqvist, 1966) would support such a hypothesis. Krantz (1946) also observed that in tetraploid potatoes "The yields obtained in the first, second and third inbred generations were higher than the calculated yields based on the reduction in heterozygosity in an amphidiploid, but lower than yields calculated on the basis of the reduction in heterozygosity of an autotetraploid." The observations by Mendiburu (1971) on potatoes also lend support to the hypothesis of Busbice and Wilsie (1966). He found unusually high performance in tetraploid progenies from crosses between tetraploids and diploid Phureja-haploid Tuberosum hybrids. This was interpreted as a manifestation of the capacity of $2n$ -pollen, formed by first meiotic division restitution, to pass onto the progeny the already heterotic diploid genotype in a largely intact array. Beneficial intra- and inter-locus interactions are presumably combined upon fertilization of an n -egg from the tetraploid female parent with a $2n$ -gamete from an unrelated diploid male parent.

Dewey (1966; 1969) suggested that an accumulation of chromosome aberrations and unfavorable gene combinations could be the major cause of an inbreeding depression which was greater than expected for autotetraploid crested wheatgrass, Agropyron cristatum (L.) Gaertn. The gametic screen would eliminate most chromosome aberrations in diploids but the buffering effect of autopoloids would permit an accumulation of such aberrations .

Rudorf (1958) obtained several S_5 generation inbreds of tetraploid potatoes, but in many cases could not go beyond the S_2 as a result of sterility, general weakness of the plants and virus infections. Thus, the same could be said of inbreeding tetraploid potatoes as Busbice (1968) said of inbreeding tetraploid alfalfa : ".....the barrier to producing homozygous alfalfa is not the time required to achieve homozygosity but the loss of fertility and vegetative vigor in the inbred progeny ".

Chase (1963) has proposed a hypothetical analytical breeding scheme in which the techniques of inbreeding and ploidy manipulations would be utilized. Haploids extracted from autotetraploids could be crossed with diploid species, after which inbreeding of the diploid F_1 crosses would be required to fix desirable genetic material. Resynthesis of the tetraploid level is possible through colchicine doubling or by the use of gametes with the unreduced chromosome number.

B. Inbreeding in diploid potatoes

No inbreeding studies have been reported in cultivated diploid potatoes, probably because the diploids are mostly self-incompatible (Cipar et al., 1964). Graham (1963) reported little or no inbreeding depression upon selfing the self-compatible wild diploid species Solanum verrucosum Schlecht. Upon selfing introductions and intraspecific

hybrids of S. verrucosum, Abdalla (1970) found a severe inbreeding depression for stem height, leaflet size, per cent stainable pollen, seeds per fruit, tuber number and tuber weight. Within S. verrucosum, F_1 hybrids performed better than inbreds. For several characters, the magnitude of heterosis in the F_1 and the degree of inbreeding depression in the F_2 were found to be associated. Although S. tuberosum Group Phureja is normally self-incompatible, self-compatible plants do occur sporadically (Dodds, 1956; Paxman, 1958; 1960) and have been used in producing inbred plants (Abdalla, 1970; Lamm, 1945).

Self-pollination of normally self-compatible cross-fertilized crops frequently results in sharply reduced seed set. In fully self-compatible crops, this can be considered to be a manifestation of inbreeding depression. In species or hybrids which are only partially self-compatible, it is difficult to distinguish if reduced seed set is due to effects of inbreeding depression, or of partial self-compatibility. Cooper and Brink (1940) considered that in alfalfa the endosperm rather than the embryo was the basis for the inbreeding depression effect. It has been suggested also that epistatic gene action may be the important factor in zygote viability in alfalfa; recessive lethals in the homozygous state at one locus may mask the effects of viability genes at all other loci (Busbice, 1968). Other examples from the literature as quoted by Brink and Cooper (1947) where selfing in a normally cross-pollinated species causes a malfunctioning of the endosperm include Zea mays L. and Secale cereale L.

Reduced seed set upon selfing has been reported in the self-compatible species S. verrucosum, but the effect of inbreeding upon the endosperm in potato is not known. However, the endosperm is known to play

an important role in the success and failure of interspecific crosses (Lee and Cooper, 1958). The importance of the endosperm in seed development in potato is also evident from the observation that the recovery of haploids from tetraploids is regularly associated with the presence of a hexaploid endosperm (Wangenheim et al., 1960).

The discovery that highly self-compatible plants occurred in hybrid families when Groups Phureja and Stenotomum were crossed with haploids of Group Tuberosum (mainly US-W 4) (Cipar, 1964; Smiley, 1963) has made inbreeding by selfing possible in cultivated diploid potatoes. Several workers (Abdalla, 1970; Cipar, 1963; Hollenback, 1966; Howard, 1960; Smiley, 1963) have reviewed the genetics of self- and cross-incompatibility in potatoes. The one locus gametophytic system is reported to occur in most Solanum species, including S. tuberosum Groups Phureja and Stenotomum, haploids of Groups Tuberosum and Andigena and hybrids between haploids of Groups Tuberosum and Andigena with Groups Phureja and Stenotomum (Cipar, Peloquin and Hougas, 1964; 1967). A two locus gametophytic system has also been reported for some Solanum species (Pandey, 1962). Such a system has been postulated for Groups Phureja and Stenotomum as well (Abdalla, 1970).

The occurrence of self-compatibility in a hybrid between two self-incompatible parents has not been fully explained. Smiley (1963) postulated a type of complementary gene action in the self-compatible hybrid US-W 1109. If the parents, Group Phureja WRF 359.9 and US-W 4, would have the respective genotypes FF and F'F', then their hybrid US-W 1109 would be FF' and self-compatible. The progeny obtained by selfing US-W 1109 would then segregate in a 1 : 1 ratio for self-compatibility and self-incompatibility. Smiley interpreted his data to fit such a hypothesis.

Hollenback (1966) proposed a scheme similar to the one postulated by Pandey (1964) for crosses between Nicotiana alata and N. langsdorffii. In this scheme a hypomorphic condition of the S allele (S_{FI}) is assumed. The diploid species, S_I·I and the haploid US-W 4, S_{FI}·FI would both be self-incompatible but the hybrids S_I·FI would be self-compatible if there are no alleles in common, i.e. S₁·2 x S_{F3}·FI. With one allele in common the progeny would segregate in a 3 : 1 ratio and with two alleles in common (such as in selfing) a 1 : 1 ratio would be expected. The homozygous S₁·FI genotype might be pseudo-self-compatible and could increase the proportion of self-compatible plants.

There are reports of an increase in S-specificities upon inbreeding (Denward, 1963; Nettancourt and Ecochard, 1969; Pandey, 1970). Nettancourt (1969) suggested that the self-incompatible plant may be equipped with a switch system or a mutagenic mechanism which enables it to display a new specificity when this specificity is needed for increasing the level of genetic polymorphism in the population. Pandey (1970) proposed that the principal mechanism of the generation of new S-alleles was intracistronic recombinations in the structural cistron(s) of the S-gene complex. These recombinations would be controlled by regulating genes. Inbreeding would produce homozygosity of these genes and consequently reduce the suppression of recombination at the S-locus.

Recessive genes are known that control chromosome pairing (Riley and Law, 1965). This suggests that various abnormalities in chromosome behavior can be expected to occur upon inbreeding cross-pollinated species. Such plants have been found upon inbreeding in several crops (Rees, 1961). Among the S₁ progeny of S. rybinii (now Group Phureja) Lamm (1945) found 1 asynaptic plant, 2 plants with a higher chiasma

frequency than the parent and several plants with two or more univalents at metaphase I. The S_1 plants also segregated for morphological characteristics and varied widely in pollen fertility. Abdalla (1970) reported severe inbreeding depression in male fertility upon selfing Group Phureja. Several inbred plants with reduced pollen stainability were found to have from 2 to 8 univalents at metaphase I (Abdalla, 1970). Wang (1971) has found segregation for several meiotic abnormalities in F_2 populations of both inter- and intra-specific crosses of wild tuber-bearing *Solanums*. She provided evidence that abnormalities such as poor pairing at pachytene, high frequencies of ring bivalents at diakinesis and high univalent frequencies at metaphase I may be inherited as simple recessives.

C. Genetic markers

There are very few genetic markers that are available for research in the common potato. Much of the work in the early part of this century was based on the assumption that Group Tuberosum had a disomic inheritance pattern and has therefore become obsolete. Frumwirth (1925) and Swaminathan and Howard (1954) have reviewed this material. However, with a tetrasomic inheritance pattern, progress in describing new markers has been very slow because of the large populations that are required (Hougas and Peloquin, unpublished). Furthermore, a large proportion of the more recently reported mutants are either semi-lethal (non-flowering) or forms of cytoplasmic male sterility that do not lend themselves to use as genetic markers. A list of recently identified or currently used genes is presented in the Appendix (A). There is no standard nomenclature for genes in potatoes; the same symbol has been used for different genes (Howard, 1970).

Hougas and Peloquin (1962) have pointed out that a genetic study of self-compatible hybrids between cultivated diploid Groups Phureja and Stenotomum and haploids of Group Tuberosum can be expected to produce several marker genes. Many recessive genes exist in Group Tuberosum which are infrequently expressed due to tetrasomic inheritance. The Groups Phureja and Stenotomum are known to be sources of great genetic diversity. Due to self-incompatibility, recessive genes of these two Groups are also infrequently expressed. Inbred progenies from self-compatible diploid hybrids, involving Groups Phureja and Stenotomum and Tuberosum haploids should therefore be a fertile source of unexplored genetic diversity.

1. Genes controlling synthesis and distribution of anthocyanins

Dodds (1962) stated that : " In cultivated potatoes there are no characters of taxonomic value based on differences of colour due to anthocyanins. The genetic system for the production and control is the same in all potatoes. They are able to develop anthocyanins but their appearance and distribution are governed by genes different from those controlling production of pigment. The same anthocyanins occur in similar patterns in plants of different ploidy but the correlations between organs of the individual have not been determined in most instances." However, since 1962 some differences have been reported in pigmentation systems between the cultivated diploids and the cultivated tetraploids (Howard, 1970; Howard et al., 1970).

Howard (1970) showed a general scheme of at least six major genes controlling anthocyanin pigmentation in Group Tuberosum. The inheritance of pigmentation in the cultivated diploid species proposed by Dodds and Long (1955) is similar but not identical with this scheme. In the diploids,

there is no distinction between periderm and phelloderm pigmentation of the tubers, whereas in Group Tuberosum this is controlled by two separate genes (Howard, 1960; 1970). It now appears that in Group Tuberosum R and E are allelic (Howard, 1968). Howard (1960) has suggested that the Group Tuberosum R may be more comparable to the R^{DW} than to the R of the cultivated diploids. There is some evidence that Group Tuberosum R is linked with F (Lunden, 1960). However, this is not true in the diploids (Howard, 1970), where F is known to be linked with B and I (Dodds and Long, 1956). On the basis of their effect on tuber pigmentation, I in the diploids seems to be identical to D in Group Tuberosum (Howard, 1969; 1970). Similar differences appear to exist between the inheritance schemes of spectacle in the cultivated diploids and Group Tuberosum. In the diploids, the genotype Ii^{SD} together with either P or R or both produces spectacle (Dodds and Paxman, 1962; Simmonds, 1969). In Group Tuberosum the situation is more complicated. The genotypes REMD or BEED can produce both hidden spotted or spectacled. M is the gene responsible for the pigment restriction and is closely linked to R (Howard, 1970). In the diploids, the gene responsible for the restriction of pigmentation (i^{SD}) is an allele of I and is not linked to R.

There is also evidence that the pigmentation systems of the wild and cultivated diploid species differ. Paxman (1960) stated : " In the wild potatoes, no pigment variation is known that corresponds to the variation associated with the R and Ac loci in the cultivated types. The blue-flowered wild forms correspond to the dominant P genotype but the white-flowered forms do not behave as though they were pp in constitution; thus crossing with white cultivated clones (pp) gives coloured hybrids." Paxman (1961) has postulated an inhibitor in several wild diploid species,

Co, which when homozygous recessive suppresses pigmentation in the corolla (similar to ff in the cultivated species). Paxman (1961) claims that Co is linked to the B I F linkage group of the cultivars.

The close linkage of the three genes B, I and F, all of which affect the distribution of anthocyanins in diploid potatoes, is very similar to the situation in eggplant (Solanum melongena L.) where three closely linked factors for anthocyanins are postulated (Tigchelaar et al., 1968). One locus is thought to have three alleles. The B locus in potato consists of a group of alleles in a hierarchical system of dominance in the following order : $\underline{B}^d > \underline{B}^c > \underline{B}^b > \underline{B}^a > b$ (Dodds and Long, 1956).

Instances of multiple alleles, "super genes" or pseudo alleles affecting anthocyanin development and distribution, are relatively common in nature (Alston, 1959). This raises some questions with regard to gene action in anthocyanin synthesis. Alston (1959) suggested that : " In Solanum, as well as in Streptocarpus, tissue culture of certain critical plant parts affected by each allelic form might prove valuable. Since in such cases the plant possesses the factors required for anthocyanin synthesis, it should be possible to initiate pigment formation in isolated parts of a plant, even though that plant does not carry the allele governing pigmentation therein. The stimulus, if found, would be a key to gene action."

Lunden (1937) postulated that the differences in segregation ratios which he obtained from the crosses Pppp x pppp and Dddd x dddd would be due to a different degree of double reduction. He suggested that the factor P would be located close to the centromere and the factor D far from the centromere. By means of the half-tetrad analysis in 4x - 2x crosses, Mendiburu and Pqloquin (1969) calculated the map distance P - centromere to be 12 - 14 units.

Peloquin and Hougas (1959) have demonstrated the value of pigmentation genes in the extraction of haploids from Group Tuberosum. The development of a line with the genotype $\underline{PPE}^d \underline{E}^d$ by Hermesen (1970) is a potential improvement in this area.

Physiologically, anthocyanin production is often the result of conditions which are injurious to the plant. Alston (1959) listed the following conditions which may be responsible for anthocyanin formation in plants genetically competent to produce the pigment : low temperature, tissue damage, plant tumors, mineral deficiency, abnormally high concentrations of growth factors and autumn coloration. Nilsen (1971) has shown that near-ultraviolet light can induce anthocyanin synthesis in the diploid potato clone US-W 5279.15.

ii. Chemogenetic markers

Biochemical techniques have been used to study several phenolic substances, especially the flavonoids, in the Solanum species (Harborne, 1967; 1968; Harborne and Simmonds, 1964). Harborne (1958; 1959) has outlined the basic methods of paper chromatography of anthocyanins and of other flavonoid pigments. Hess and Meyer (1962) have demonstrated the separation of anthocyanins with thin layer chromatography (hereafter abbreviated as TLC). Stahl (1969) gives the most comprehensive treatment of the voluminous literature which has appeared to-date in relation to the TLC method. Structures of some phenolic compounds of potatoes are given in Fig. 1.

The chemical activity of the R locus appears to be rather complex. Dodds and Long (1955) listed 3 components produced by the R locus and concluded that "...it becomes a matter for speculation whether this locus is in fact three closely linked genes or a compound structure of subunits

not classifiable as genes within their own rights." These authors also noted that the activity of the subunit of the R locus concerned with floral pigment is not suppressed by P, whereas the subunit of R responsible for tuber pigment is suppressed in the presence of P. Thus epistasis occurs in the tubers only.

The P locus seems to be less complicated. In Group Phureja, P controls the production of delphinidin in both flowers and tubers (Harborne, 1960 a). The biochemical pathway of pigment synthesis as it relates to P and R is not clear although some pathways have been proposed (Dodds and Long, 1955; Howard et al., 1970). Any hypothesis must take into account the fact that epistasis of P over R does occur in the tubers but not in the flowers.

The Ac locus is concerned with the acylation of the anthocyanins with p-coumaric acid, the attachment of a glucose residue in the 5-position and the methylation of delphinidin and cyanidin derivatives. On the basis of these various biochemical effects, Harborne (1967) has suggested that, like the R locus, the Ac locus may be compound. Both acylation and methylation are not completely effective in plants with Ac- genotypes. Thus often both acylated and unacylated forms of the above mentioned pigments are found in the presence of Ac-. Plants with the genotype acac have only the unacylated pigments.

Tubers of several varieties of Group Tuberosum contain only single pigments which contrasts with the mixtures of pigments in flowers and tubers of cultivated diploids reported by Dodds and Long (1955) and Harborne (1960 a). Tubers of Group Andigena examined by Howard et al. (1970) did contain mixtures of pigments similar to the cultivated diploids. They speculated that " The differences between the Andigena potatoes with their

mixed pigments and the Tuberosum varieties with single pigments may be correlated with the fact that Tuberosum varieties are descended from a very small number of introductions from South America and that variability in the Tuberosum group was further cut down by the extensive inbreeding following the blight epidemics of the 1840's."

In Group Tuberosum tubers, the gene P adds an OCH_3 group to peleragonidin to produce peonidin. The fact that the genes E and R of Tuberosum produce the same anthocyanin in the periderm and phelloderm respectively has been considered additional evidence that these genes are allelic (Howard et al., 1970).

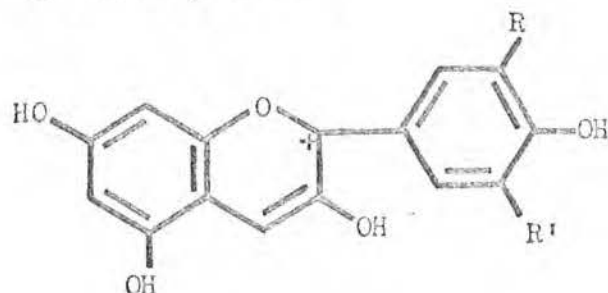
Samorodova-Bianki (1965) has analyzed the anthocyanidins and flavonoids in tubers and flowers of several Solanum species. Rutin was found to be the main flavonol of potato flowers and varied in per cent of corolla dry weight from 2.5 in S. tarijense to 12.5 in certain Group Tuberosum varieties.

The distribution of a number of phenolic glycosides in potato species appears to have taxonomic significance. The berries of 19 wild tuber-bearing Solanum species have been found to contain a caffeic acid derivative which was absent from the berries of cultivated potatoes and of non tuber-bearing Solanums. In contrast, berries of cultivated potatoes are known to contain a coumaric acid derivative and a coumarin which are not present or are very rare in the wild species (Harborne and Corner, 1961).

