Fortschritte der Pflanzenzüchtung
Beihefte zur Zeitschrift für Pflanzenzüchtung
13

Advances in Plant Breeding
Supplements to Journal of Plant Breeding

Hans Ross

Potato Breeding – Problems and Perspectives

Verlag Paul Parey
Berlin und Hamburg

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Potato Breeding – Problems and Perspectives

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und Pflanzenbau, München

With 22 figures and 19 tables



1986

Verlag Paul Parey · Berlin and Hamburg

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Fortschritte der Pflanzenzüchtung

Beihefte zur Zeitschrift für Pflanzenzüchtung · Heft 13

Advances in Plant Breeding

Supplement 13 to Journal of Plant Breeding

Edited by
W. Horn, Freising / G. Röbbelen, Göttingen

Anschrift des Verfassers:
Dompfaffenweg 33, D-5000 Köln 30

CIP-Kurztitelaufnahme der Deutschen Bibliothek

Ross, Hans:
Potato breeding : problems and perspectives / Hans Ross. – Berlin ; Hamburg : Parey, 1986.
(Advances in plant breeding ; 13)
ISBN 3-489-61110-1
NE: Fortschritte der Pflanzenzüchtung

Gedruckt mit Unterstützung der Deutschen Forschungsgemeinschaft

Cover design: Jochen Goks, Berlin

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Set and printed by Saladruck, Steinkopf & Sohn, D-1000 Berlin 36. Bound by Berliner Buchbindekunst Thomas Fuhrmann KG, D-1000 Berlin 36.

ISSN 0301-2727 · ASTM-Coden: FSPZAR (Fortschritte der Pflanzenzüchtung / Advances in Plant Breeding)

ISBN 3-489-61110-1 · Printed in W. Germany

Preface

The rapid and astonishing progress in the field of biology and biochemistry has not left plant breeding untouched. However, only few cultivated plants, like the potato, can profit by the new knowledge, methods and techniques. The potato possesses the advantages of vegetative propagation; it is tetraploid, can be regenerated from tissue and protoplasts, and is amenable to transformation. Among the objects of plant breeding, it is above all to the potato that the new results of research can be applied.

Until recently the new techniques were branded as unconventional and considered only in opposition to conventional or classical methods. However, such a distinction is no longer justified because in the meantime almost every sphere of potato breeding has adopted them as natural steps in its development. In the area of variety breeding, genetic engineering with its new techniques of plasmid transfer of genes and asymmetric protoplast fusion with distantly related species possessing outstanding resistance, opens up entirely new vistas. Selection for resistance in axenic cell- and protoplast cultures is being already performed.

There are good reasons why variety breeders stick to old proved methods. On the other hand, there is little readiness among pure researchers to make themselves familiar with practical problems and their implications. This Supplement should help to further the dialogue between scientists and practical breeders, which is essential for a continuous progress. Without a close co-operation a speedy translation of research results into practice is not possible. The intention here is to inform the reader about the present state of potato breeding which has been reached mainly by pursuing the classical breeding methods. Also discussed, however, is not only the progress which has already been made through the application of new technology in the different fields of potato breeding, but also that which can already be foreseen for the future.

The Supplement was planned together with my colleague Dr. habil. Werner HUNNIUS. Dr. HUNNIUS died unexpectedly August 3rd, 1983. He left behind a first, more or less detailed outline of the Sections 7.8, 8, 9, and 10. They had to be rewritten to include the newest research results and I must, therefore take the full responsibility for their contents, though I am greatly indebted to Werner HUNNIUS for his valuable cooperation.

Cologne, autumn 1985

Hans Ross

Vorwort

Die stürmische Entwicklung der Biologie und Biochemie haben auch die Pflanzenzüchtung nicht unberührt gelassen. Aber nur wenige Kulturpflanzen konnten, wie die Kartoffel, von den neuen Kenntnissen und Methoden profitieren. Die Kartoffel besitzt die Vorteile der vegetativen Vermehrung, sie ist tetraploid, aus Protoplasten und Geweben regenerierbar und unter bestimmten Bedingungen sogar einer Transformation zugänglich. Bei ihr können neue Forschungsergebnisse in bevorzugter Weise Anwendung finden.