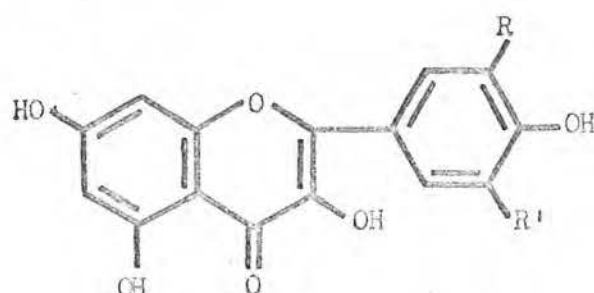
Harborne (1962) found a total of 9 flavonol glycosides in flowers of 60 wild species belonging to 12 different Series and 39 cultivars. Kaempferol 3-glucoside was found only in S. santolallae. All species in the three Series, Conicibaccata, Piurana and Demissa which are known to be closely related, were found to be particularly rich in kaempferolglyco-

Fig. 1. Structures of some phenolic compounds of potatoes

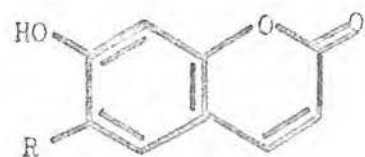
A. Anthocyanidins

Pelargonidin, $R = R' = H$ Cyanidin, $R = OH, R' = H$ Peonidin, $R = OCH_3, R' = H$ Delphinidin, $R = R' = OH$ Petunidin, $R = OH, R' = OCH_3$ Malvidin, $R = R' = OCH_3$

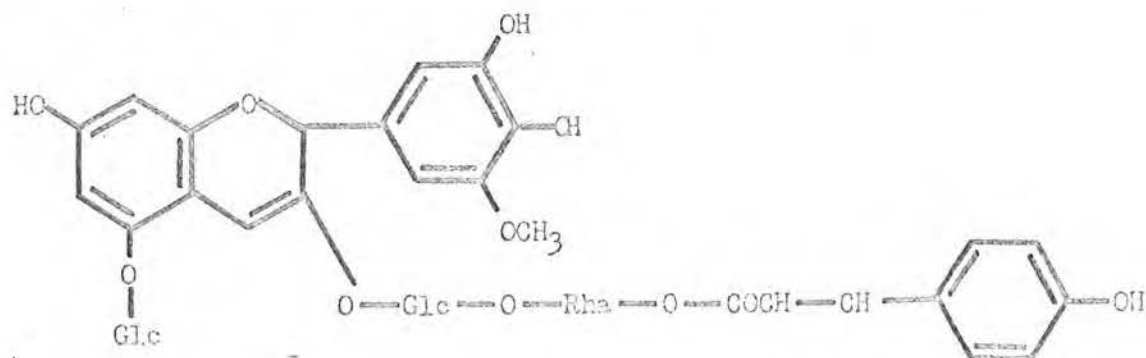
B. Flavonols

Kaempferol, $R = R' = H$ Quercetin, $R = OH, R' = H$ Myricetin, $R = R' = OH$

C. Coumarins

Aesculetin, $R = OH$ Scopoletin, $R = OCH_3$

D. Petanin (Petunidin 3-(p-coumaroyl rutinoside) 5-glucoside)



sides. Luteolin 7-glucoside was found only in S. stoloniferum and in S. x vallis-mexici which is considered to be a hybrid between S. stoloniferum and S. verrucosum (Marks, 1958). Two potato seed glycosides appeared to be specific to the tuberous Solanum species. A gene which controls the transfer of glucose to quercetin 3-rhamnosylglucoside, G1, was found in S. chacoense and S. stoloniferum (Harborne, 1960). The species S. pinnatisectum, S. jamesii and S. sambucinum, all of which belong to the Series Pinnatisecta (Hawkes, 1963) contain three coumarins which do not occur in 70 other species (Harborne, 1962).

iii. Genes controlling morphological and physiological characters

Simmonds (1965) listed 12 mutants which could be distinguished on a morphological or physiological basis. Of these 12 mutants, 4 are reported to have reached the flowering stage : nlnl, ymym (Dodds and Paxman, 1962), drdr and shsh (Simmonds, 1965). Of these, only droopy (drdr) plants are known to have been used successfully in crosses (Simmonds, 1966). The dr locus is linked to the S locus (Simmonds, 1965; 1966). The Ym (yellow margin) locus is sometimes linked to a recessive lethal in the repulsion phase. Heterozygous Ymym plants yield significantly more than homozygous YmYm. It has been suggested that the heterotic effect on yield of that part of the chromosome which contains the Ym locus has led to the preferential selection of heterozygotes and hence to their high frequency in cultivation (Dodds and Paxman, 1962). A search for auxotrophic mutants by Simmonds (1965) proved unsuccessful.

A large number of somatic mutations have been reported. In a review Heiken (1958) listed 14 different types of somatic mutations, including changes in leaf type, flower color and tuber color. Howard (1970) has

reviewed more recent cases. None of these mutants have been reported to flower and are therefore of little use as genetic markers.

A considerable number of genes have been described which confer disease resistance (Appendix, A). Tests for the presence of most of these genes are rather complicated because often many independent genes can confer resistance and the expression of resistance is frequently a matter of degree. The group of genes which produce the resistant reactions to potato viruses A, B, C, X, Y and S require relatively less complicated procedures for their detection. It appears that these reactions are controlled by single dominant genes. However, disturbed segregation ratios, due to as yet undetermined causes, are sometimes encountered (Cockerham, 1970). Howard and Fuller (1965) reported that in Group Tuberosum Nx, Na and Nc were located on 3 different chromosomes of the same homologous group of 4. Nb was found to be independent of Nx and Nc. In another family of different origin, Nx, Nb and Nc were found to be independent of each other (Howard and Fuller, 1965). The same genes are also present in several diploid species (Ganguly, 1963).

Cockerham (1970) provided evidence from crosses between the Group Tuberosum cultivars Craigs Defiance (Nx, Nb, Na, Nc) and Flourball (nx, nb, na, nc) that Nx and Na are closely linked in the coupling phase and that Nb segregates independently of Nx, Na and Nc. The relationship between the linkage group on which Nx and Na are located and Nc is still not clear (Cockerham, 1970). Because the old system of symbolism for genes affecting resistance to viruses X and Y was not sufficiently discriminating in distinguishing genes with similar effect but located in different species or at different loci, a new symbolism in which the basic type, origin and effect of these genes is easily apparent has been pro-

posed (Cockerham, 1970).

In a consideration of the general genetic system of virus resistance, Cockerham (1970) states " The evidence available indicates that reactions to virus X are conditioned by six genes situated at four, or possibly five, different loci (There are no data on the relationship of R_x and Nb_{tbr}). Two of these loci, Nx_{tbr} and Nx_{chc}, appear to be associated in a linkage group which also contains the loci of genes controlling reactions to Y viruses, namely, Ma_{tbr}, Ny_{chc} and, possibly, Nc_{tbr}. A significant feature of this association is that each of the genes concerned controls a lethal necrotic reaction and it may well be that the whole group of genes is situated within a section of chromosome which is in overall control of this response to infection irrespective of the virus evoking the response."

In addition to the above mentioned dominant genes, some recessive genes are known to confer resistance to some virus diseases. Bagnall and Young (1968) reported this type of resistance to virus S in the Group Tuberosum variety Saco. Kishore and Riemann (1968) found a recessive gene for immunity to virus X which seems to act independent of the other known dominant and complementary genes for resistance to virus X.

MATERIALS AND METHODS

The field experiments were conducted at the Interregional Potato Introduction Station, Sturgeon Bay, Wisconsin. The initial plant materials used in these investigations are listed in Table 1. During the course of these studies several inbred lines and clones with known genotypes for certain genes have been derived.

A. Vigor and yield

In each generation, plants were self-pollinated and the seed produced by self-compatible plants bulked to produce subsequent generations. Observations were made on populations grown from true seed and from tubers. Families grown from true seed were started in the greenhouse and transplanted to the field. Two replicates of seedling populations were obtained by dividing a bulked population of seed into 2 equal lots. All plants producing tubers were saved for evaluation in tuber plantings. Replicated trials were planted in the field in 2 years.

The following characteristics were measured :

1. Plant height - in cm when the plants had reached maximum height.
2. Vigor index - based on total amount of foliage. Individual plants were rated on a scale from 1 (very weak or small) to 5 (very vigorous).
3. Number of tubers per hill.
4. Tuber weight - g per hill.

B. Fertility

In 1968 a fertility study was conducted on all replicates planted from tubers. The data were obtained by using the decapitation technique (Peloquin and Hougas, 1959), and methods similar to those employed by Hollenback (1966). For each flowering inbred clone to be tested, 3 stems with 4 buds each were collected at random from a large population of Group Phureja plants, and these 12 flowers were then used as females to test the pollen-fertility. Up to 2 stems, also with 4 buds each, were cut from each flowering clone of the inbred populations. On each stem, 3 flowers were used to test the self-compatibility, and one flower was emasculated and pollinated with a mixture of pollen from the Group Phureja population to measure the female-fertility. A total of 3651 pollinations were made; of these 935 were made to measure self-compatibility, 2375 to measure pollen-fertility and 341 to measure female-fertility. The data obtained were also used to classify individual clones in families US-W 5309 and US-W 5328 for their compatibility behavior.

The progenies obtained by selfing the two self-compatible S_0 clones, US-W 5328-11 and US-W 5328-20, were analyzed for self-compatibility in 1970. The pollinating procedures were the same as used in the fertility study in 1968. In contrast to 1968 when the populations used were derived from bulked lots of seed, the populations of the 1970 study were S_1 progenies from 2 known parental clones. A total of 2036 pollinations were made in this study. Within these 2 S_1 lines, each flowering plant was classified for its self-compatibility as well as for its pollen- and female-fertility. The following rules were set up in the classification :

1. Plants which produced no fruit when less than 3 pollinations were made in a particular test were not classified with the exception of

- the cases mentioned under Rule 3.
2. Only plants which were pollen-fertile and highly female-fertile were classified for self-compatibility.
 3. Plants which produced seed upon selfing but not upon crossing with the tester plants were classified as both pollen-fertile and female-fertile.
 4. A distinction was made between self-compatibility and pseudo-self-compatibility at the level of ≥ 15 seeds per fruit.
 5. A similar distinction as under Rule 4 was made between plants with a high female-fertility and those with a low female-fertility at the level of ≥ 15 seeds per fruit.

C. Genetic markers

i. General procedure

During the growing seasons of 1968, 1969 and 1970 the inbred lines were examined for qualitative traits which might be controlled by single genes. This examination was done both by visual inspection and by chromatographic analysis of flower pigments of sample plants. Plants which appeared to have potentially useful genotypes were self-pollinated and where possible crossed with other plants with and without the trait in question. In addition to the inbred lines used for studies on vigor and yield, additional plant material was incorporated in this part of the research (Table 2).

ii. Chemogenetic markers

All analytical chromatography was done by means of TLC. Several recently developed materials and methods are described by Stahl (1969). Many techniques were tried before the methods described below were chosen

(See : Preliminary survey of materials and methods for the detection of chemogenetic marker genes, Appendix, B).

The adsorbent was mixed with distilled water to a slurry and applied to 20 x 20 cm glass plates with a Quickfit spreader. The plates were allowed to dry for 12 hours at room temperature before use. The developing solvent was the upper phase of 4 parts n-butanol : 1 part acetic acid : 5 parts water (on a volume basis). The solvent was aged for approximately 2 days after mixing.

Plant tissue to be analyzed for anthocyanins was extracted by the procedure of Harborne (1958). Extracts containing chlorophyll were washed with petroleum ether before applying a sample to the TLC plate. Generally the samples of the extracts were applied directly to the plates without prior purification other than the elimination of chlorophyll. In the case of potato flowers, petals of 3 - 5 flowers were usually sufficient to produce enough extract which could be analyzed. The amount of extracting solvent added to the tissue was kept to a minimum so no further concentration of the extract was necessary. For the extraction of flavonoids from white flowers, the general method of Harborne (1959) was used.

The brush type sample applicator described by Tamura (1965) was used to apply a sample from the extract to the plate. Since this was a survey for qualitative differences, the quantities and the concentrations of the extracts from the various genotypes applied to the plates were only approximately equal. Spots of reference compounds were placed on each plate for comparison. As soon as the sample spots were dry, the plates were placed in the developing solvent in a Camag S-chamber or in a Quickfit developing tank. The developed plates were dried at room temperature. Anthocyanin bands were clearly visible in daylight but several other

phenolic bands were only visible in ultraviolet light in a UV viewing box (Chromatovue Model CC 20) or upon treatment with certain chromogenic spray reagents (Harborne, 1959).

Table 1. Initial plant materials used

Family	Pedigree	Used for studies on			
		Fertility			Marker genes
		Vigor and yield	Effect of inbrdg.	Segreg. for self-c.	
US-W 5309	Phu PI 195191 x US-W 4	+	+		+
" 5314	Phu PI 225708.1 x US-W 4	+	+		+
" 5315	Phu PI 225675.1 x US-W 4	+	+		+
" 5328	Stn PI 230513.1 x US-W 4	+	+	+	+
" 7139	Phu PI 320382.3 x US-W 5315.9				+
" 7143	US-W 8030.3 x US-W 8818.1				+
" 9175	US-W 5312 x self				+
" 9568	US-W 7875.9 x US-W 8818.1				+
" 9569	US-W 7875.9 x US-W 8818.2				+
" 9570	US-W 8030.9 x US-W 8818.2				+
" 9571	US-W 8030.7 x US-W 8818.1				+
" 9586	Stn PI 230513.1				+
" 9587	US-W 8640.4 x US-W 8646.1				+
" 9588	US-W 8646.1 x US-W 8640.4				+
PI 365375	Sib ₃ of Phu PI 225702 x Phu PI 225682 (from Hermsen)				+

Table 2. Materials and equipment used in chemogenetic study

Materials and equipment	Source
A. Adsorbents	
Avicel TG 104, (22 g Avicel + 50 ml water for five 20 x 20 cm plates for a coating of 0.25 mm).	American Viscose Division, FMC Corporation, Newark, Delaware.
Sigmacell Type 19. Microcrystalline cellulose, approx. particle size is 19 microns (20 g Sigmacell + 50 ml water for five 20 x 20 cm plates for a coating of 0.25 mm).	Sigma Chemical Company, St. Louis, Missouri.
B. Equipment	
Chromatovue Model CC 20 viewing box	Ultra-violet Products Inc., San Gabriel, California.
Quickfit TLC apparatus, Model 8 CR.	Matheson Scientific, Chicago, Illinois.
Sandwich chamber for a 20 x 20 cm plate	Canag Inc., Milwaukee, Wisconsin.
C. Reference materials	
Antirrhinin (Cyanidin 3-rhamnosylglucoside).	i. Red flowers of <u>Streptocarpus</u> . Seed was obtained from the Park Seed Company Inc., Greenwood, South Carolina.
	ii. Flowers of <u>Antirrhinum majus</u> , cultivar Padre. Seed was obtained from the Olds Seed Company, Madison, Wisconsin.
Petanin (Petunidin 3-(p-coumaroyl rutinoside)-5-glucoside)	Ripe fruits of the garden huckleberry, <u>Solanum guineense</u> . Seed was obtained from the Olds Seed Company, Madison, Wisconsin.
Rutin (Quercetin 3-rhamnosylglucoside).	Mann Research Laboratories, New York, New York.

RESULTS

A. Vigor and yield

Inbreeding depression was expressed by all measured characters. When averaged over the 4 families, the inbreeding depression generally followed the rate expected based on reduction of heterozygosity. For tuber weight per hill for the populations grown from tubers in 1968, it was possible to calculate a curve which agreed very well with the observed data (Fig. 2). Considerable variation in response to inbreeding existed between families (Table 3). Progenies obtained by selfing from family US-W 5328 exhibited an inbreeding depression at each generation of inbreeding. Such progenies from family US-W 5309 had a sharp depression from the S_0 to the S_1 , but the subsequent S_2 generation was not depressed. The performance of such progenies from families US-W 5314 and US-W 5315 respectively, was slightly lower and slightly higher in the S_2 than in the S_1 (Table 3).

The inbreeding depression of populations obtained by clonal reproduction was nearly always less than in the parent population (Tables 3 and 4; Fig. 3). Failure of tuberization in parent populations caused a reduction in the number of genotypes in the subsequent populations. The percentage of tuberizing plants decreased with each level of inbreeding (Table 5).

A severe inbreeding depression was observed from the S_0 to the S_1 for all characters for all families when grown from true seed. There was

Table 3. Effect of inbreeding on four diploid potato families

First clonal generation (1968)															
Family	Number of clones			Plant height (cm)			Vigor index			Tubers/hill			Tuber weight g/hill		
	S ₀	S ₁	S ₂	S ₀	S ₁	S ₂	S ₀	S ₁	S ₂	S ₀	S ₁	S ₂	S ₀	S ₁	S ₂
US-W 5309	12	16	16	43	25	27	3.5	2.3	2.5	21	16	15	563	248	269
US-W 5328	9	12	8	41	27	17	3.6	2.4	1.6	19	15	8	390	425	167
US-W 5314	0	12	11		27	23		2.4	2.2		13	7		287	88
US-W 5315	0	10	9		25	29		2.6	2.7		20	21		297	415
Total	21	50	44												
Weighted Average				42	26	25	3.5	2.4	2.2	20	16	14	499	304	181
% of S ₀				100	62	60	100	69	63	100	80	70	100	61	36
Second generation (1969)															
US-W 5309	12	10	11	31	25	31	3.6	2.8	3.7	10	14	12	198	165	217
US-W 5328	7	11	4	37	28	14	4.0	3.3	2.0	20	13	5	370	330	13
US-W 5314	0	10	4		22	22		2.7	2.6		13	13		100	95
US-W 5315	0	9	7		20	25		2.5	3.5		13	14		202	237
Total	19	40	26												
Weighted Average				33	24	25	3.7	2.9	3.2	14	13	12	260	211	175
% of S ₀				100	73	76	100	78	86	100	93	86	100	81	67

Table 4. Effect of inbreeding on potato populations grown from true seed and the first clonal generation.