Noch vor wenigen Jahren pflegte man die neuen Techniken als unkonventionelle Züchtung der konventionellen oder klassischen gegenüberzustellen. Aber eine solche Trennung erscheint wenig gerechtfertigt, denn inzwischen ist fast jeder Bereich der Kartoffelzüchtung von grundlegenden Änderungen betroffen. Der Neuzüchtung eröffnet die Gentechnologie durch Gentransfer mittels Plasmiden oder durch asymmetrische Protoplastenfusion völlig neue Wege. Die Erhaltungszüchtung ist durch den Einsatz einer kontinuierlichen, sterilen in-vitro Vermehrung sowie eines verbesserten Virusnachweises mittels der enzymatischen ELISA-Methode bzw. des cDNA-Tests in Umwandlung begriffen. Für die klassische Kreuzungszüchtung steht mit der Erscheinung der somaklonalen Variation der Protoplastenregenerate eine vielleicht neuartige genetische Variabilität zur Verfügung. Die Möglichkeit einer Haploiden Passage macht die Züchtung planmäßiger und erlaubt es im Grundsatz, einen heteroallelen Genotyp gezielt aus vier verschiedenen Einzelgenomen aufzubauen. Neue elektrophoretische und kolorimetrische Methoden finden in die Selektion von Qualitätsmerkmalen Eingang. Möglichkeiten der Resistenzauslese an in-vitro kultivierten Einzelzellen deuten sich an.

Aus guten Gründen neigt der praktische Pflanzenzüchter dazu, für seine Arbeit die seit langem bewährten Methoden vorzuziehen. Andererseits fehlt es in der reinen Forschung zuweilen an Einsicht, die Probleme der Praxis zu begreifen. Das vorliegende Heft soll dazu beitragen, den Dialog zwischen Wissenschaftlern und Züchtern zu fördern, ohne den ein erfolgreicher Fortschritt nicht möglich ist. Die Darstellung beschreibt den Stand der Kartoffelzüchtung, wie er heute mit den klassischen Züchtungsmethoden erreicht wurde. Zugleich wird erörtert, welche Fortschritte sich aus Anwendungen neuer Methoden in den einzelnen Bereichen der Kartoffelzüchtung ergeben haben und noch ergeben könnten.

Das Beiheft wurde zusammen mit Herrn Kollegen Dr. habil. Werner Hunnius geplant. Herr Hunnius verstarb im August 1983; er hinterließ erste Niederschriften der Kapitel 7.8, 8, 9 und 10. Da diese längere Zeit zuvor angefertigt waren, wurde vor Abschluß des Manuskripts eine fast vollständige Neufassung erforderlich, um auch die neuesten Forschungsergebnisse zu berücksichtigen. Mir fiel daher die gesamte Verantwortung für den Inhalt des Heftes zu. Für seine wertvolle Mitarbeit gebührt Werner Hunnius besonderer Dank.

Köln, im Herbst 1985

Hans Ross

(Eine erweiterte deutsche Zusammenfassung kann durch den Bundesverband Deutscher Pflanzenzüchter Abt. Kartoffel, D-3300 Bonn, Kaufmannstraße 71, bezogen werden.)

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1 Introduction

The potato is one of the world's most valuable basic food crops. It is superior to all others in protein production per unit area and time, and to most others in terms of energy production *(Tab. 1)*. Its protein is particularly valuable because of the high content of those essential amino acids (EAA index 76–79) occuring more rarely in other plant proteins. It is similar to milk protein in this respect. It is outstanding in the way that complements other protein mixtures (*Tab. 2,* KOFRANYI 1972).