Character	Seedling populations (1968)				First clonal generations (1969)			
	S ₀	S ₁	S ₂	S ₃	S ₀	S ₁	S ₂	S ₃
Number of plants	29	163	184	163	11	41	48	35
Plant height (cm)	25	16	16	15	46	21	20	20
% of S ₀	100	64	64	59	100	46	43	42
Vigor index	4.7	2.4	2.7	2.7	4.8	3.0	3.0	3.0
% of S ₀	100	51	57	57	100	60	60	60
Tubers/hill	10	6	5	5	23	16	20	16
% of S ₀	100	60	50	50	100	70	87	70
Tuber weight (g/hill)	61	20	16	14	426	236	234	193
% of S ₀	100	33	26	23	100	55	55	45
% tuberizing	69	81	80	71	97	93	94	83
% with ≥ 1 viable tubers after storage	62	70	72	63				
% with ≥ 4 viable tubers after storage	38	33	32	25				

Table 5. Effect of inbreeding on asexual reproduction

Year	Number of clones planted		
	S_0	S_1	S_2
1968	21	50	44
1969	19	40	26
% replanted in 1969	90	80	59

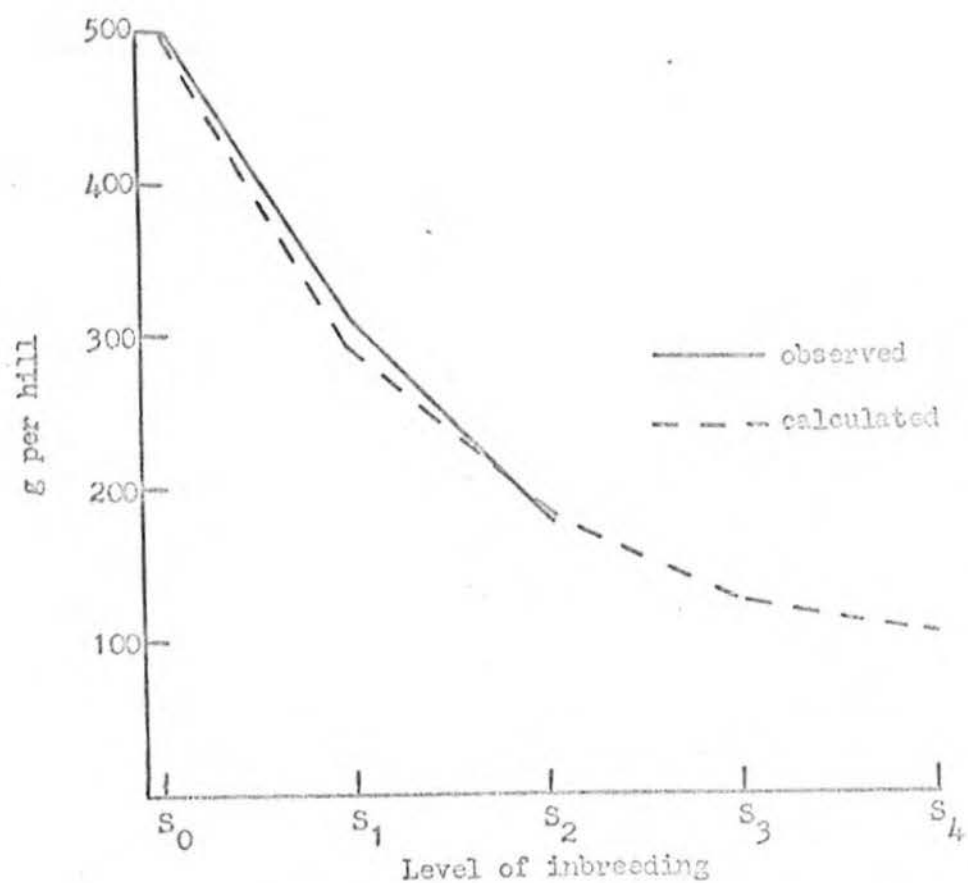


Fig. 2. Average tuber weight in g per hill, first clonal generation, 1968

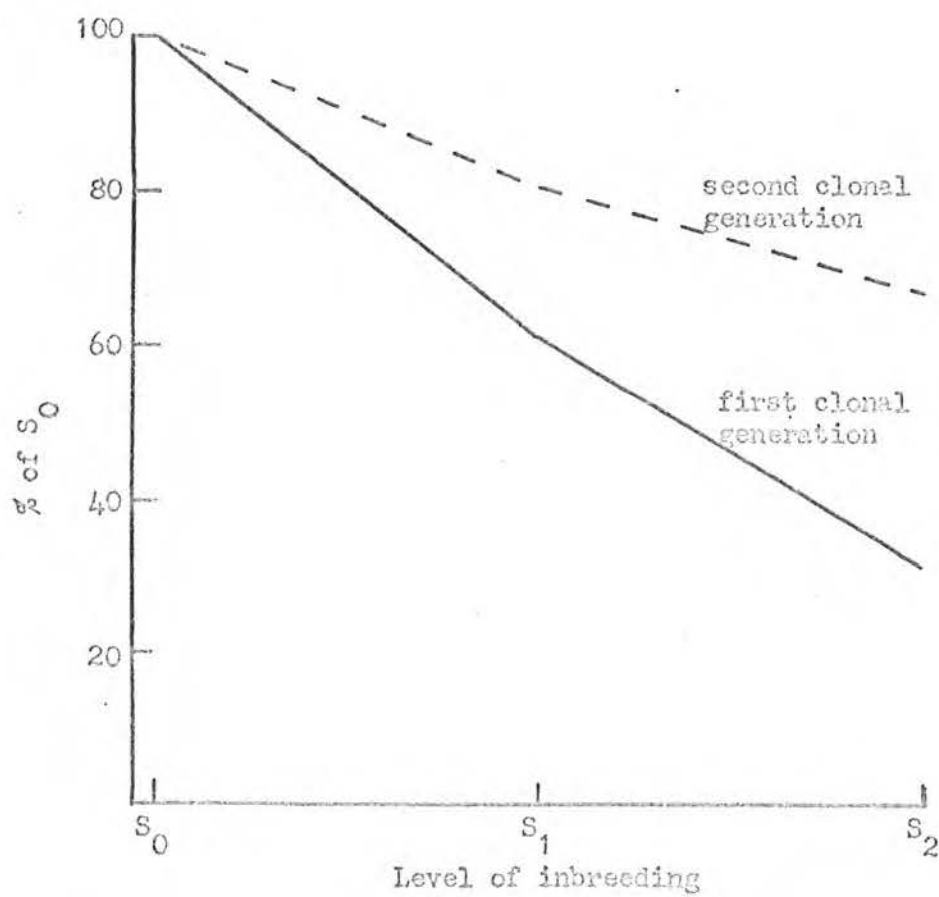


Fig. 3. Average tuber weight in per cent of S_0 for first and second clonal generation, 1968

little if any depression in the subsequent S_2 and S_3 generations. These populations tuberized fairly well in the field even in the advanced generations of inbreeding. Most of these tubers were very small, however, and were not viable the following spring after standard winter storage conditions (Table 4).

B. Fertility

All S_0 clones flowered but generally the per cent of flowering clones decreased rapidly from the S_0 to the S_1 , but not from the S_1 to the S_2 (Table 6). There was no apparent depression in the number of fruit per pollination associated with inbreeding, but the average number of seeds per fruit decreased with each level of inbreeding for self-compatibility and pollen- and female-fertility. The average self-compatibility was much lower than either pollen- or female-fertility at all levels of inbreeding when expressed as seeds per fruit. The inbreeding depression, especially from the S_0 to the S_1 , was much less severe for self-compatibility than for pollen- and female-fertility (Table 7; Fig. 4).

The following abbreviations were used in classifying individual clones :

SC = self-compatibility

SI = self-incompatibility

PSC = pseudo-self-compatibility (< 15 seeds per fruit)

HFF = high female-fertility

LFF = low female-fertility (< 15 seeds per fruit)

FS = female-sterility

PS = pollen-sterility

U = unclassified

Both families US-W 5309 and US-W 5328 contained self-incompatible, pseudo-self-compatible and self-compatible clones. All classifiable clones of both families were highly female-fertile and pollen-fertile (Tables 8 and 9). The observations on the relatively small sample of plants from family US-W 5328 are in general agreement with those of Hollenback (Table 10).

In the two S_1 lines, US-W 9547 and US-W 9550, the majority of the flowering clones could not be classified for self-compatibility due to the high number of clones which were classified as either female-sterile, low female-fertile or pollen-sterile (Tables 11 and 12). A progeny test revealed differences between the two full sibs, US-W 5328-11 and US-W 5328-20, in relation to both the female-fertility and self-compatibility reaction of these two clones. US-W 5328-11 was classified as a pseudo-self-compatible clone and exhibited only a medium degree of female-fertility. With the exception of one clone, all of its progeny (US-W 9547) which set at least some seed upon selfing was also pseudo-self-compatible. This is close to expectation since according to the model presented by Hollenback (p. 81 of this thesis) a pseudo-self-compatible clone can upon selfing produce only pseudo-self-compatible or self-incompatible progeny. In contrast, US-W 5328-20, which was a highly self-compatible clone produced a progeny (US-W 9550) with a relatively large proportion of self-compatible clones. Of the clones classified for female-fertility in family US-W 9547, only 6 of 19 (32 %) of the classifiable clones were highly female-fertile whereas in family US-W 9550, 25 of 39 (64 %) of the classifiable clones were highly female-fertile (Table 13; Fig. 5).

Table 6. Effect of inbreeding on flowering

Family	Level of inbrdg.	Number clones planted	Average number of clones emerged	% stand	Clones flowering number	% of clones planted
US-W 5309	S ₀	12	12.0	100	12	100
"	S ₁	16	16.0	100	6	38
"	S ₂	16	13.7	85	12	75
US-W 5328	S ₀	9	8.0	88	9	100
"	S ₁	12	10.0	83	9	75
"	S ₂	8	4.3	54	4	50
US-W 5314	S ₁	12	11.0	92	7	58*
"	S ₂	11	6.7	61	5	45*
US-W 5315	S ₁	10	8.7	87	5	50*
"	S ₂	9	8.0	89	3	33*
Weighted avg	S ₀	21	20.0	95	21	100
"	" S ₁	50	45.7	91	27	54
"	" S ₂	44	32.7	74	24	54

* Is a relatively low estimate since the data from these clones were collected rather late in the flowering season.

Table 7. Effect of inbreeding on fertility

Family	Level of inbrdg	Number of flowering clones	Number of pollinations			Fertile clones in per cent of flowering clones			Fruits per pollination			Seeds per fruit		
			self	female	pollen	self	female	pollen	self	female	pollen	self	female	pollen
US-W 5309	S ₀	12	254	90	501	63	97	89	.38	.62	.11	11.4	72.8	42.9
"	S ₁	6	68	24	179	10	15	19	.32	1.00	.16	10.3	32.7	40.3
"	S ₂	12	151	62	450	15	61	51	.60	.65	.11	6.1	31.9	37.9
US-W 5328	S ₀	9	149	52	367	25	71	83	.35	.64	.14	13.2	82.3	64.5
"	S ₁	9	114	41	299	17	30	57	.48	.45	.17	11.6	44.7	41.7
"	S ₂	4	36	13	121	10	—	20	.50	—	.12	2.7	—	5.5
US-W 5314*	S ₁	7	58	20	167	7	21	21	.33	1.00	.13	1.2	16.3	29.5
"	S ₂	5	32	12	101	5	10	10	1.00	.67	.42	.8	9.0	41.2
US-W 5315*	S ₁	5	40	16	114	16	23	38	.67	.17	.22	6.0	12.0	19.2
"	S ₂	3	33	11	76	38	63	38	.52	.52	.07	9.6	14.9	3.1
Total pollinations			935	341	2375									
Weighted avg	S ₀					47	86	85	.37	.63	.12	11.8	75.9	53.7
"	"	S ₁				16	23	34	.43	.59	.18	8.7	34.4	35.6
"	"	S ₂				16	47	41	.60	.63	.13	6.3	29.9	37.6

* Data collected late in flowering season

Table 8. Classification of self-compatibility, female- and pollen-fertility of family US-W 5309

Self-comp.				Female-fert.			Pollen-fert.			Classification		
Clone number	No. poll.	No. frts	No. seeds per fruit	No. poll.	No. frts	No. seeds per fruit	No. poll.	No. frts	No. seeds per fruit	Self-comp.	Female fert.	Pollen fert.
1	23	11	12	8	6	68	33	3	69	PSC	HFF	PF
2	22	6	10	8	4	52	30	4	4	U	HFF	U
3	18	5	15	5	4	75	36	9	44	SC	HFF	PF
4	19	7	8	7	6	47	34	3	73	PSC	HFF	PF
5	21	0	-	7	7	46	33	6	35	SI	HFF	PF
6	22	11	10	8	3	42	35	0	-	U	HFF	U
7	23	8	4	7	5	65	33	7	52	PSC	HFF	PF
8	24	8	19	14	6	154	36	3	22	SC	HFF	PF
9	24	0	-	8	4	54	32	3	32	SI	HFF	PF
10	10	2	32	4	2	75	22	1	78	SC	HFF	PF
11	24	3	3	8	6	108	36	2	38	PSC	HFF	PF
12	<u>24</u>	<u>5</u>	11	<u>6</u>	<u>2</u>	72	<u>33</u>	<u>2</u>	24	PSC	HFF	PF
Total	254	66		90	55		393	46				
Aver. no. seeds/fruit			11				73				43	
Median no. seeds/fruit			11				67				38	

Table 9. Classification of self-compatibility, female- and pollen-fertility of family US-W 5328

Self-comp.				Female-fert.			Pollen-fert.			Classification		
Clone No.	No. poll.	No. frts	No. seeds per fruit	No. poll.	No. frts	No. seeds per fruit	No. poll.	No. frts	No. seeds per fruit	Self-comp.	Female fert.	Pollen fert.
1	18	7	2	6	4	51	34	7	60	PSC	HFF	PF
2	17	0	-	6	4	128	35	10	46	SI	HFF	PF
3	14	3	23	6	2	96	34	3	78	SC	HFF	PF
4	8	2	49	4	4	105	24	2	71	SC	HFF	PF
5	18	1	60	6	3	101	35	6	121	SC	HFF	PF
6	12	6	4	4	3	37	35	8	47	PSC	HFF	PF
7	10	0	-	4	4	91	23	5	46	SI	HFF	PF
8	17	1	0	6	4	51	35	4	79	SI	HFF	PF
9	<u>11</u>	<u>0</u>	-	<u>2</u>	<u>0</u>	-	<u>11</u>	<u>1</u>	59	U	U	PF
Total	125	20		44	28		266	46				
Aver. no. seeds/fruit			13				82				64	
Median no. seeds/fruit			14				94				60	

Table 10. Summary of self-compatibility, female- and pollen-fertility of families US-W 5309 and US-W 5328 and comparison with data from other investigators

Family	Self-comp. no. plants				Female-fert. no. plants		Pollen-fert. no. plants	
	SC	PSC	SI	U	HFF	U	PF	U
US-W 5309	3	5	2	2	12	0	10	2
US-W 5328	3	2	3	1	8	1	9	0
US-W 5328 (Hollenback, 1966)	13	5	2	2				
US-W 5328 (Smiley, 1963)	17			0				

Table 11. Classification of self-compatibility, female-fertility in the S₁ family US-W 9547

Seed- ling no.	Self-comp.			Female-fert.			Pollen-fert.			Classification		
	No. poll.	No. frts	No. seeds per fruit	No. poll.	No. frts	No. seeds per fruit	No. poll.	No. frts	No. seeds per fruit	Self-comp.	Female fert.	Pollen fert.
6	8	2	20	15	1	4	0	-	-	SC	HFF	PF
8	1	0	-	1	1	26	0	-	-	U	HFF	U
9	3	0	-	1	0	-	20	2	28	U	U	PF
10	4	0	-	3	0	-	19	0	-	U	U	PS
14	18	4	1	5	1	7	20	6	44	U	LFF	PF
15	3	0	-	3	1	1	13	0	-	U	LFF	PS
18	3	0	-	12	2	15	10	0	-	U	HFF	PS
19	7	4	5	11	3	68	24	7	75	PSC	HFF	PF
21	1	0	-	2	2	13	0	-	-	U	LFF	U
23	4	0	-	2	0	-	13	1	24	U	U	PF
24	11	1	12	3	0	-	11	1	64	PSC	LFF	PF
29	11	3	4	4	0	-	15	3	38	U	LFF	PF
35	5	4	1	10	2	12	5	1	57	PSC	LFF	PF
37	7	3	3	3	0	-	13	5	15	U	LFF	PF
38	12	0	-	2	1	2	31	0	-	U	LFF	PS
39	11	0	-	1	0	-	15	0	-	U	U	PS
40	1	0	-	2	0	-	13	0	-	U	U	PS
41	7	5	7	2	1	35	11	3	125	PSC	HFF	PF
42	7	1	7	3	2	100	0	-	-	U	HFF	U
43	15	0	-	4	0	-	18	3	30	U	FS	U

Table 11. Continued

		Self-comp.		Female-fert.		Pollen-fert.				Classification		
Seed-		No.		No.		No.		No.		Self-	Female	Pollen
ling	No.	No.	seeds	No.	seeds	No.	seeds	No.	seeds	comp.	fert.	fert.
no.	poll.	frts	per fruit	poll.	frts	poll.	frts	poll.	frts			
44	24	12	4	5	2	4	13	0	-	U	LFF	U
50	5	4	6	3	1	13	11	0	-	PSC	LFF	U
51	5	0	-	2	1	5	14	0	-	U	LFF	PS
52	0	-	-	0	-	-	15	1	175	U	U	PF
55	12	3	4	3	0	-	28	7	67	U	U	PF
56	3	0	-	2	0	-	12	3	112	U	U	PF
57	14	0	-	3	1	1	25	2	38	U	LFF	PF
58	2	0	-	2	0	-	12	2	32	U	U	PF
Total	204	46		109	22		381	47				
Aver. no.			5			27			56			
seeds/fruit												
Median no.			5			12			41			
seeds/fruit												

Table 12. Classification of self-compatibility, female- and pollen-fertility in the S₁ family US-W 9550

Self-comp.				Female-fert.			Pollen-fert.			Classification		
Seed- ling no.	No. poll.	No. frts	No. seeds per fruit	No. poll.	No. frts	No. seeds per fruit	No. poll.	No. frts	No. seeds per fruit	Self- comp.	Female fert.	Pollen fert.
12	13	2	2	5	0	-	11	0	-	PSC	LFF	U
13	2	0	-	1	0	-	11	0	-	U	U	PS
16	11	0	-	4	0	-	14	0	-	U	FS	PS
25	25	14	19	5	3	32	23	2	55	SC	HFF	PF
26	5	0	-	2	0	-	27	0	-	U	U	PS
27	5	3	7	4	1	85	10	0	-	U	HFF	U
28	11	6	3	3	1	9	20	9	70	U	LFF	PF
33	15	2	1	3	0	-	11	6	74	U	FS	PF
34	8	0	-	6	0	-	28	0	-	U	FS	PS
38	16	2	25	5	2	202	28	10	43	SC	HFF	PF
39	11	1	15	2	2	28	33	3	46	SC	HFF	PF
41	5	0	-	0	-	-	15	0	-	U	U	PS
43	16	10	3	6	4	2	19	0	-	U	LFF	PS
45	13	4	1	11	1	132	14	0	-	U	HFF	U
48	4	1	14	2	0	-	34	20	44	PSC	LFF	PF
51	0	-	-	2	0	-	9	3	127	U	U	PF
56	24	7	19	3	2	60	10	8	70	SC	HFF	PF
64	14	0	-	2	2	3	14	5	44	U	LFF	PF
66	27	5	37	4	4	78	36	7	30	SC	HFF	PF
69	6	1	5	3	3	36	10	0	-	U	HFF	U