Today the potato ranks fifth in area among crop plants grown for human consumption, and fourth in yield *(Tab. 3)*. Its area has decreased in developed countries, in part due to the labour intensive demands of planting, harvesting, transportating and preparation in the kitchen, and in part due to changes in eating habits resulting from consumer resistance to

Tab. 1: Comparison between energy and protein production per day and per hectare of some tropical food crops and potatoes in all countries between 30° N. Lat. and 30° S. Lat.
Energie und Eiweißproduktion per Tag und Hektar bei einigen tropischen Nahrungspflanzen in Ländern zwischen 30° nördl. und 30° südl. Breite (VAN DER ZAAG 1976)

	Energy production (10^3 kJ)	Protein production (kg)
Potatoes – *Kartoffel*	166	1.1
Sweet potatoes – *Süßkartoffel*	167	0.5
Cassave – *Kassawa*	154	0.2
Maize – *Mais*	133	0.8
Yams – *Yams*	121	0.6
Rice – *Reis*	120	0.6
Chick pea – *Kichererbse*	67	0.9
Dry beans – *Trockenbohnen*	40	0.6

Tab. 2: Biological value of protein of the egg and some selected foods
Biologischer Wert des Eiweißes des Eies und einiger ausgewählter Nahrungsmittel (KALDY 1972)

Egg – *Ei*	96	Soybean – *Sojabohne*	72	Wheatflour – *Weizenmehl*	53
Potato – *Kartoffel*	73	Maize – *Mais*	54	Pea – *Erbsen*	48
				Bean – *Bohnen*	46

The biological value indicates the part of the N-intake which is retained for sustainment and growth
Der biologische Wert zeigt den Anteil des aufgenommenen und absorbierten Stickstoffs an, der im Körper für Wachstum und Erhaltung zurückgehalten wird

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Tab. 3: World production of the potato and other important food crops 1983
Weltproduktion von Kartoffeln und anderen wichtigen Nahrungspflanzen 1983 (FAO Production Yearbook **38,** 1984)

	million hectares	million tons
Wheat – *Weizen*	231.9	521.7
Rice – *Reis*	147.5	469.9
Maize – *Mais*	129.6	449.3
Potato – *Kartoffel*	20.3	312.2
Sweet potatoes – *Süßkartoffel*	7.7	117.3
Sorghum – *Sorghumhirse*	49.0	71.7

carbohydrate rich food. On the more positive side have been improvements in the full mechanisation of planting and harvesting and the development of a processing industry (HUNNIUS 1981).

The potato is especially valuable when the nutritional problems of the future are considered (HORTON 1981). Up to now, the potato has been almost unknown in many developing countries. However, its great adaptability to different climates, not only to the highlands of the tropics but also to the lowlands, has become apparent and this is leading to greater expansion in a variety of countries (VAN DER ZAAG & HORTON 1983). When newly introduced, the potato adapts well and can be assimilated into many different cropping systems. The International Potato Center (CIP) established in 1972, is engaged in investigating the problems associated with potato cultivation in developing countries with diverse climates, pathogens etc.

The potato as a subject for breeding has some interesting characteristics. Potato cultivars (cvs.) are propagated vegetatively. Any selected genotype can be maintained with all its intra- and interlocus interactions and, when satisfactory, can be multiplied and released as a new variety. There is no further meiosis to disturb its genetic composition. As an autotetraploid, the potato possesses four interchangeable alleles at a given locus. This gives the possibility of breeding tetraallelic (heteroallelic) genotypes. The importance of tetraallelism for yield and other polygenic characters has become evident, mainly through the work of PELOQUIN, MENDIBURU, MENDOZA & HAYNES (Section 3.3).

The potato is outstanding in having the richest genetic resources of any cultivated plant and in the facility with which the germ plasm can be incorporated into cvs. Almost all of the related wild species can be crossed with *S. tuberosum*. Additionally, there is a wealth of semicultivated primitive cvs. in South America. A disadvantage, characteristic of autotetraploids is the difficulty of elucidating the genetics of the various traits. As a vegetatively propagated crop, it is affected by numerous pathogens transmitted via the tubers.

The genetic potential for yield is far from being exhausted. According to estimates, yield could perhaps be doubled if breeders paid more attention to the morphological and physiological preconditions for yield (VAN DER ZAAG & BURTON 1978).

Current research in potato breeding is characterized by the exploration of unconventional methods to complement traditional approaches. The use of dihaploids and axenic multiplication in seed production is already practiced. For most rapid progress in potato breeding it is essential that the dialogue between breeders and scientists is better developed than at present.

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2 Basic breeding material

2.1 Wild species, their properties and cytology

About 180 different tuber bearing wild species are known (CORRELL 1962, OCHOA 1962, HAWKES & HJERTING 1969, 1983, HAWKES 1978 b; for acronyms see SIMMONDS 1963). They comprise the subsection *Potatoe* (formerly *Hyperbasarthrum*) of the section *Petota* (D'ARCY 1972) (formerly *Tuberarium*) and are divided into 18 series. They form, with few exceptions, stolons with terminally born tubers. Most of the series contain only diploid species (2n = 24). However, cytotypes are not uncommon. Some series have developed polyploid chains up to the hexaploid level. The series *Tuberosa* comprises 68 wild and 8 cultivated species, most of these closely related to *S. tuberosum*. They are distributed from the central parts of Southern Chile as far north as the Southern States of the USA. They occur from sea level up to 4,500 m a.s.l. and are to be found in nearly every geographic plant association within this area. They possess a broad spectrum of resistances to parasites and to frost and drought and have other valuable traits. The following wild species occur in the pedigree of very many



Fig. 1: S. chacoense Bitt



Fig. 2: S. phureja Juz. et Buk. (syn. *S. rybinii* Juz. et Buk.)

cvs.; genes from several wild species are often incorporated in the genome of one and the same cv. (*Tab. 4* and *14*).

1. *S. demissum* Lindl., Series *Demissa,* 6x, selffertile, Mexico, resistant to infection with PVY and PLRV, hypersensitive and field resistant to late blight, resistant to pathotypes of wart and both *Globodera* species; alkaloids confer resistance to Colorado beetle and other insects, moderately resistant to frost. Genes of *S. demissum* are incorporated into more than 50 % of the world's cvs. mainly because of the resistance of *S. demissum* to late blight and PLRV, and its good combining ability.

2. *S. acaule* Bitt., *Acaulia,* 4x, 6x, selffertile, high Andes of Argentine as far as Peru. It comprises proveniences with extreme resistance to PVX, resistance to infection with PLRV and PSTV, to pathotypes of wart, to both *Globodera* species and to frost.

3. *S. chacoense* Bitt. *(Fig. 1). Commersoniana,* 2x, Argentine, Southern Brazil, Bolivia, Paraguay and Uruguay. This species comprises proveniences with extreme resistance to PVA and PVY, resistance to late blight, Colorado beetle, tuber moth and other insects. *S. chacoense* has been used mainly as a hybrid with *S. phureja* (2x) *(Fig. 2).* This hybrid served as a partner to *S. demissum* to create tetraploid hybrids for the smooth euploid transfer of genes into ssp. (= subspecies) *tuberosum.*

4. *S. spegazzinii* Bitt. (syn. *S. famatinae* Bitt. et Wittm.), *Tuberosa,* 2x, Northwest Argentine, resistant to *Fusarium,* pathotypes of wart and *Globodera* species, low content of reducing sugars. This species became of interest to the breeder because of its resistance to nematodes.

5. *S. stoloniferum* Schlechtd. et Bché. *(Fig. 3), Longipedicellata,* 4x, selffertile, Mexico. Some proveniences have extreme resistance to PVA and PVY, hypersensitivity and field resistance to late blight or resistance to *Empoasca.* About 20 cvs. carry the gene Ry for extreme resistance to PVA and PVY derived from *S. stoloniferum.*

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Fig. 3: S. stoloniferum Schlechtd. et Bouché

6. *S. vernei* Bitt. et Wittm., *Tuberosa,* 2x, Northwest Argentine, outstanding for protein and starch content, resistant to late blight and both *Globodera* species. Its content of reducing sugars is low. *S. vernei* has been used in breeding mainly as a source of resistance to nematodes and because of its high starch content.