Table 12. Continued

Self-comp.			Female-fert.			Pollen-fert.			Classification			
Seed- ling no.	No. poll.	No. frts	No. seeds per fruit	No. poll.	No. frts	No. seeds per fruit	No. poll.	No. frts	No. seeds per fruit	Self- comp.	Female fert.	Pollen fert.
75	6	0	-	4	0	-	13	7	74	U	FS	PF
76	7	4	28	3	2	31	11	3	73	SC	HFF	PF
79	3	3	28	0	-	-	0	-	-	SC	HFF	PF
80	5	1	12	2	1	18	0	-	-	U	HFF	U
84	4	0	-	4	0	-	11	1	16	U	FS	PF
87	12	5	17	4	3	51	13	0	0	SC	HFF	PF
90	5	0	-	7	5	9	30	2	29	U	LFF	PF
96	9	7	30	3	1	31	9	2	259	SC	HFF	PF
98	5	5	11	2	2	26	9	3	75	PSC	HFF	PF
99	6	0	-	5	0	-	10	3	52	U	FS	PF
104	31	7	3	12	0	-	5	1	18	PSC	LFF	PF
105	10	2	25	3	0	-	17	4	48	SC	HFF	PF
108	22	0	-	9	1	60	18	0	-	U	HFF	FS
109	11	2	55	3	3	90	31	12	25	SC	HFF	PF
112	5	0	-	2	1	23	0	-	-	U	HFF	U
113	9	0	-	2	2	63	24	2	28	SI	HFF	PF
118	3	0	-	2	0	-	13	0	-	U	U	FS
119	21	1	3	3	1	15	8	0	-	U	HFF	U
120	15	4	3	2	0	-	13	1	165	U	U	PF
121	10	7	6	1	0	-	14	4	89	U	U	PF

Table 12. Continued

Self-comp.				Female-fert.			Pollen-fert.			Classification		
Seed- ling no.	No. poll.	No. frts	No. seeds per fruit	No. poll.	No. frts	No. seeds per fruit	No. poll.	No. frts	No. seeds per fruit	Self- comp.	Female fert.	Pollen fert.
123	13	10	18	3	2	24	16	14	78	SC	HFF	PF
124	19	8	26	7	3	112	10	1	205	SC	HFF	PF
129	21	0	-	7	2	6	15	3	18	U	LFF	PF
133	7	1	30	6	1	25	24	1	37	SC	HFF	PF
134	<u>19</u>	<u>5</u>	22	<u>5</u>	<u>1</u>	310	<u>12</u>	<u>1</u>	12	SC	HFF	PF
Total	509	130		174	56		703	138				
Aver. no. seeds/fruit			16				47				58	
Median no. seeds/fruit			15				31				52	

Table 13. Summary of self-compatibility, female- and pollen-fertility data in the clones US-W 5328-11, US-W 5328-20 and their respective progenies obtained by selfing

US-W number	Self-compatibility					Female-fertility				Pollen-fertility			
	No. clones	No. poll.	No. frts	No. seeds per fruit		No. poll.	No. frts	No. seeds per fruit		No. poll.	No. frts	No. seeds per fruit	
				Avg.	Median			Avg.	Median			Avg.	Median
5328-11	1		31	12			11	25			4	28*	
9547 (5328-11 ⊗)	28	204	46	5	5	109	22	27	12	381	47	56	41
5328-20	1		28	61			6	52			4	39*	
9550 (5328-20 ⊗)	45	509	130	16	15	174	56	47	31	703	138	58	52

Classification (Number of plants)

	Self-compatibility				Female-fertility				Pollen-fertility		
	SC	PSC	SI	U	HFF	LFF	FS	U	PF	PS	U
5328-11		+			+				+		
9547	1	5	0	22	6	12	1	9	16	7	5
5328-20	+				+				+		
9550	15	4	1	25	24	8	6	7	30	8	7

* Based on sib-pollinations and therefore probably a low estimate

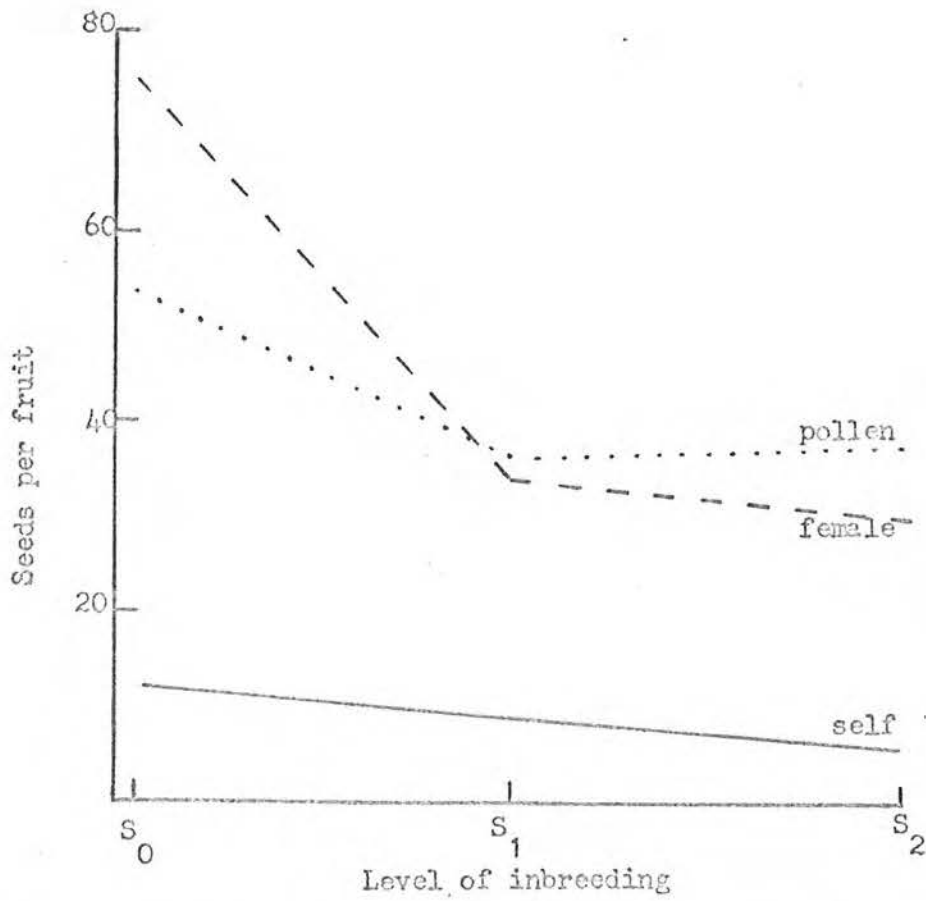


Fig. 4. Average fertility in seeds per fruit upon inbreeding

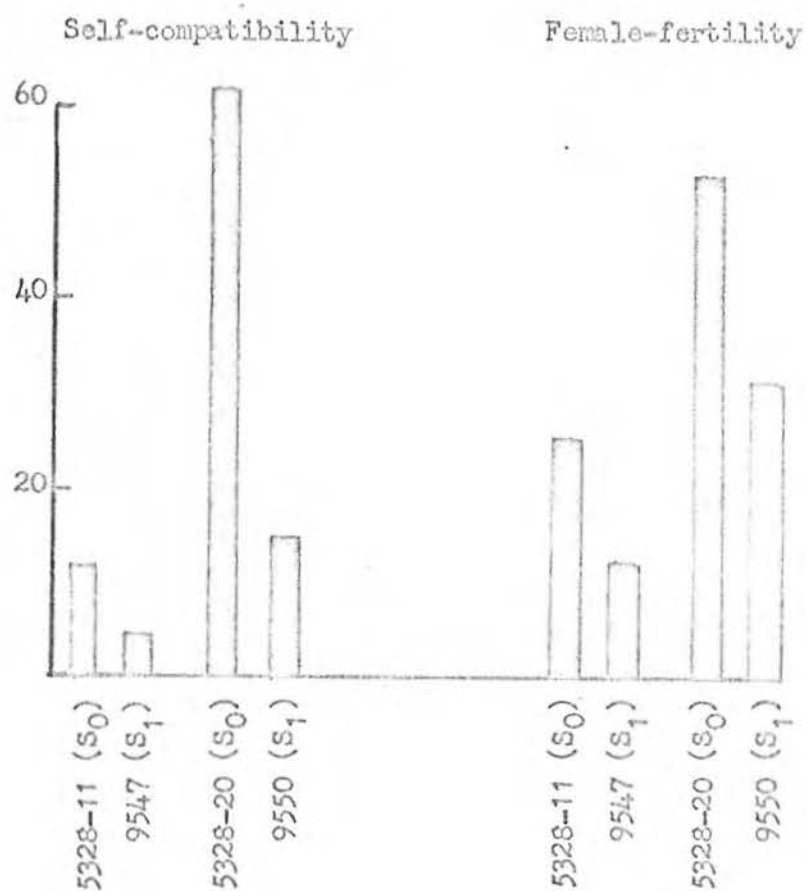


Fig. 5. Median number of seeds per fruit of 2 S_0 clones and their S_1 progenies

C. Genetic markers

i. Genes controlling synthesis and distribution of anthocyanins

Progenies obtained from selfing self-compatible clones were analyzed for previously described marker genes such as those listed below:

- P - Controls synthesis of blue pigment (delphinidin and petunidin derivatives).
- R - Controls synthesis of red pigment (cyanidin and pelargonidin derivatives).
- Ac - Controls acylation of the anthocyanin molecules by p-coumaric acid (Fig. 1 D and Fig. 8).
- B - Is a multi-allelic locus. The highest known allele, $\underline{B}^d$, controls the distribution of pigment to the stem nodes and to the cotyledonary node of the embryo (seed spot, Fig. 6).
- I - Controls distribution of pigment to the tuber skin and stem.
- F - Controls distribution of pigment to the flower.

A search for new marker genes was also undertaken. Considerable attention was paid to two traits not previously described or analyzed: pigmented leaves and pigmented ovary wall. The two clones US-W 8818.1 and US-W 8818.2 exhibit extremely heavy pigmentation in the mature leaves when grown under normal growing conditions in the field at Sturgeon Bay. In the case of US-W 8818.1 the leaves turn purple and with US-W 8818.2 they become red. The pigmented ovary wall is illustrated in Fig. 9. The phenotype has previously been described (Pushkarnath, 1960) but no information on its inheritance was available from the literature. The pigment synthesis in the ovary wall seems to stop when the flowers are open, because the pigment is gradually diluted when fruits develop and enlarge.

Fig. 6.
Seed spot

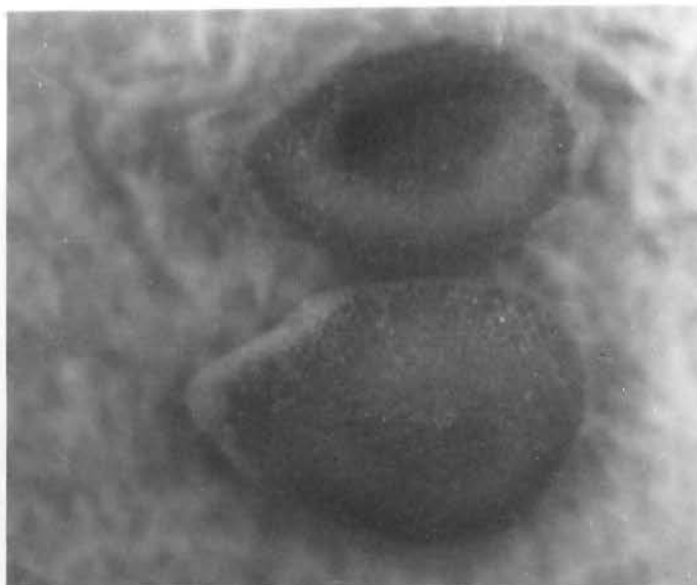


Fig. 7.
Pigmented stigma

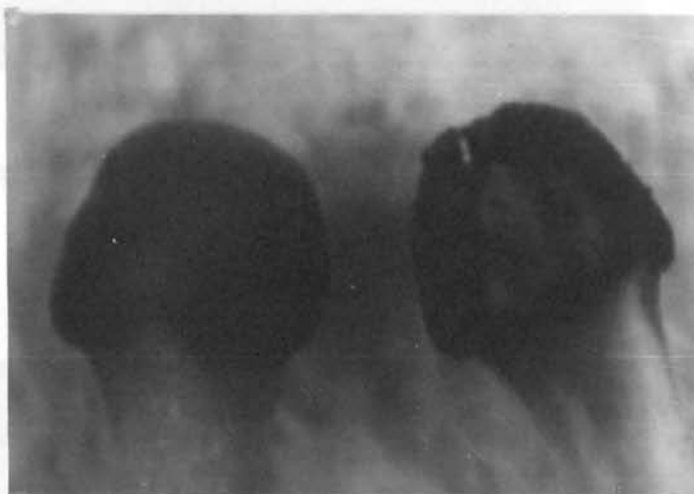


Fig. 8.
TLC chromatogram
in long wave
ultraviolet light

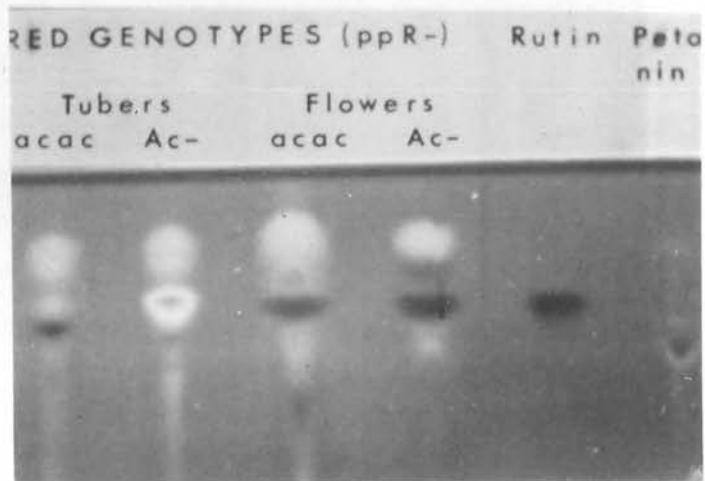
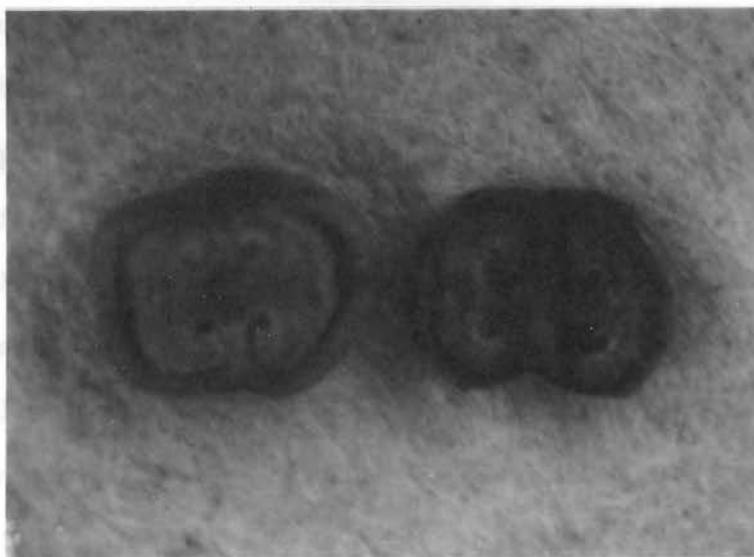


Fig. 9. Pigmented ovary wall



A. Longitudinal dissection



B. Transverse dissection

This trait appears to be inherited as a single dominant gene (see following discussion) and the gene symbol Cm has tentatively been assigned.

Another trait which may have value as a genetic marker is the pigmented stigma (Fig. 7). Populations with segregation for this trait were small, but the preliminary data indicate that this trait is recessive. The number of genes involved in the control of pigmented stigma has not yet been determined.

When the clones with pigmented leaves (US-W 8818.1 and US-W 8818.2) were crossed to clones with non-pigmented leaves, approximately 23 % of the total number of plants from all progenies were found to have at least some leaf pigmentation varying from light to heavy (Table 14). When non-pigmented or very light pigmented plants from the progenies described above (The pigmented plants in these progenies did not produce sufficient flowers or were sterile) were backcrossed to their respective pigmented parent, the resulting progenies contained several dwarf plants with small leaves and abnormal leaflets. Two types of leaflets were observed: a. small and round; b. small and narrow with a mottled appearance. Nearly all the pigmented plants in these progenies were also dwarf plants, but not all dwarf plants were pigmented. This was especially true in the backcross progeny from US-W 9571-1 x US-W 8818.1. All of the heavily pigmented plants from this progeny could be classified under either of the two types of dwarf plants. Several of the lighter pigmented plants from this progeny had a normal plant type and most of the non-pigmented plants were also of the normal plant type. However, several non-pigmented dwarf plants were observed as well (Table 15).

The factors controlling the basic pigmentation of the flowers (F) and of the tuber skin (I) are known to be closely linked (Dekkers and Long,

1956). This is confirmed by the segregation pattern of I and f in families US-W 9547 and US-W 9581 (Tables 16 C and 17 C). The factor controlling pigmentation of the ovary wall (Ow) also segregates jointly with the I f linkage group (Tables 16 A and B and 17 A and B).

The linkage of Ow with I is further supported by the data from 5 other S_1 progenies where no deviation from the expected phenotypes occurred when the parental genotypes were assumed to be $\frac{Ow}{ow} \frac{I}{i} \frac{f}{f}$ and no crossing over between the Ow and I loci (Table 18). In this case only plants with either fully pigmented or completely white tubers were considered because of the difficulties of assigning genotypes to the plants which produce tubers with various degrees of pink skin color. In families US-W 9550 and US-W 9554 nearly all plants with pink tubers had the same phenotype for ovary wall pigmentation as did the plants with white tubers. The pink color in this case may have been the result of an incomplete suppression of tuber pigmentation in certain ii genotypes. In family US-W 9552, all of the plants with pink tubers correspond with the plants with fully pigmented tubers in respect to the phenotype for ovary wall pigmentation (Table 18). In this instance, the pink color could possibly have resulted from reduced activity of the basic pigment producing gene R in I- genotypes. This is known to be true in plants with $\frac{R^{EW}}{R^{EW}} \frac{R^{EW}}{R^{EW}}$ genotypes which, in the presence of F and I, produce white flowers and pink tubers (Dodds and Long, 1956). Since all plants in this family already had white flowers, presumably as a result of the ff genotype, a correlation between tuber and flower pigmentation could not be made. Thus it was not possible to determine whether the pink tuber color in this family was the result of a reduced activity at the R locus such as in $\frac{R^{DM}}{R^{DM}} \frac{R^{EW}}{R^{EW}}$, or due to some other cause.

The genotypes for 31 self-compatible or pseudo-self-compatible clones at various levels of inbreeding have been determined on the basis of a progeny test. Several progenies exhibited a high degree of uniformity not only for genes controlling the synthesis and distribution of anthocyanins but also for several other traits for which the inheritance has not yet been solved such as tuber shape, tuber flesh color and leaf type (Table 21).

ii. Chemogenetic markers

The routine laboratory technique which was developed as part of this investigation permitted screening of families segregating for genes affecting flavonoid synthesis. In the families US-W 9545 and US-W 9559, segregation for dominant and recessive genotypes at the Ac locus have been detected. The segregation ratios in both families very closely approximated a 3 : 1 ratio (Table 21).

All plants in family US-W 9559 had red flowers and white tubers, so that the determination was done on the flower pigments only. Family US-W 9545 was segregating for flower and tuber pigmentation, so that in this family the pigment analysis was done on the red flowers and the red tubers. The few plants which had both pigmented flowers and pigmented tubers exhibited internal consistency, i.e. where the flower pigment had been classified as unacylated, the tuber pigment was also unacylated.

With some experience it was quite easy to distinguish between acylated red and unacylated red flower pigments by a visual inspection of the flowers in the field (The unacylated pigment is more intense). When visual scoring was done, uncertain cases were always analyzed in the laboratory.

However, a distinction between Ac- and acac genotypes could not be made upon a visual inspection of the tuber pigments. The S_3 clone, US-W 9187-2, produced only a few flowers in the summer of 1970 and has tentatively been designated as acac on the basis of a visual inspection. The few plants in the S_4 progeny of this clone which produced some flowers also all appear to have the unacylated red flower pigment upon visual inspection.

Plant material containing the gene P was very limited in this study. Consequently no families segregating for acylated and unacylated blue pigments were detected. The laboratory method was very effective in distinguishing the acylated red pigments in either flowers or tubers from blue pigments. The acylated red pigments were frequently misclassified as blue or purple pigments when inexperienced persons made a visual scoring.

Several differences and similarities due to the absence or presence of certain bands could be observed in the chromatograms of flowers from different Solanum species growing in the field at the Potato Introduction Station. Differences within species or within inbred lines were also observed. No simple segregation ratios have as yet been found for such bands in families exhibiting segregation for these traits.

iii. Genes controlling tuber shape

The following classification for tuber shape was used :

- a. Compressed, longitudinal axis (stem to apical end) shorter than the diameter axis.
- b. Round.
- c. Oval.

d. Long.

e. Very long, tubers often curved or hooked.

No attempts were made to subdivide the oval shapes any further into round-oval, long-oval, etc. In evaluating tuber shape, it must be taken into account that nearly all of the tubers classified were harvested from plants grown from true seed. The tubers were therefore smaller than they might have been if the observations had been made on tubers from subsequent clonal generations. On the other hand, it has been shown in this study that a considerable number of genotypes derived by inbreeding are usually lost in the first several asexual reproduction cycles. The best representative sample of genotypes is therefore probably available in the seedling year.

Self-compatible clones with oval tubers produced progenies upon selfing with relatively more long tubers than did similar clones with round tubers. In progenies from parents with round tubers and from parents with oval tubers, the tuber shapes were not randomly distributed between plants with white and pigmented tubers. Most of the white tubers were long and most of the pigmented tubers were round (Tables 19 and 20). Clones with long tubers produce only progeny with long tubers upon selfing whereas clones with oval or round tubers can produce progeny with all shapes upon selfing (Table 21). At least one clone (US-W 9537-7) has been found which produces progeny with only round and compressed tubers upon selfing. Clones US-W 9185-33 and US-W 9190-15 which have round tubers also do not produce any progeny with long tubers upon selfing, although plants with oval tubers do occur. All the self-compatible clones which have been found to breed true for long tubers are white, whereas the above mentioned clones (US-W 9537-7, US-W 9185-33 and US-W 9190-15) all have pigmented tubers (Table 21).

Table 14. Segregation for leaf pigmentation in F_1 hybrid families

Parents				Progeny				
Female		Male		US-W no.	Leaf pigmentation Number of plants			
US-W no.	Phenotype	US-W no.	Phenotype		none	light	medium	heavy
8030.3	non-pigm.	8818.1	heavy blue pigm.	7143	47	2	3	7
7875.9	"	8818.1	" " "	9568	30	11	2	2
7875.9	"	8818.2	heavy red pigm.	9569	10	1	1	1
8030.9	"	8818.2	" " "	9570	45	0	9	6
8030.7	"	8818.1	heavy blue pigm.	9571	32	1	2	0
Total					164	15	17	16
% of total no. plants					77	7	8	8
Pigmented plants of all classes in % of total					23			

Table 15. Segregation for leaf pigmentation in progenies from backcrosses between F_1 hybrids and the pigmented parents

Parents				Progeny				
Female		Male		Pigmen- tation	Plant type			
US-W no.	Phenotype	US-W no.	Phenotype		Normal	Dwarf plants Leaflet type		
						round	narrow, mottled	
9568-1	very light blue pign.	8818.1	heavy blue pign.	none	31	29	2	0
				light	5	0	5	0
				heavy	0	-	-	0
9570-2	light red pign.	8818.2	heavy red pign.	none	111	111	0	0
				light	25	25	0	0
9571-1	no pign.	8818.1	heavy blue pign.	none	78	63	15	0
				light	30	7	17	6
				heavy	14	0	2	12
9571-2	no pign.	8818.1	heavy blue pign.	none	68	68	0	0
				light	11	9	0	2
				heavy	1	0	0	1

Table 16. Segregation of the gene Ow with the genes I and F in family US-W 9547 (Proposed genotype of selfed parent is $\frac{Ow\ I\ f}{ow\ i\ F}$)

A. Segregation of Ow and f

		Observed				Expected*
		Ovary wall				
Flowers		Unclas-	Non-	Pigm.		
		sified	pigm.			
White (<u>ff</u>)	18	1	1	16		0 : 17
Pigm. (<u>F-</u>)	25	0	8	17		8.33 : 16.66
Unclassif.	6	6	0	0		
Total	49	7	9	33		10.50 : 31.50

B. Segregation of Ow and I

		Observed				Expected*
		Ovary wall				
Tubers		Unclas-	Non-	Pigm.		
		sified	pigm.			
White (<u>ii</u>)	6	2	4	0		4 : 0
Pigm. (<u>I-</u>)	41	5	5	31		0 : 36
Unclassif.	2	0	0	2		
Total	49	17	9	33		10.50 : 31.50

Table 16. Continued

C. Segregation of I and f

		Observed				Expected*
		Tubers				
Flowers		Unclassified	Non-pigm.	Pigm.		
White (<u>ff</u>)	18	1	0	17		0 : 17
Pigm. (<u>F-</u>)	25	1	4	20		8 : 16
Unclassif.	6	0	2	4		
Total	49	2	6	41		10.25 : 30.75

* Only plants classified for the respective character(s) are considered. Complete linkage without crossing-over was assumed in calculating the expected values.

Table 17. Segregation of the gene Ow with the genes I and F in family US-W 9581 (Proposed genotype of selfed parent is $\frac{Ow}{ow} \frac{I}{i} \frac{F}{f}$)

A. Segregation of Ow and f

		Observed				Expected*	
		Ovary wall					
Flowers		Unclas- sified	Non- pigm.	Pigm.			
White (<u>ff</u>)	7	0	0	7		0	: 7
Pigm. (<u>F-</u>)	22	4	7	11		6	: 12
Unclassif.	19	13	3	3			
Total	48	17	10	21		7.75	: 23.25

B. Segregation of Ow and I

		Observed				Expected*	
		Ovary wall					
Tubers		Unclas- sified	Non- pigm.	Pigm.			
White (<u>ii</u>)	7	5	2	0		2	: 0
Pigm. (<u>Ii</u>)	27	11	1	15		0	: 16
Unclassif.	14	1	7	6			
Total	48	17	10	21		7.75	: 23.25

Table 17. Continued

C. Segregation of I and f

		Observed				Expected*	
		Tubers					
Flowers		Unclas- sified	Non- pigm.	Pigm.			
White (<u>ff</u>)	7	4	0	3		0	: 3
Pigm. (<u>F-</u>)	22	7	4	11		5	: 10
Unclassif.	19	3	3	13			
Total	48	14	7	27		8.50	: 25.50

* Only plants classified for the respective character(s) are considered. Complete linkage without crossing-over was assumed in calculating the expected values.

Table 18. Segregation of the gene Om with the gene I (Proposed genotype of all selfed parents is $\frac{Om\ I\ P}{Om\ I\ I}$)

Family US-W no.	Tubers	Ovary wall				Expected (assuming complete linkage)
		Unclas- sified	Non- pigm.	Pigm.		
9548	White (<u>ii</u>)	13	9	4	0	4 : 0
	Pigmented (red) (<u>I-</u>)	19	4	0	15	0 : 15
	Unclassified	7	0	0	7	
9549	White (<u>ii</u>)	9	2	7	0	7 : 0
	Pigmented (red) (<u>I-</u>)	24	3	0	21	0 : 21
	Unclassified	4	0	1	3	
9550	White (<u>ii</u>)	34	14	20	0	20 : 0
	Pigmented (pink)	18	5	12	1	?
	Pigmented (red) (<u>I-</u>)	66	11	0	55	0 : 55
	Unclassified	14	0	6	8	
9552	White (<u>ii</u>)	19	11	8	0	8 : 0
	Pigmented (pink)	10	5	0	5	?
	Pigmented (red) (<u>I-</u>)	56	17	0	39	0 : 39
	Unclassified	9	0	0	9	
9554	White (<u>ii</u>)	13	4	9	0	9 : 0
	Pigmented (pink)	4	2	2	0	?
	Pigmented (red) (<u>I-</u>)	34	11	0	23	0 : 23
	Unclassified	10	0	2	8	
Total	White (<u>ii</u>)	88	40	48	0	48 : 0
	Pigmented (pink)	32	12	14	6	?
	Pigmented (red) (<u>I-</u>)	199	46	0	153	0 : 153
	Unclassified	44	0	9	35	
		363	98	71	194	66.25 : 198.75

Table 19. Segregation of factors controlling tuber shape and tuber pigmentation in progenies from parents with round tubers

Selfed parent			Progeny						
Phenotype			Tuber pigmentation			Tuber shape			
US-W no.	Tuber pigmentation	Tuber shape	US-W no.	Color	Total no. tubers	Round	Oval	Long	
5328-11	pigm. (red)	round	9547	white	6	1	1	4	
				pigm. (red)	41	13	24	4	
5328-19	pigm. (red)	round	9549	white	9	1	2	6	
				pigm. (red)	24	5	16	3	
5328-25	pigm. (red)	round	9554	white	13	0	7	6	
				pigm. (pink)	4	1	2	1	
				pigm. (red)	34	20	14	0	
Total				white	28	2	10	16	
				pigm. (pink)	4	1	2	1	
				pigm. (red)	99	38	54	7	
					131	41	66	24	
						Per cent of			
						Total	Round	Oval	Long
that were white						21	5	15	67
" " pigm. (pink)						3	2	3	4
" " pigm. (red)						76	93	82	29
" " round						31			
" " oval						51			
" " long						18			

Table 20. Segregation of factors controlling tuber shape and tuber pigmentation in progenies from parents with oval tubers

Selfed parent				Progeny					
Phenotype				Tuber pigmentation		Tuber shape			
US-W no.	Tuber pigmentation	Tuber shape	US-W no.	Color	Total no. tubers	Round	Oval	Long	
5328-12	pigm. (red)	oval	9548	white	13	2	0	11	
				pigm. (red)	19	2	17	0	
5328-20	pigm. (red)	oval	9550	white	34	5	4	25	
				pigm. (pink)	18	4	9	5	
				pigm. (red)	66	31	30	5	
5328-21	pigm. (red)	oval	9552	white	19	0	4	15	
				pigm. (pink)	10	6	4	0	
				pigm. (red)	56	26	27	3	
Total				white	66	7	8	51	
				pigm. (pink)	28	10	13	5	
				pigm. (red)	141	59	74	8	
					235	76	95	64	
Per cent of									
Total						Round	Oval	Long	
that were white						28	9	8	80
" " pigm. (pink)						12	13	14	8
" " pigm. (red)						60	78	78	12
" " round						32			
" " oval						41			
" " long						27			

Table 21. The assignment of genotypes to self-compatible clones based on segregation ratios for their selfed progeny

Progeny			Segregation ratios			Selfed parent		
US-W no.	Derived from family	Level of inbreeding	Character	Observed ¹⁾ ²⁾	Expected	US-W no.	Pheno- type ²⁾	Geno- type assigned
9500	5309	S ₁	flower pigmentation	31 W : 0 P	$\infty : 0$	5309-1	W	<u>ff</u>
"	"	"	tuber skin "	31 W : 0 P	$\infty : 0$	"	W	<u>ii</u>
"	"	"	tuber shape	29 L : 2 Ov : 0 R		"	L	
9509	5309	S ₁	flower pigmentation	56 W : 0 P	$\infty : 0$	5309-7	W	<u>ff</u>
"	"	"	tuber skin "	56 W : 0 P	$\infty : 0$	"	W	<u>ii</u>
"	"	"	tuber flesh color	11 W : 7 LY : 36 Y : 5 DY		"	Y	
"	"	"	tuber shape	52 L : 4 Ov : 0 R		"	L	
9509	5309	S ₂	flower pigmentation	35 W : 0 P	$\infty : 0$	8762-17	W	<u>ff</u>
"	"	"	tuber skin "	35 W : 0 P	$\infty : 0$	"	W	<u>ii</u>
"	"	"	tuber flesh color	19 W : 16 LY		"	LY	
"	"	"	tuber shape	35 L : 0 R		"	L	
9513	5309	S ₂	flower pigmentation	13 P : 5 W	13.50 : 4.50	9504-1	P	<u>Pp</u>
"	"	"	flower color	13 Red : 0 Pu	$\infty : 0$	"	Red	<u>pp</u>

Table 21. Continued

Progeny			Segregation ratios			Selfed parent		
US-W no.	Derived from family	Level of inbreeding	Character	Observed ¹⁾ ²⁾	Expected	US-W no.	Pheno-type ²⁾	Geno-type assigned
9513	5309	S ₂	tuber skin pigmentation	42 W : 0 P	∞ : 0	9504-1	W	<u>ii</u>
"	"	"	tuber flesh color	6 W : 35 LY : 1 Y		"	LY	
"	"	"	tuber shape	35 L : 0 R		"	L	
9516	5309 (?)	S ₂	flower pigmentation	43 W : 0 P	∞ : 0	8762.7(?)	?	<u>ff</u>
"	"	"	ovary wall "	43 P : 0 NP	∞ : 0	"	?	<u>Ow Ow</u>
"	"	"	tuber skin "	26 P : 0 W	∞ : 0	"	?	<u>II</u>
"	"	"	tuber shape	4 R : 21 Ov : 1 L		"	?	
9521	5309	S ₄	flower pigmentation	10 P : 0 W	∞ : 0	9187-2	P	<u>FF</u>
"	"	"	flower color	10 Red : 0 Pu	∞ : 0	"	Red	<u>ppR-</u>
"	"	"	acylation	no data, probably all UA		"	UA	<u>acac(?)</u>
"	"	"	tuber pigmentation	34 W : 0 P	∞ : 0	"	W	<u>ii</u>
"	"	"	tuber shape	34 VL : 0 R		"	VL	
"	"	"	tuber flesh color	34 W : 0 Y		"	W	

Table 21. Continued

Progeny			Segregation ratios			Selfed parent		
US-W nb.	Derived from family	Level of inbreeding	Character	Observed ¹⁾ ²⁾	Expected	US-W no.	Pheno-type ²⁾	Geno-type assigned
9523	5309(?)	S ₄	flower pigmentation	23 P : 1 W		9187-9(?)	?	
"	"	"	ovary wall "	24 P : 0 NP	∞ : 0	"	?	<u>Ow</u> <u>Ow</u>
"	"	"	tuber skin "	10 Red : 11 Spect : 0 W		"	?	
"	"	"	tuber shape	20 R : 1 Ov		"	?	
"	"	"	chromat. band Rf .62	9 Yes: 5 No		"	?	
9525	5312	S ₂	Flower pigmentation	43 : 0 W	∞ : 0	9175-3	P	<u>FF</u>
"	"	"	flower color	34 Pu : 9 Red	32.25 : 10.75	"	Pu	<u>PuR-</u>
"	"	"	stigma pigmentation	24 P : 17 NP		"	P	
"	"	"	flower variegation	10 Var : 31 NVar		"	Var	
9527	5314	S ₂	flower pigmentation	45 W : 0 P		9177-7	W	<u>ff</u>
"	"	"	tuber skin "	34 W : 37 Pi		"	W(?)	

Table 21. Continued

Progeny			Segregation ratios			Selfed parent		
US-W no.	Derived from family	Level of inbreeding	Character	Observed ¹⁾ ²⁾	Expected	US-W no.	Pheno-type ²⁾	Geno-type assigned
9528	5314	S ₃	flower pigmentation	38 W : 0 P	∞ : 0	9185-33	P	<u>ff</u>
"	"	"	tuber skin "	9 W : 29 Pi		"	Pi	
"	"	"	tuber shape	20 R : 18 Ov		"	R	
9535	5315	S ₁	flower pigmentation	15 P : 0 W	∞ : 0	9190-15	P	<u>FF</u>
"	"	"	ovary wall "	15 NP : 0 P	∞ : 0	"	NP	<u>ow ow</u>
"	"	"	tuber skin "	45 P : 0 W	∞ : 0	"	P	<u>II</u>
"	"	"	tuber skin color	45 Red : 0 Pu	∞ : 0	"	Red	<u>ppR-</u>
"	"	"	tuber shape	25 R : 20 Ov : 0 L		"	R	
9541	"	S ₅	flower pigmentation	7 P : 3 W	7.50 : 2.50	9537-7	P	<u>FF</u>
"	"	"	ovary wall "	3 P : 10 NP		"	NP	
"	"	"	tuber skin "	30 P : 0 W	∞ : 0	"	P	<u>II</u>
"	"	"	tuber skin color	30 P(25 Red + 5 Pi) : 0 Pu	∞ : 0	"	Red	<u>ppRR^{DW}</u>
"	"	"	tuber shape	30 R ³⁾ : 0 L		"	R	

Table 21. Continued

Progeny			Segregation ratios			Selfed parent		
US-W no.	Derived of family	Level of inbreeding	Character	Observed ¹⁾ 2)	Expected	US-W no.	Pheno-type ²⁾	Geno-type assigned
9545	5328	S ₁	flower pigmentation	11 P : 4 W	10.25 : 3.75	5328-4	P	<u>Ff</u>
"	"	"	flower color	11 Red : 0 Pu	11 : ∞	"	Red	<u>ppR-</u>
"	"	"	ovary wall pigmentation	10 P : 4 NP	10.50 : 3.50	"	P	<u>Ow ow</u>
"	"	"	tuber skin "	12 P : 6 W	13.50 : 4.50	"	P	<u>Ii</u>
"	"	"	acylation	9 A : 5 UA	10.50 : 3.50	"	A	<u>Acac</u>
"	"	"	tuber shape	5 L : 4 Ov : 9 R		"	R	
9547	5328	S ₁	flower pigmentation	25 P : 18 W	32.25 : 10.75	5328-11	P	<u>Ff</u> (?)
"	"	"	flower color	25 Red : 0 Pu	∞ : 0	"	Red	<u>ppR-</u>
"	"	"	ovary wall pigmentation	33 P : 9 NP	31.50 : 10.50	"	P	<u>Ow ow</u>
"	"	"	tuber skin "	41 P : 6 W	35.25 : 11.75	"	P	<u>Ii</u> (?)
"	"	"	tuber shape	2 V1 : 6 L : 25 Ov : 14 R		"	R	
9548	5328	S ₁	flower pigmentation	26 W : 0 P	∞ : 0	5328-12	W	<u>ff</u>