Genes of the following wild species have been introduced into only a few cvs., mostly as a result of using the species as a crossing partner with *S. demissum* (see above) or to broaden the genetic base: *S. commersonii* (2x, 3x), *S. maglia* (2x, 3x) and *S. microdontum* (2x) in Great Britain; *S. fendleri* (4x) and *S. maglia* (2x, 3x) in the USA; *S. kurtzianum* (2x), *S. microdontum* (2x) and *S. toralapanum* (2x) in Canada and *S. verrucosum* (2x) in the Federal Republic of Germany.

Breeding with the following wild species is under way but has not yet reached the of variety production *S.* x *berthaultii* (2x), carries pheromones and is covered with a glandular pubescence which confer resistance to aphids (Section 7.2.1); *S. capsicibaccatum* (2x) and *S. gourlayi* (2x, 4x) which are resistant to *Globodera pallida; S. etuberosum* (2x) which is not tuber bearing but resistant to PLRV; *S. bulbocastanum* (2x), *S. fendleri* (4x), *S. hjertingii* (4x) and *S. pinnatisectum* (2x) resistant to late blight and *S. sparsipilum* (2x), resistant to PVX, *Pseudomonas solanacearum, Synchytrium endobioticum, Fusarium, Globodera pallida* and *Phthorimaea;* low content of reducing sugars.

Resistance, as described above, is often limited to only a few proveniences of the respective species. High protein and starch contents are found in nearly every wild species.

The diploid wild species are self incompatible with few exceptions. Crossed with oneanother in either direction they readily form fertile hybrids. These are met with only occasionally in nature. Natural hybrids between wild and cultivated species are called weed species (Section 2.5).

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Meiotic studies with diploid wild species hybrids have led to the assumption that most species possess a common basic genome which varies only slightly in structural composition (SWAMINATHAN & HOWARD 1953, VON WANGENHEIM et al. 1957, HOWARD 1960, 1970). Such differences do not hinder gene exchange during subsequent back crossing, important for the elimination of unwanted genes.

The tetra- and hexaploid species are self compatible. As to their meiotic behaviour there is a sharp contrast between the tetraploid wild species and the tetraploid cultivated species *S. tuberosum*. *S. acaule* and *S. stoloniferum* form bivalents and show disomic inheritances (ROSS 1954, 1958c, COCKERHAM 1970, EVERHART & ROWE 1974). *S. tuberosum,* however, forms quadrivalents, and dominant genes segregate tetrasomically. Accordingly it is assumed that the two or three subgenomes of the polyploid wild species are rather more differentiated, one from another and from the basic genome than have those of the diploid species (HAWKES 1978b, RAMANNA & HERMSEN 1979). The cryptic structural differences may have arisen due to the action of a selection pressure for higher fertility.

2.2 Primitive cultivated species and their properties

HAWKES (1978b, 1981) allocates the cultivated potatoes to four species and four hybrid species *(Fig. 4* and *5)*. DODDS (1962) classifies *S. phureja* (syn. *S. rybinii*) *(Fig. 2)*, *S. stenotomum, S.* × *chaucha,* ssp. *andigena* and ssp. *tuberosum* as »Groups« of the one species, *S. tuberosum*. The ssp. *andigena* and *S. phureja* are cultivated in the mountainous regions of South America. The other species and hybrids occupy distinct areas: *S. goniocalyx* occurs in the Andes of Central Peru, *S.* × *ajanhuiri* in the high altitudes of Central Bolivia, *S. stenotomum, S.* × *juzepczukii, S.* × *chaucha* and *S.* × *curtilobum* in the Andes of both countries. All of these forms are being rapidly replaced by modern cvs. of ssp. *tuberosum* or hybrids with it.

The primitive Andean cvs. are distinguished from those of ssp. *tuberosum* by smaller leaves and leaflets, many more stalks, smaller and more irregular tubers with deep eyes and often red and violet coloured skin *(Fig. 5)* and by giving lower yields. Most cvs. have highly fertile pollen. Their short-day character is expressed in the production of longer stolons and by being late maturing when grown under long day conditions of higher latitudes. Cvs. of the ssp. *andigena* (4x) are superior to the diploid cultivated species by showing greater phenotypic stability, better adaptability and higher productivity.