Table 21. Continued

Progeny			Segregation ratios			Selfed parent		
US-W no.	Derived of family	Level inbreeding	Character	Observed 1) 2)	Expected	US-W no.	Pheno-type 2)	Geno-type assigned
9548	5328	S ₁	ovary wall pigmentation	22 P : 4 NP	19.50 : 6.50	5328-12	P	<u>Ow ow</u>
"	"	"	tuber skin pigmentation	19 P : 13 W	24 : 8	"	P	<u>Ii</u>
"	"	"	tuber skin color	19 Red : 0 Pu	∞ : 0	"	Red	<u>ppR-</u>
"	"	"	tuber shape	4 R : 17 Ov : 11 L		"	Ov	
9549	5328	S ₁	flower pigmentation	32 W : 0 P	∞ : 0	"	W	<u>ff</u>
"	"	"	ovary wall "	24 P : 8 NP	24 : 8	"	P	<u>Ow ow</u>
"	"	"	tuber skin "	24 P : 9 W	24.75 : 8.25	"	P	<u>Ii</u>
"	"	"	tuber skin color	24 Red : 0 Pu	∞ : 0	"	Red	<u>ppR-</u>
"	"	"	tuber shape	6 R : 18 Ov : 9 L		"	R	
9550	5328	S ₁	flower pigmentation	102 W : 0 P	∞ : 0	5328-20	W	<u>ff</u>
"	"	"	ovary wall "	64 P : 38 NP	76.50 : 25.50	"	P	<u>Ow ow</u>
"	"	"	tuber skin "	34 W : 16 Pi : 66 Red		"	Red	<u>Ii</u>

Table 21. Continued

Progeny			Segregation ratios			Selfed parent		
US-W no.	Derived from family	Level of inbreeding	Character	Observed ¹⁾ ²⁾	Expected	US-W no.	Pheno-type ²⁾	Geno-type assigned
9550	5328	S ₁	tuber skin color	84 (Red + Pi) : 0 pu	∞ : 0	5328-20	Red	<u>ppR-</u>
"	"	"	tuber shape	35 L : 43 Ov : 40 R		"	Ov	
9552	5328	S ₁	flower pigmentation	61 W : 0 P	∞ : 0	5328-21	W	<u>ff</u>
"	"	"	ovary wall "	53 P : 8 NP	45.75 : 15.25	"	P	<u>OwOw</u>
"	"	"	tuber skin "	19 W : 10 Pi : 56 Red		"	Red	<u>Ii</u> (?)
"	"	"	tuber skin color	66 (Red + Pi) : 0 Pu	∞ : 0	"	"	<u>ppR-</u>
"	"	"	tuber shape	18 L : 35 Ov : 32 R		"	Ov	
9554	5328	S ₁	flower pigmentation	44 W : 0 P	∞ : 0	5328-25	W	<u>ff</u>
"	"	"	ovary wall "	31 P : 13 NP	33 : 11	"	P	<u>OwOw</u>
"	"	"	tuber skin "	13 W : 4 Pi : 34 Red		"	Red	<u>Ii</u> (?)
"	"	"	tuber skin color	38 (Red + Pi) : 0 Pu	∞ : 0	"	"	<u>ppR-</u>
"	"	"	tuber shape	7 L : 23 Ov : 21 R		"	R	
9559	5328	S ₂	flower pigmentation	47 P : 0 W	∞ : 0	8775-34	P	<u>FF</u>

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Table 21. Continued

Progeny			Segregation ratios			Selfed parent		
US-W no.	Derived from family	Level of inbreeding	Character	Observed ¹⁾ ²⁾	Expected	US-W no.	Pheno-type ²⁾	Geno-type assigned
9559	5328	S ₂	flower color	47 Red : 0 Pu	∞ : 0	8775-34	Red	<u>ppR-</u>
"	"	"	ov. wall pigmentation	0 P : 47 NP	0 : ∞	"	NP	<u>owow</u>
"	"	"	tuber skin "	0 P : 47 W	0 : ∞	"	W	<u>ii</u>
"	"	"	acylation	36 A : 11 UA	35.25 : 11.75	"	A	<u>Acac</u>
"	"	"	tuber shape	47 L : 0 R		"	L	
"	"	"	tuber flesh color	29 LY : 18 Y		"	Y	
9560	5328	S ₂	flower pigmentation	26 W : 0 P	∞ : 0	9546-1	W	<u>ff</u>
"	"	"	ov. wall pigmentation	20 P : 6 NP	19.50 : 6.50	"	P	<u>owow</u>
"	"	"	tuber skin "	11 P : 8 W	14.25 : 4.75	"	P	<u>ii</u>
"	"	"	tuber skin color	11 Red : 0 Pu	∞ : 0	"	Red	<u>ppR-</u>
9572	7139	S ₁	flower pigmentation	16 P : 13 W	21.75 : 7.25	7139-2	P	<u>Ff</u>
"	"	"	flower color	4 Red : 12 Pu	4 : 12	"	Pu	<u>PpRR</u>
"	"	"	ovary wall pigmentation	10 P : 23 NP		"	P	

Table 21. Continued

Progeny			Segregation ratios			Selfed parent		
US-W no.	Derived from family	Level of inbreeding	Character	Observed ¹⁾ ²⁾	Expected	US-W no.	Pheno-type ²⁾	Geno-type assigned
9572	7139	S ₁	albino seedlings	14 Alb : 57 NAlb	17.75 : 52.25	7139-2	NAlb	<u>Alal</u>
9574	7139	S ₁	flower pigmentation	50 : P 4 W		7139-7	P	
"	"	"	flower color	43 Pu : 7 Red	37.50 : 12.50	"	Pu	<u>PpR-</u>
"	"	"	ovary wall pigmentation	23 P : 34 NP		"	P	
"	"	"	tuber skin "	31 P : 11 W	31.50 : 10.50	"	P	<u>Ii</u>
9575	7139	S ₁	albino seedlings	17 Alb : 42 NAlb	14.75 : 44.25	7139-8	NAlb	<u>Alal</u>
9577	7139	S ₁	flower pigmentation	48 W : 0 P	∞ : 0	7139-19	W	<u>ff</u>
"	"	"	ovary wall "	27 P : 21 NP		"	P	
"	"	"	tuber skin "	3 W : 11 P	3.50 : 10.50	"	?	<u>Ii</u>
9578	7139	S ₁	flower pigmentation	30 W:0 P	∞ : 0	7139-20	W	<u>ff</u>
"	"	"	ovary wall "	23 P : 7 NP	22.50 : 7.50	"	P	<u>Owow</u>
"	"	"	tuber pigmentation	14 P : 4 W	13.50 : 4.50	"	?	<u>Ii</u>
"	"	"	albino seedlings	23 Alb : 62 NAlb	21.25 : 63.75	"	NAlb	<u>Alal</u>

Table 21. Continued

Progeny			Segregation ratios			Selfed parent		
US-W no.	Derived from family	Level of inbreeding	Character	Observed ¹⁾ 2)	Expected	US-W no.	Pheno-type ²⁾	Geno-type assigned
9579	7139	S ₁	flower pigmentation	21 P : 7 W	21 : 7	7139-22	P	<u>Ff</u>
"	"	"	ovary wall "	0 P : 25 NP	0 : ∞	"	NP	<u>owow</u>
"	"	"	albino seedlings	18 Alb : 59 NAlb	19.25 : 57.75	"	NAlb	<u>Alal</u>
9580	7139	S ₁	flower pigmentation	20 P : 9 W	21.75 : 7.25	7139-23	P	<u>Ff</u>
"	"	"	ovary wall "	0 P : 29 NP	0 : ∞	"	NP	<u>owow</u>
9581	"	S ₁	flower pigmentation	22 P : 7 W	21.75 : 7.25	7139-32	P	<u>Ff</u>
"	"	"	ovary wall "	21 P : 10 NP	23.25 : 7.75	"	P	<u>OwOw</u>
"	"	"	tuber skin "	28 P : 7 W	26.25 : 8.75	"	?	<u>Ii</u>
9582	7139	S ₁	flower pigmentation	31 P : 12 W	32.25 : 10.75	7139-36	P	<u>Ff</u>
"	"	"	ovary wall "	20 : 24 NP		"	P	
9583	Stn	S ₁	flower pigmentation	19 W : 0 P	∞ : 0	Stn PI 230513.1	?	<u>ff</u>
"	"	"	tuber skin "	19 P : 0 W	∞ : 0	"	?	<u>II</u>
"	"	"	ovary wall "	19 P : 0 NP	∞ : 0	"	?	<u>OwOw</u>

Table 21. Continued

Progeny			Segregation ratios		Selfed parent			
US-W no.	Derived from family	Level of inbreeding	Character	Observed ¹⁾ ²⁾	Expected	US-W no.	Pheno- type ²⁾	Geno- type assigned
9586	Stn	S ₁	tuber skin color	19 Red : 0 Pu	∞ : 0	Stn PI 230513.1	?	<u>ppRR</u>
"	"	"	acylation	19 UA : 0 A	∞ : 0	"	?	<u>acac</u>

¹ Since not all flowering plants tuberized and vice versa, the ratios for flowers and tubers of the same family do not necessarily correspond.

² Code of abbreviations used:

A = Acylated	Pi = Pink	LX = Light Yellow	VL = Very Long	Var = Variegated
UA = Unacylated	Pu = Purple	Y = Yellow	L = Long	NVar = Non-variegated
P = Pigmented	NP = Non-pigmented	DY = Dark Yellow	Ov = Oval	Alb = Albino
	W = White		R = Round	NAlb = Non-albino

³ Included 6 "compressed" types

DISCUSSION

A. Vigor and yield

Generally the average performance of the 4 families decreased with each subsequent level of inbreeding. The inbreeding depression measured for yield for the first clonal generation in 1968 coincided with a curve calculated on the basis of heterozygosity in a diploid organism (Table 3; Fig. 2). In another sample from the same population, in both the seedling populations and the first clonal generation, the inbreeding depression for yield and other characters related to vigor exhibited a very sharp decrease from the S_0 to the S_1 but only a slight depression or no depression at all from the S_1 to the S_3 (Table 4). A considerable inbreeding depression is expected in crops that are normally highly heterozygous and cross-pollinated. A depression from the S_0 to the S_1 which is greater than can be expected on the basis of a reduction in heterozygosity could be interpreted as an indication of the presence of non-additive genetic variance. Significant non-additive variance has been reported in potatoes (Mendiburu, 1971).

Upon inbreeding tetraploid potatoes, Krantz (1946) obtained an S_2 that yielded 82 % of the S_0 . In this experiment with diploids the first clonal generation S_2 yielded only 36 % of the S_0 (Table 3). The difference can be attributed to the more rapid increase in homozygosity at the diploid level. The 4 families responded differently to inbreeding. The fluctuations in response were partly due to reproductive difficulties (both

sexual and asexual) which increase with inbreeding. The genetic differences between the original parent populations (S_0) could also have contributed to differences in response to inbreeding. This was observed at the tetraploid level by Krantz and Hutchins (1929).

Attempts to obtain each subsequent level of inbreeding from a representative sample of parents at the preceding generation were not always successful. Only the more vigorous plants flowered, and only a portion of these were self-fertile in the advanced generations of inbreeding (Table 7). Selection for vigor in the sexual cycle has also been observed upon inbreeding at the tetraploid level (Krantz, 1946; Krantz and Hutchins, 1929). Rudorf (1958) reported similar difficulties upon inbreeding tetraploid potatoes. He could not obtain the S_3 in most inbred lines due to their lack of vigor, and to virus infection and reduced fertility. Approximately 45 % of the genotypes were lost between the time of planting the seed and the transplanting stage. Similar losses were reported by Engel (1957).

Several genotypes were unable, due to lack of tuberization, to produce the second clonal generation. When the performance of the first clonal generations was compared with the asexual progenies, the depression in relation to the respective S_0 's was smaller for most progenies than for their respective asexual parental populations (Tables 3 and 4; Fig. 3). This can probably be attributed, in part, to failures of asexual reproduction. Many plants that were classified as "tuberizing" in the seedling year, nevertheless only produced small immature tubers which were not viable the following spring after standard storage conditions. The per cent of seedlings with 4 or more viable tubers the following spring was considerably lower in the S_3 than in the S_0 .

although the per cent of plants classified as "tuberizing" was approximately the same for the S_0 and S_3 (Table 4). The reduced asexual reproduction indicates that harmful recessive genes may have been uncovered, the expression of which is delayed until after the first clonal generation, or until an unfavorable reaction between the environment and the weaker genotypes occurs. The genotypes in which lethal or semi-lethal genes are expressed are automatically excluded from contributing to the subsequent generation, which in turn affects the rate of inbreeding. In spite of these problems in reproduction, several fairly vigorous S_5 lines have been obtained.

Assuming no selection for heterozygosity, the diploid S_5 generation is approximately 97 % homozygous. In comparison, the autotetraploid S_5 generation, assuming random chromosome segregation, would only be 53 % homozygous (Bartlett and Haldane, 1934). The unavoidable selection for vigor during reproduction, although kept to a minimum, probably resulted to a certain extent in some selection for heterozygosity as well. However, the diploid S_5 generation probably represents the greatest degree of inbreeding ever imposed upon potatoes.

The rapid decline of vigor upon inbreeding suggests that hybrid vigor may be obtained when two inbred clones with a high combining ability are crossed. Considering the success with hybrid vigor in other crops, this area certainly warrants further investigation in potatoes, especially on the diploid level where advanced inbred lines can be obtained in a relatively short time. A broader genetic base will probably be necessary to develop more diverse inbred lines that can be used as parents to produce maximum hybrid vigor.

B. Fertility

Self-compatibility was considerably lower than either pollen- or female-fertility for all levels of inbreeding when expressed as percent of flowering clones or as seeds per fruit. A reduction in seed set for self-compatibility, pollen-, and female-fertility could be demonstrated with each subsequent level of inbreeding (Table 7; Fig. 4). In these experiments no study was made of the growth of the pollen tubes in the styles. It is therefore difficult to separate clearly the effects of inbreeding upon the level of self-compatibility from that of various degrees of breakdown of the self-incompatibility reaction since both phenomena can be expected to affect the number of seeds per fruit. In addition, the level of self-compatibility can be obscured by either the level of pollen- or female-fertility or both.

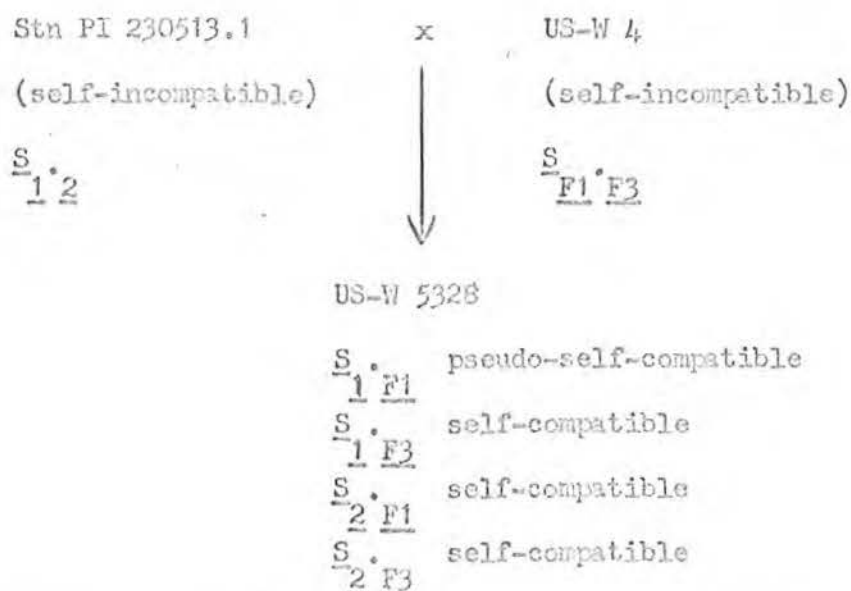
The data obtained on self-compatibility for family US-W 5328 agree with those obtained by Hollenback (1966) for this family (Table 10). In both instances self-incompatible and pseudo-self-compatible plants occur in the S_0 population. These data contradict the results obtained by Smiley (1963) who reported only self-compatible plants in this family. Unfortunately Smiley (1963) did not present any detailed data of his experimental results so that a further comparison with his methods of classification is not possible.

The data reported herein and by Hollenback (1966) indicate no female-sterility in family US-W 5328 (Tables 8, 9 and 10). Hollenback (1966) did observe some plants with a reduced level of female-fertility in this family. However, the progenies obtained by selfing US-W 5328-11 and US-W 5328-20 contained several plants which were classified as low female-

fertile or female-sterile (Tables 11, 12 and 13). Thus it appears that inbreeding has uncovered recessive genes for female-sterility. Information from the progeny tests of the two clones from family US-W 5328 indicates that the difference between these two clones in the degree of female-fertility is genetically controlled (Table 13; Fig. 5). This could be interpreted as an additive effect of the genes for female-sterility. In that case, the clone US-W 5328-11 which was classified as pseudo-self-compatible may have been misclassified for self-compatibility since the reduced level of female-fertility would also reduce the seed set upon selfing.

The information obtained on self-compatibility in the S_0 generation seems to fit the scheme presented by Hollenback (1966). This hypothesis, which is based on the one proposed by Pandey (1964) for *Nicotiana glauca* and *N. langsdorffii* assumes two basic forms of incompatibility alleles at the S locus: S_I and S_{FI} (I represents the allele number). The hypothesis was formulated to account for the presence of pseudo-self-compatibility as well as self-compatibility and self-incompatibility in the F_1 hybrids between the diploid Groups Phureja and Stenotomum and the Group Tuberosum haploid US-W 4 when both parents are self-incompatible. The effect of the S_{FI} form is to slow pollen tube growth, affecting S_I pollen less than S_{FI} pollen. Reciprocal differences can therefore be explained. The S_{FI} alleles differ from the S_I alleles in that they have less pollen specificity than the S_I alleles. Both the $S_{I \cdot I}$ and the $S_{FI \cdot FI}$ genotypes are self-incompatible. Genotypes with common alleles such as $S_{I \cdot FI}$, $S_{I \cdot F2}$ etc. are pseudo-self-compatible and genotypes with no common alleles such as $S_{I \cdot F2}$, $S_{I \cdot F3}$ etc. are fully self-compatible.

According to Hollenback's (1966) hypothesis the following scheme can then be applied to fit the behavior of family US-W 5328 :



Due to environmental factors pseudo-self-compatible clones might occasionally be classified as either self-incompatible or as self-compatible. A pseudo-self-compatible clone would upon selfing produce self-incompatible and pseudo-self-compatible progeny.