The collection of primitive cultivated species at the International Potato Center in Lima (CIP) comprise in 1979 12,500 introductions. About 3000 clonal samples, were analyzed for

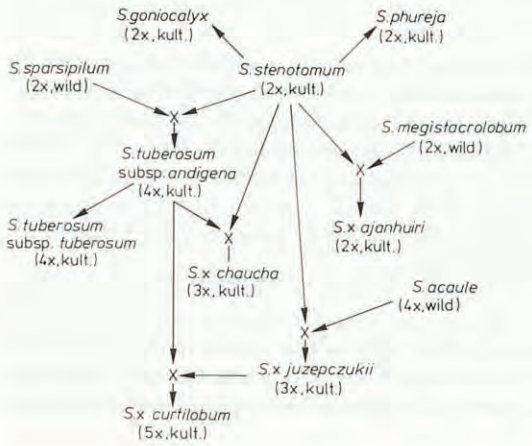


Fig. 4: Cultivated species and their origin
Die Kulturarten und ihre Abstammung
(HAWKES 1981)

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Fig. 5: Tubers of Andean cultivated species
Knollen andiner Kulturarten (by courtesy of N. W. SIMMONDS)

their morphological and gel-electrophoretic properties (OCHOA 1981, STEGEMANN et al. 1973). The studies resulted at that time in the identification of 588 different genotypes (cvs.) belonging to the ssp. *andigena,* 20 to *S. stenotomum,* 15 to *S.* × *juzepczukii,* 2 to *S.* × *chaucha,* and 2 to *S.* × *curtilobum.* The remaining forms which were analyzed proved to be duplicates.

2.3 Utilization of primitive and wild species in breeding

The various primitive cultivated species and wild species represent diverse gene-pools and possess great variability for many traits. They are of great importance in breeding for heterotic genotypes. It is generally assumed that the combination of breeding parents carrying alien genes produce more hybrid vigour in yield than the combination of parents without alien genes would do. Primitive cultivated and wild species, moreover, are indispensable sources for resistance to pathogens and for other traits. The genetic base of potato breeding needs a permanent broadening (HAWKES 1979, GLENDINNING 1979 a, ROSS 1979 a). Cvs. of the ssp. *andigena* should not be neglected in so doing. Intensive studies during the last decades have disclosed that nearly all traits found in wild species can also be detected in primitive cultivars. But fewer back cross generations are needed after using cultivated species. We agree with HOWARD (1970) when he says that the search for newly required genes should proceed in the sequence: ssp. *tuberosum* cvs. and breeding parents, ssp. *andigena* and other cultivated species and finally wild species.

Crossing and gene transfer between the two tetraploid subspecies of *S. tuberosum* offers no problems. However, two to three generations of back crosses to ssp. *tuberosum* are usually necessary for adaptation to long days (RASCO et al. 1980). Yields then often surpass those of ssp. *tuberosum* families (Section 3.3).

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Among the diploid cultivated species *S. phureja (Fig. 2)* should be mentioned in particular. It lacks tuber dormancy and shows a good combining ability when crossed with ssp. *tuberosum*. Although it has long been utilized in breeding, some important specific traits have only recently come to light. *S. phureja* has an above average tendency to form unreduced gametes by a meiotic process deviating from what is normal. The unreduced gametes produced by a First Division Restitution (FDR) transmit the almost intact and valuable genotype to tetraploid progeny following 4x–2x-crosses, so giving a heterotic push. This heterotic tendency is also observed in diploid parents (McHALE & LAUER 1981 a). Moreover, pollen of *S. phureja* acts as an inducer of parthenogenesis in tetraploids (Section 5.1.2.1).