Abdalla (1970) reported female-sterility in Groups Phureja and Stenotomum which he considered to be a manifestation of female-incompatibility. The author postulated a two locus gametophytic incompatibility system in these two groups. The regular S locus is proposed to be epistatic over the second locus, R. The latter locus is postulated to have an allele, R_{fi} (female-incompatibility), which when present in the homozygous condition would lead to female-incompatibility. Until the cause of female-sterility is further elucidated, it seems advisable not to ascribe this to an incompatibility system. The data from Abdalla (1970) could be explained equally well by a hypothesis of recessive genes for female-sterility which are not necessarily related to an incompatibility mechanism.

Inbreeding by selfing imposes selection for self-compatibility. Lines homozygous for this trait have not yet been detected in the limited scope of this investigation. Of the total number of plants of the S_1 progenies investigated, nearly 50 % of the plants which were classified for their female-fertility behavior were classified as either low female-fertile or as female-sterile and could therefore not be classified for their self-compatibility reaction. Thus it appears that a better understanding of the female-fertility system and possible ways of producing inbred lines with a high degree of both female-fertility and pollen-fertility may be required before a successful search for lines homozygous for self-compatibility may be completed.

Future investigations could focus on the cause of the reduced seed set by studying pollen tube growth in the style as well as the proportions of functional endosperms in non-inbred parents and their progenies obtained through selfing. Linkage relations with the gene(s) for self-compatibility will also be of special interest. Simmonds (1966) reported a case of linkage of a harmful recessive gene to the S locus in potato. If the gene(s) for self-compatibility are linked to recessive lethals, it may be difficult to develop true breeding self-compatible clones.

C. Genetic markers

Self-compatible diploid hybrids between the self-incompatible Groups Phureja and Stenotomum and haploids of Group Tuberosum have been predicted to be excellent sources of unexplored genetic variability (Hougas and Peloquin, 1962). The selfing of such hybrid clones has produced progenies segregating for a large number of traits. Monogenic segregation ratios of previously described marker genes was confirmed and the

inheritance of the new trait, pigmented ovary wall, could be determined.

The data indicate that the gene Ow is closely linked with the B I F linkage group. It appears that in the material used in this study Ow is linked with I in the coupling phase and with F in the repulsion phase, such that a plant heterozygous for the 3 genes would have the following linkage constitution : $\frac{Ow \ I \ f}{ow \ i \ F}$. The linear order of these genes has not yet been determined. Without crossing-over, all white flowering plants in a progeny derived by selfing a plant which is heterozygous for Ow, I and F are expected to have pigmented ovary wall and pigmented tubers (assuming no other genes inhibiting flower pigmentation to be present). Similarly, all plants in such a progeny which have white tubers are expected to have non-pigmented ovary wall and pigmented flowers.

In several progenies which were derived from selfed $\frac{Ow \ I \ f}{ow \ i \ F}$ genotypes, no cross-overs between Ow and i or between ow and I could be detected (Table 18), but such cross-overs apparently did occur in the parents of families US-W 9547 and US-W 9581 (Tables 16 and 17 respectively). In cases where viable tubers are still available, those plants now considered as having been derived from "cross-over gametes" will be re-examined to verify these results. More data on crossing-over will be needed to determine the linear order of these genes. A large number of cross-overs cannot be detected in progenies obtained through selfing and therefore a test cross to a tester clone which is homozygous recessive for these 3 loci will be necessary.

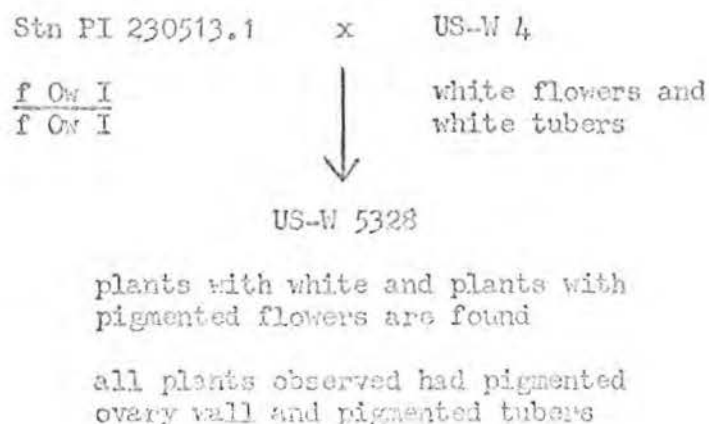
It might be argued that the gene Ow is an allele at the B locus which is known to control the distribution of anthocyanins in various plant organs. Since the B locus is also closely linked to I and F such a hypothesis is highly attractive. However, preliminary evidence

indicates that this is not the case. Genetic material received from Hermesen (1970) known to be homozygous $\underline{B}^{\underline{d}}\underline{B}^{\underline{d}}$ did not exhibit pigmented ovary wall. Likewise, none of the stocks used in the genetics of pigmented ovary wall had seed spot, which can only be conferred by the two highest alleles known to exist at the $\underline{B}$ locus ($\underline{B}^{\underline{c}}$ and $\underline{B}^{\underline{d}}$). Thus, if the pigmented ovary wall was the result of the activity of an until now undiscovered allele at the $\underline{B}$ locus higher than $\underline{B}^{\underline{d}}$, then one could expect plants with $\underline{Ow}$ -genotypes to exhibit the effects of $\underline{B}^{\underline{d}}$ as well. If, on the other hand, $\underline{Ow}$ would be equivalent to an allele which, in hierarchical dominance, would fall somewhere between $\underline{B}^{\underline{c}}$ or $\underline{B}^{\underline{d}}$ and $\underline{b}$ then one could expect all plants grown from seeds with seed spot to have pigmented ovary wall. Since neither appears to be the case it is tentatively concluded that $\underline{Ow}$ is not an allele of the $\underline{B}$ locus. However, considering the three loci $\underline{B}$, $\underline{I}$ and $\underline{F}$, Dodds and Long (1956) concluded that : " The closeness of these loci evidently facilitates intergenic cooperation either in reaction or timing sequences of the development of anthocyanins and the production of pattern effects." This statement can now probably be interpreted to include $\underline{Ow}$ as well.

In addition to having the factors $\underline{B}$, $\underline{I}$, $\underline{F}$ and $\underline{Ow}$ in one linkage group, it now appears that a major gene controlling tuber shape is also located on this chromosome. Upon selfing clones which were of the genotype $\underline{Ii}$ with either round or oval tubers, most of the long tubers were white ($\underline{ii}$) and most of the round tubers were pigmented ($\underline{I-}$) (Tables 19 and 20). Howard (1970) has reported similar results. It is apparent that the long shape is recessive since the long form breeds true upon selfing, but segregation for shape often occurs upon selfing clones with round or oval tubers (Table 21). Clones with round tubers which upon selfing do not produce any progeny with long tubers can then be considered homozygous for the dominant gene(s).

Crosses between plants with long white and round pigmented tubers have already been made and seed is available for analysis.

From the breeding behavior of Stn 230513.1 and of several S_0 clones in family US-W 5328 we can now make some inferences about the genetic constitution of the haploid US-W 4. This haploid is the Tuberosum parent of the hybrid family US-W 5328, as indicated below :



Since plants with pigmented as well as plants with white flowers were found in family US-W 5328, we can conclude that US-W 4 is heterozygous for $\underline{F}$. All progenies obtained by selfing clones from family US-W 5328 were segregating for $\underline{Ow}$. It appears therefore that US-W 4 is homozygous recessive for pigmented ovary wall. Since US-W 4 has white tubers we may assume that it is homozygous recessive at the $\underline{I}$ locus as well. Thus, disregarding the linear order of the genes involved, US-W 4 would be $\frac{ow \text{ } \underline{i} \text{ } \underline{F}}{ow \text{ } \underline{i} \text{ } \underline{f}}$.

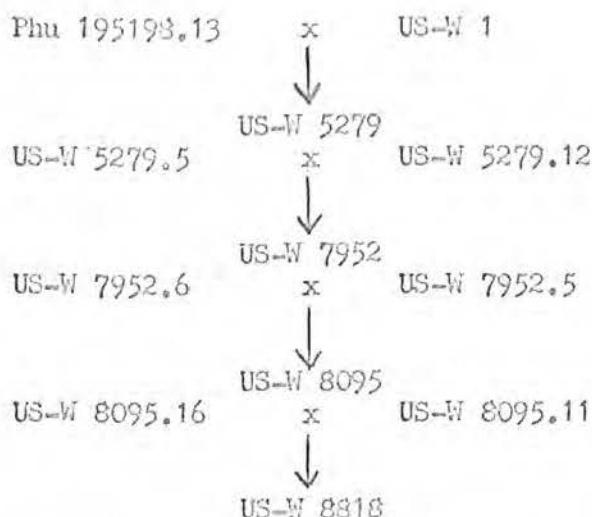
If the gene $\underline{F}$ is present, then we can expect pigmented flowers if the genes for anthocyanin synthesis are present. Because US-W 4 has white flowers we can postulate that it is probably homozygous recessive at the $\underline{P}$ locus and can at most have a very weak $\underline{R}$ allele such as $\underline{R}^{\underline{UW}}$. The anthocyanin synthesis of such an allele would be suppressed in the tuber by the $\underline{ii}$ constitution. On the basis of the phenotype of a clone with a postulated genetic

constitution such as this one, it is impossible to distinguish between rr and $R^{DW}r$ or $R^{DW}R^{DW}$. This may be detected upon crossing a clone such as US-W 4 to a clone with the $\frac{f}{f} \frac{I}{I} \frac{r}{r}$ genotype. Unfortunately $\frac{r}{r}$ clones are not yet available. Howard (1960) has suggested that Group Tuberosum R may be more comparable to R^{DW} than to R of the cultivated diploids. The occurrence of some pink tubers in several progenies from US-W 5328 clones (Table 18) could be the result of certain progeny plants being homozygous for Group Tuberosum R in the presence of I .

Considerably more work needs to be done to determine the genetics of the leaf pigmentation of clones US-W 8818.1 and US-W 8818.2. The phenomenon is nevertheless very interesting, and may well be a secondary effect as a result of some physiological disorder. The conditions which encourage anthocyanin formation are often injurious to the plant (Alston, 1959). Thus what is inherited may be a barrier in the metabolism, which in turn causes an excessive anthocyanin synthesis in the leaves. If this is the case then several different, but genetically determined, physiological disorders may each induce anthocyanin synthesis. The cause of leaf pigmentation in the semi-lethal dwarf mutants which occurred in the progenies of backcrosses to the pigmented parents (Table 15) may therefore be different from the cause of leaf pigmentation in the parents US-W 8818.1 and US-W 8818.2. Whatever causes the leaf pigmentation in these two clones, it is probably not restricted to any physiological disorder in the root system since scions of these clones grafted onto tomato rootstocks eventually showed the same symptoms.

The discovery by Nilssen (1971) that the diploid potato clone US-W 5279.15 exhibits severely stunted growth and considerable leaf pigmentation when exposed to near-ultraviolet light is of interest in relation

to the leaf pigmentation observed in the clones from family US-W 8818. This family was derived from family US-W 5279 by means of 3 subsequent sib-pollinations as indicated in the pedigree below :



Thus the possibility exists that the same genes affecting leaf pigmentation in US-W 5279.15 under the above described conditions may be present in the clones US-W 8818.1 and US-W 8818.2. The difference between the pigmented clones from families US-W 8818 and US-W 5279 may be due to a lowering of a physiological threshold in US-W 8818 as a result of inbreeding. US-W 5279.15 will only synthesize anthocyanins in the leaves when it is exposed to near-ultraviolet light, but US-W 8818.1 and US-W 8818.2 exhibit this trait when planted in the field in Sturgeon Bay or in the greenhouse. A study of the latter 2 clones under different light conditions may provide further clues.

These clones may also be excellent source material for a study of gene action. Alston (1959) has proposed the use of tissue culture for this purpose. The various environmental factors which induce anthocyanin synthesis in cultures of leaf tissues of US-W 8818.1 and US-W 8818.2 may provide further insight into the physiology, biochemistry and genetics of anthocyanin synthesis.

The chemogenetic study has enabled the utilization of one well defined marker gene (Ac). In the families which were analyzed, the laboratory technique could be used to supplement and substantiate visual scoring for flower pigments for this locus. Classification of the tuber pigments was only possible with the laboratory method.

Many differences in flavonoid substances were observed between Solanum species growing in the field at Sturgeon Bay. Such differences may be exploited in evaluations of interspecific hybrids and may have taxonomic value as well. Although taxonomy is beyond the scope of this thesis, the laboratory technique developed to detect chemogenetic markers may also prove to be a useful tool in future taxonomic studies.

It is of interest that several of the loci which have been defined thus far in potatoes appear to be complex as such or to be part of a gene complex affecting similar functions. The loci thought to be complex include R (Dodds and Long, 1955) and Ac (Harborne, 1967). Examples of gene complexes affecting similar functions are the B I F O w linkage group which affects the distribution of anthocyanins and the linkage group which controls the hypersensitive reaction to virus X and virus Y (Cockerham, 1970). As the methods used in genetic studies are improved, more loci of a complex nature will probably be discovered in potatoes. The above mentioned cases of gene complexes can be considered as evidence of some type of functional genetic integration in potatoes. Several cases of gene complexes have already been reported in other crops (Alston, 1959; Lewis, 1967). Genetic investigations of complex loci in higher organisms usually depend upon a high resolution experimental procedure. For further study of such loci controlling the synthesis of anthocyanins or other flavonoids in potatoes, the technique of TLC seems ideally suited.

SUMMARY

The cultivated diploid potatoes Solanum tuberosum Groups Stenotomum and Phureja ($2n = 2x = 24$) are normally self-incompatible and highly heterozygous. Crossing these stocks with the Group Tuberosum haploid ($2n = 2x = 24$), US-W 4, produced the self-compatible stocks for an investigation of inbreeding in cultivated diploid potatoes. Inbreeding such hybrids would be expected to uncover several recessive genes not normally expressed. When compared with autotetraploids, diploids have segregation ratios which are relatively simple and the rate of progress towards homozygosity upon selfing is much faster. Thus stocks homozygous for marker genes of potential value for future genetic investigations can be obtained much faster by selfing self-compatible diploid hybrids than by selfing the tetrasomic polyploid Group Tuberosum. The objectives of this study were: (a) to measure the inbreeding depression for several quantitatively inherited characters; (b) to search for marker genes which can be used in future genetic studies; and (c) to develop clones and inbred lines with known genotypes for such marker genes.

The inbreeding depression for several characters, which are assumed to be quantitatively inherited, was measured in 4 hybrid families. Subsequent inbred generations were obtained by selfing the flowering self-compatible clones of the preceding generation, and bulking equal amounts of seed from each parental clone. The progenies from individual self-compatible clones were scored for segregation of previously described marker genes, mostly those controlling anthocyanin synthesis and distri-

bution, and potentially useful new markers. A routine laboratory technique, using thin-layer chromatography, was developed for screening families segregating for genes affecting the synthesis of anthocyanins and other flavonoids.

For a number of characters measured, the average inbreeding depression for the 4 families coincided with a curve calculated on the basis of loss of heterozygosity in a diploid organism. The 4 families responded differently to inbreeding. This can, in part, be attributed to genetic differences between the original parent population and to selection for vigor in both the sexual and asexual reproduction cycles. Only the more vigorous plants flowered, and only a small portion of these were self-fertile in the advanced generations of inbreeding. Several genotypes were unable, due to lack of tuberization, to produce the second clonal generation. Reduced asexual reproduction indicates that harmful recessive genes may have been uncovered, the expression of which was delayed until an unfavorable reaction between the environment and the inbred genotypes occurred. Several fairly vigorous S_5 lines were obtained, many of which exhibited a high degree of uniformity. The rapid decline in vigor upon inbreeding suggests that hybrid vigor may be obtained from crosses between advanced inbred clones.

Since inbreeding by selfing imposes selection for self-compatibility, this procedure might produce some homozygous self-compatible lines which would be very useful for an investigation of the genetics of self-compatibility. Scoring for segregation of self-compatibility was hampered by the occurrence of female- and pollen-sterility in the inbred progenies. Inbreeding apparently uncovered recessive genes for various degrees of female-sterility. As a result of these difficulties, no clones homozygous

for self-compatibility were identified.

Progenies obtained by selfing hybrid diploid clones segregated for many traits. The genotypes for several known and potential marker genes were established for 31 self-compatible diploid clones. A gene, which controls the pigmentation of the ovary wall, was found and assigned the gene symbol Cw. This gene appears to be closely linked with several other genes controlling the distribution of anthocyanins. One of the genes affecting tuber shape apparently is also associated with this linkage group. Cultivated diploid stocks with known genotypes for marker genes have great potential value for future genetic investigations in potatoes.

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APPENDIX

A. List of recently assigned or currently used gene symbols

Gene symbol	Character	Source ¹⁾	Reference
<u>Ac</u>	Acylation. Controls acylation of anthocyanins with p-coumaric acid	Phu	Vide Harborne, 1960 a
<u>AF</u>	Anther-style fusion	Tbr	Grun, 1970
<u>al</u>	albino. White seedling lethal	Stn	Simmonds, 1965
<u>B</u>	Has at least 5 alleles which control pigmentation of floral abscission layer, tuber eyebrow, seed-spot and nodal band. Linked to <u>I</u> and <u>F</u>	Phu	Dodds and Long, 1956
<u>Cl</u>	Compound leaf	Phi	Abdalla, 1970
<u>cl</u>	Curled leaf	Phu	Simmonds, 1965
<u>Co</u>	Corolla pigmentation, <u>coco</u> suppresses pigmentation of the corolla in wild diploids. Linked to <u>E I F</u>	med	Paxman, 1961
<u>cr</u>	crumpled	Phu	Simmonds, 1965
<u>D</u>	Basic gene for color. According to Howard (1961, 1970) similar to gene <u>I</u> of Dodds and Long (1955)	Tbr	Salaman, 1910
<u>df</u>	Deformed flowers. Plasmon sensitive male sterile.	chc	Grun <u>et al.</u> , 1962
<u>dr</u>	Droopy. Linked to <u>S</u> .	Phu	Simmonds, 1965, 1966
<u>dx</u>	Defective xylem	Phu	Simmonds, 1965
<u>E</u>	"Red pigment no. 1." (in periderm of tuber, eyes, stems and flowers). Closely linked to <u>H</u> (Howard, 1966). Probably an allele of Tuberousum <u>H</u> (Howard, 1963)	Tbr	Lunden, 1937

A. Continued

Gene symbol	Character	Source ¹⁾	Reference
<u>El</u>	Lethal, Plasmon sensitive, linked with <u>I</u>	Phu	Frandsen, 1967
<u>F</u>	Controls pigmentation of flower in <u>S. tuberosum</u> . Possibly linked to <u>Tuberosum R</u> (Lunden, 1960)	Tor	Lunden, 1937, 1960
<u>F</u>	Controls pigmentation of flower in diploid cultivated species. Possibly same as <u>Tuberosum F</u> , but not linked to <u>R</u> in diploids (Howard, 1970)	Phu	Dodds and Long, 1956
<u>Fa</u>	Resistance to <u>Heterodera rostochiensis</u>	spg	Ross, 1966
<u>Fb</u>	Resistance to <u>H. rostochiensis</u>	spg	Ross, 1966
<u>FM</u>	Female-sterility	Tbr	Grun, 1970
<u>G</u>	Green plants. <u>gg</u> plants produce chlorotic foliage. Linked with a factor controlling anthocyanin pigment	vrn	Plaisted and Peterson, 1967
<u>GI</u>	Glycosylation of flavonols, but not of anthocyanins	sto, chc	Harborne, 1962
<u>HI</u>	Resistance to <u>H. rostochiensis</u>	Adg	Ross, 1966
<u>H2</u>	Resistance to <u>H. rostochiensis</u>	mlt	Ross, 1966
<u>hd</u>	Hairy dwarf	Stn	Simmonds, 1965
<u>I</u>	Basic gene for tuber skin color.	Phu	Dodds and Long, 1956
<u>In</u>	Indehiscence of anthers. Plasmon	chc, Tbr	Grun et al., 1962, 1970
<u>I^{SP}</u>	Spectacle of tuber color. Allele of <u>I</u> . Only <u>I^{SP}</u> plants produce spectaclled tubers.	Phu	Dodds and Paxman, 1962; Simmonds, 1969
<u>K</u>	Resistance to <u>H. rostochiensis</u>	ktz	Ross, 1966
<u>K</u>	Round tuber shape, (linked to O?)	Tbr	Maris, 1962

A. Continued

Gene symbol	Character	Source ¹⁾	Reference
<u>L</u>	Compound leaf	ktz	Choudhuri, 1944; Swaminathan, 1956
<u>Lb</u>	Lobed microspores. Plasmon sensitive, male-sterile	chc	Grun et al., 1962
<u>Ld₁</u>	Lobed pollen grains. Presence of	US-W 42,	Abdalla, 1970
<u>Ld₂</u>	Lobed pollen grains. any 2 genes	ver	
<u>Ld₃</u>	Lobed pollen grains. in ver cytoplasm leads to lobed pollen grains		
<u>lr</u>	Leafroll. Similar to infection by leafroll virus.	Phu	Simmonds, 1965
<u>M</u>	Restricts tuber periderm pigmentation to areas around the eyes. Closely linked to <u>E</u> (Howard, 1966)	Tbr	Kelly, 1924
<u>ms₁</u>	Male-sterile, non plasmon sensitive	US-W 42,	Abdalla, 1970
<u>ms₂</u>		"	
<u>ms₃</u>		"	
<u>Na_{sto}</u>	Non-lethal necrosis to virus A. Identical with <u>Rym</u> (Ross, 1960). Independent of <u>Rysto</u> and <u>Rysto</u> .	sto	Cockerham, 1970
<u>Na_{tbr}</u>	Lethal necrosis to virus A. Identical to gene formerly designated as <u>Na</u> . Linked to <u>Nx_{tbr}</u> and possibly <u>Nctbr</u> . Independent of <u>Nb_{tbr}</u> .	Tbr	Cockerham, 1970
<u>Nb_{tbr}</u>	Lethal necrosis to virus X, Groups 1 and 2. Identical to gene formerly designated as <u>Nb</u> . Independent of <u>Nx_{tbr}</u> , <u>Natbr</u> , <u>Nctbr</u> and <u>Rx_{act}</u> .	Tbr	Cockerham, 1970
<u>Nc_{tbr}</u>	Lethal necrosis to virus strain Y ^c . Identical with gene formerly designated as <u>Nc</u> . Possibly linked to <u>Nx_{tbr}</u> and <u>Na_{tbr}</u> .	Tbr	Cockerham, 1970

A. Continued

Gene symbol	Character	Source ¹⁾	Reference
<u>Nx_{tbr}</u>	Lethal necrosis to strains of virus X within Groups 1 and 3. Linked to <u>Na_{tbr}</u> , and possibly also to <u>Nc_{tbr}</u> , but independent of <u>Nb_{tbr}</u> .	Tbr	Cockerham, 1970
<u>Nx_{tbr}</u> ^{spl}	Allelic and probably identical with <u>Nx_{tbr}</u> . Linked to <u>Nx_{chc}</u> and <u>Ny_{chc}</u> .	spl	Cockerham, 1970
<u>Nx_{chc}</u>	Lethal necrosis to all strains of virus X. Linked to <u>Nx_{tbr}</u> ^{spl} and <u>Ny_{chc}</u> .	chc,	Cockerham, 1970
<u>Ny_{chc}</u>	Lethal necrosis to all strains of virus Y and virus A. Linked to <u>Nx_{chc}</u> and <u>Nx_{tbr}</u> ^{spl} .	chc, med	Cockerham, 1970
<u>Ny_{das}</u>	Lethal necrosis to all strains of virus Y and virus A. Identical with gene formerly designated as <u>Ny</u> . Independent of <u>Ry_{hou}</u> .	das	Cockerham, 1970
<u>Ns</u>	Necrotic reaction to virus S.	Adg	Baerecke, 1967
<u>ns₁</u> <u>ns₂</u> <u>ns₃</u> <u>ns₄</u>	Non shedding anthers, plasmon sensitive, male sterile. Same phenotype as <u>pl</u> .	Phu	Abdalla, 1970
<u>Ng₁</u> <u>Ng₂</u>	Normal growth. <u>Ng₁</u> and <u>Ng₂</u> are complementary	Phu	Abdalla, 1970
<u>nl</u>	Narrow leaflet	Phu	Dodds and Paxman, 1962
<u>O</u>	Deep eyes. (Linked to <u>K</u> ?)	Tbr	Maris, 1962
<u>O₁</u> <u>O₂</u> <u>O₃</u>	Resistance to <u>H. rostochiensis</u>	opl	Ross, 1966

A. Continued

Gene symbol	Character	Source ¹⁾	Reference
<u>P</u>	Purple pigmentation of tubers, stems, flowers	Tbr	Salaman, 1910
<u>pl</u>	Pollenless. Plasmon sensitive male sterile	chc	Grun <u>et al.</u> , 1962
<u>py</u>	Pink and yellow. Leaflets are pink underneath and have yellow margins	Adg	Simmonds, 1965
<u>Ps</u>	Partly stained sterility. Plasmon sensitive, male sterile.	US-W 42,	Abdalla, 1970
<u>R</u>	Red pigmentation of tuber phelloderm in tetraploids. Allelic with <u>E</u> (Howard, 1968). Possibly linked to <u>F</u> (Lunden, 1960)	Tbr	Lunden, 1937
<u>R</u>	Red pigmentation of tuber, stems, flowers in diploid species. Allele <u>RDW</u> produces pink tubers and white flowers.	Phu, Stn	Dodds and Long, 1956
<u>R</u>	Second incompatibility locus in two locus system	arp	Pushkarnath, 1953
<u>R</u> _(n)	Hypersensitive reaction to <u>Phytophthora infestans</u> (n=1 - ?).		Black <u>et al.</u> , 1953
<u>Rx_{acl}</u>	Extreme resistance to all strains of virus X. Independent of <u>Nx_{tbr}</u> and <u>Rx_{adg}</u> .	acl	Cockerham, 1970
<u>Rx_{acl}</u> ⁿ	Allele of <u>Rx_{acl}</u> . Lethal necrosis to all strains of virus X. Independent of <u>Nb_{tbr}</u> .	Adg	Cockerham, 1970
<u>Rx_{adg}</u>	Extreme resistance to all strains of virus X. Independent of <u>Nx_{tbr}</u> and <u>Rx_{acl}</u> .	Adg	Cockerham, 1970
<u>Ry_{dms}</u> ^a	Allele of <u>Ny_{dms}</u> . Lethal necrosis to virus A. <u>dms</u>	dms	Cockerham, 1970
<u>Ry_{hou}</u>	Extreme resistance to all strains of viruses Y and A. Independent of <u>Ny_{dms}</u> .	hou	Cockerham, 1970

A. Continued

Gene symbol	Character	Source ¹⁾	Reference
<u>Ry_{sto}</u>	Extreme resistance to all strains of viruses Y and A. Identical with <u>Ry</u> (Ross, 1960).	sto	Cockerham, 1970
<u>Ry_{storna}</u>	Extreme resistance to virus Y and lethal necrosis to Virus A. Probably allele of <u>Ry_{sto}</u> . Confirmed allele of <u>Ry_{storna}</u> .	sto	Cockerham, 1970
<u>Ry_{ston1}</u>	Lethal necrosis to virus Y, coupled with susceptibility to virus A. Identical with <u>Ryn</u> (Ross, 1960). Allele of <u>Ry_{sto}</u> . Independent of <u>Ry_{storna}</u> , <u>Ry_{ston2}</u> , and <u>Na_{sto}</u> .	sto	Cockerham, 1970
<u>Ry_{storna}</u>	Extreme resistance to virus Y and non-lethal necrosis to virus A. Allele of <u>Ry_{storna}</u> and <u>Ry_{ston2}</u> . Independent of <u>Ry_{sto}</u> , <u>Ry_{ston1}</u> and <u>Na_{sto}</u> .	sto	Cockerham, 1970
<u>Ry_{ston2}</u>	Identical in effect with <u>Ry_{ston1}</u> . Independent of <u>Ry_{ston1}</u> , <u>Ry_{sto}</u> and <u>Na_{sto}</u> . Allele of <u>Ry_{storna}</u> .	sto	Cockerham, 1970
<u>s</u>	Resistance to virus S.	tbr	Bagnall and Young, 1968
<u>S</u>	Self-incompatibility		see: Abdalla, 1970 Cipar, 1963
<u>sh</u>	Shorty	Stn	Simmonds, 1965
<u>SM</u>	Shrivelled microspores	Tbr,	Grun, 1970
<u>Sp</u>	Sporads	chc Tbr,	
<u>TA</u>	Thin anther	chc Tbr	
<u>Tr</u>	Tetrad sterility	" "	ver Abdalla, 1970
<u>un₁</u> <u>un₂</u>	Undivided microsporocyte	US-M 42, "	Abdalla, 1970

A. Continued

Gene symbol	Character	Source ¹⁾	References
<u>y</u>	Yellow-green seedling	Tbr	Lunden, 1937
<u>V₁</u> - <u>V₄</u>	Resistance to <u>H. rostochiensis</u>	vrn	Ross, 1966
<u>vc</u>	Veinal chlorosis	gon	Simmonds, 1965
<u>w</u>	White (albino)	Tbr	Lunden, 1937
<u>W</u>	Winged stem	mcd	Choudhuri, 1944; Swaminathan, 1956
<u>X</u>	Resistance to <u>Synchytrium endo-</u> <u>bioticum</u>	Tbr	Lunden, 1937, 1950
<u>Y</u>	Resistance to <u>S. endobioticum</u> .	Tbr	Lunden, 1937, 1950
<u>Z</u>	Two complementary genes	Tbr	" " "
<u>ym</u>	Yellow margin	Adg	Dodds, 1956; Dodds and Parman, 1962

¹⁾ For code of abbreviations to potato names see Simmonds (1963).

B. Preliminary survey of materials and methods for the detection of chemogenetic marker genes

i. Concentration of the extract

The extracts were generally not concentrated prior to application to the plates. Only in cases when it became apparent that a particular extract was too dilute and only if no more fresh tissue was available would the extract be concentrated in a flash evaporator at room temperature. If concentration of the extract was anticipated then the extraction would be done with approximately 8 % acetic acid in methanol rather than with 1 % hydrochloric acid in methanol which was used in all other cases for anthocyanin extraction. Concentration of an anthocyanin solution in hydrochloric methanol often results in a partial acid hydrolysis due to the increasing acidity of the solvent upon concentration (Von Elbe and Schaller, 1968; Mullick, 1966).

ii. Adsorbents

Several adsorbents were tried in a preliminary survey, with only a few giving satisfactory results. The adsorbents which were screened are listed in Table 22. Excellent results were obtained with Avicel TG 104 cellulose; unfortunately the production of this material has been discontinued by the manufacturer. Good resolutions were also obtained with the Signacell Type 19 cellulose. The Camag cellulose did not seem to produce the degree of resolution as did the Avicel or Signacell but this material could still be used successfully with pigments containing relatively few components. In general the celluloses with the smaller particles sizes (<25 microns) provided the best resolution. These materials required a

longer developing time than the coarser adsorbents, but the higher degree of resolution far outweighed the time factor.

iii. Application of the extract sample to the plate

Many techniques have been described by Stahl (1969). Only simple inexpensive instruments were considered for our purpose. When the sample was applied with a capillary micropipette, the layer of adsorbent was often damaged at the point of application. This resulted in an uneven solvent flow across the plate and consequently in "tailing" of the pigment and unsatisfactory separation. These problems were eliminated with the brush type sample applicator described by Tamura (1965).

iv. Developing solvents

Many solvents which are used in paper chromatography (PC) can also be used with thin-layer chromatography (TLC). The choice of solvent often depends upon the materials to be separated. The solvents that were screened are listed in Table 23. The best general solvent which can be used for the separation of both anthocyanins and flavonol glycosides is solvent 14 which is also widely used in PC. Solvents 4 and 9 also performed fairly well as general solvents. Solvents 12 and 10 did well in the two dimensional TLC as first and second solvents respectively (See Mullick, 1969, for procedure). The other solvents were not satisfactory for use as general solvents on TLC although some others, especially solvent 15 did well on PC. Solvent 7 has been used effectively in detecting flavonol glycosides. Harborne's (1959) observation that alcohol - acetic acid mixtures (such as solvent 14) alter in composition with age was confirmed in our laboratory. As the mixture aged an aqueous layer continued to form. This resulted in lower R_F

values with the older solvent as compared with a "fresh" 2-day old solvent. Fuleki and Francis (1967) have shown that the polarity of a solvent can be altered by changing the amount of water in the organic phase.

v. Reference materials

Most anthocyanins and flavonol glycosides cannot be purchased commercially, although several anthocyanidin chlorides are available. Most of the naturally occurring flavonoids are also difficult to obtain from other investigators. Even when refrigerated in a slightly acid medium most anthocyanins become contaminated with breakdown products after 1 - 2 months. Thus co-chromatography with known anthocyanins has become a standard procedure. Harborne (1967) has listed the major sources of a large number of anthocyanins. However, most of these sources contain several other pigments as well, and it is usually necessary to separate these pigments chromatographically (preparative chromatography), identify the pigment band which is desired and then elute that band and concentrate the eluate. This is very time consuming and requires additional equipment.

Two exceptions were the sources of petanin and of rutin. The best source of petanin was the garden huckleberry, Solanum ruiginosum. Francis and Harborne (1966) reported that 93 % of the pigment of these berries is petanin. Our results confirm this to the extent that only one major band appeared upon chromatography of the extract of frozen huckleberries. Thus no further purification was necessary in this case. Rutin was available commercially.

vi. Preparatory chromatography

Occasionally it was necessary to purify a pigment chromatographically. For example, when no ripe huckleberries were available, the petals of Petunia hybrida 'Flandring Velvet' were our source of petanin. In order to establish on the basis of chemical tests which of the several bands present was petanin, it was necessary to separate the bands and elute them separately.

Preparatory chromatography was done by means of PC as well as TLC. The method described by Harborne (1958) was used for preparatory PC. The substances were eluted from the paper strips with the technique described by Ballinger and Maness (1969). Several methods described for preparatory TLC have been attempted (Asen, 1965; Bennett and Heftmann, 1963; Connolly et al., 1964; Klein and Rapoport, 1970). None of these methods were completely satisfactory, although the method of Connolly et al. (1964) has been used with a certain amount of success. This was still not any better than what could be achieved with heavy filter paper (Whatman 3MM) and proper equipment for PC. Since the eluate from preparatory work always had to be concentrated, the pigment was eluted with 8 % acetic acid in methanol.

vii. Visualization and recording of chromatograms

Since spots of anthocyanins on chromatograms gradually fade away, the plates should be examined on the same day as they are developed. For a routine check it was generally sufficient to make an inspection in visible and under ultraviolet light in the chromatoview chamber. When flavonoids previously described in the literature were identified for the first time with TLC, several visualization reagents as described by Harborne (1959)

were used in addition to the examination under ultraviolet light.

In addition to recording the Rf values of the various spots, it may be helpful for future reference to have a general idea about the color and the fluorescence of the spots. The system for marking fluorescent spots under ultraviolet light developed by Nybom (1966) was used for this purpose.

Photography of chromatograms under ultraviolet light was performed successfully by using Kodachrome II Day film and a series of filters as described by Jackson (1965) and the Eastman Kodak Company (Anonymous).

Table 22. Adsorbents screened

Material	Brand name
Silica gel	Silicar TLC-7 GF
" "	" TLC-4 GF
" " factory coated plates. .	Chromaplate 4 GF
" " " " " . .	" 4 G
" " " " " . .	" 7 G
Microcrystalline cellulose	Avicel TG 104 (≤ 25 microns)
" "	Baker TLC Reagent (≤ 100 microns)
" "	Camag (no size indicated)
" "	Sigmacell Type 19 (≤ 19 microns)
Microcrystalline cellulose factory coated plates	Mannogram, 0.25 mm thick layer
Filter paper strips	Gelman A Chromatography Media
" " "	" SG " "

Table 23. Solvents screened

Sol-vent	Ingredients	Volumetric proportions	Reference
1	Ethyl acetate, formic acid, N HCl	85 : 6 : 9	Harborne, 1967
2	Formic acid, conc. HCl, H ₂ O ("Forestal solvent")	5 : 2 : 3	Harborne, 1958
3	Formic acid, conc. HCl, H ₂ O . . .	10 : 1 : 3	Nybom, 1964
4	Ethyl acetate, formic acid, H ₂ O . . .	8 : 2 : 3	Harborne, 1958
5	Isopropanol, 2 N HCl	1 : 1	Harborne, 1958
6	H ₂ O, acetic acid	7 : 3	Harborne, 1958
7	H ₂ O, acetic acid	85 : 15	Harborne, 1962
8	Ethyl acetate, formic acid, H ₂ O . . .	70 : 15 : 15	Hess and Meyer, 1962
9	n-Butanol, formic acid, H ₂ O . . .	85 : 5 : 10	Hess and Meyer, 1962
10	Amyl alcohol, acetic acid, H ₂ O . . .	2 : 1 : 1	Nybom, 1964
11	Conc. HCl, formic acid, H ₂ O . . .	4 : 1 : 8	Nybom, 1964
12	Methanol, conc. HCl, H ₂ O	190 : 1 : 10	Mullick, 1969
13	n-Butanol, acetic acid, H ₂ O . . .	6 : 1 : 2	Harborne, 1959; Nybom, 1964
14	n-Butanol, acetic acid, H ₂ O . . .	4 : 1 : 5	Harborne, 1958
15	n-Butanol, benzene, formic acid, H ₂ O	100 : 19 : 10 : 25	Fuleki and Francis, 1967

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