Most of the other diploid wild and cultivated species when crossed with ssp. *tuberosum* give some tetraploid progeny by means of unreduced gametes. However, it is recommended that combination breeding is continued for as long as possible at the diploid level using dihaploids as recurrent parents. After appropriate back-crossing and selection the elite interdihaploid genotypes can then be finally crossed with a complementary tetraploid ssp. *tuberosum* genotype with complementary characteristics (Section 5.1.3.2). Another possibility is the mitotic tetraploidization of the species or, for better, of hybrids following interdihaploid breeding. Mitotic tetraploidisation can be allowed to happen spontaneously by the tissue culture of leaf explants (Section 5.1.3.1). In addition a way often practised in practical breeding is to combine the diploid species with *S. demissum* to give a tetraploid hybrid that is easily crossed with ssp. *tuberosum*. The possibility should also be mentioned of receiving triploid hybrids after crossing diploid wild or cultivated species with ssp. *tuberosum* as the female parent. Although more or less sterile, these may yield some fertile tetraploid hybrids after crossing with ssp. *tuberosum* (JACKSON et al. 1978). Otherwise, the triploids can be polyploidized and crossed with ssp. *tuberosum* to give tetraploid progeny.

Some diploid wild species cannot be crossed directly with *S. tuberosum*. However, some have been combined by means of a bridge species (DIONNE 1963). *S. bulbocastanum*, resistant to late blight, formed a hybrid with *S. acaule*. This hybrid after treatment with colchicine could be crossed with *S. phureja*. Also cross compatible with *S. tuberosum* is the late-blight resistant hybrid *S. verrucosum* × *S. bulbocastanum* (HERMSEN 1982). A successful combination of the colchicine doubled hybrid of PLRV-resistant *S. etuberosum* × the late-blight resistant *S. pinnatisectum* with *S. tuberosum* was obtained using *S. stoloniferum, S. polytrichon* or *S. verrucosum* as the bridge species (HERMSEN & TAYLOR 1979, HERMSEN 1982, 1984, HERMSEN et al. 1981).

Another way of combining *S. brevidens*, a PLRV-resistant species closely related to *S. etuberosum*, with ssp. *tuberosum*, was found by HANNEMAN Jr. & EHLENFELDT (1984). A triploid hybrid ($2n = 3x = 36$) between tetraploid *S. brevidens* (induced by colchicine) and diploid *S. chacoense* was crossed as the female parent with tetraploid ssp. *tuberosum* genotype. It resulted in a single pentaploid hybrid ($2n = 5x = 60$) that yielded many seeds when subsequently crossed with ssp. *tuberosum*. The progeny formed tubers. For somatic hybridisation see Section 5.3.2.

Direct hybridization of tetraploid species such as *S. acaule* and *S. stoloniferum* with ssp. *tuberosum* is seldom successful, because the development of the endosperm is hampered (Section 3.4). However, this failure can be overcome by the spontaneous formation of unreduced egg cells by the wild species or by colchicine treatment. Octoploid forms of both species cross easily with ssp. *tuberosum* to produce fertile hexaploid hybrids (VON WANGENHEIM 1954, 1957 a). In addition, the tetraploid hybrid, *S. acaule* × *S. stoloniferum*, forms bivalents and can be crossed directly with ssp. *tuberosum*, to give fertile hybrids, due to the functioning of unreduced embryo sacks (VON WANGENHEIM et al. 1957, Ross 1958 a). Tetraploid wild species can also be crossed with diploid cvs. or dihaploids. Some triploids will result which after colchizining can be combined with ssp. tuberosum. Fertile tetraploid and ± sterile triploid hybrids arise from crosses of the pentaploid *S.* × *curtilobum* with ssp. *tuberosum*.

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Tab. 4: List of European cultivars carrying genes from *S. acaule (acl), S. chacoense (chc), S. microdontum, S. sparsipilum, S. spegazzinii (spg), S. stoloniferum (sto), S. vernei (vrn)* and *S. verrucosum*. Nearly all enumerated cvs. carry in addition genes from ssp. *andigena, S. demissum* and *S. phureja*

Liste europäischer Sorten mit Genen der Wildarten S. acaule (acl), S. chacoense (chc), S. microdontum, S. sparsipilum, S. spegazzinii (spg), S. stoloniferum (sto), S. vernei (vrn) und S. verrucosum. Fast alle aufgeführten Sorten enthalten außerdem Gene von ssp. andigena, S. demissum und S. phureja

cultivar *Sorte*	[1]	[2]	cultivar *Sorte*	[1]	[2]	cultivar *Sorte*	[1]	[2]
Aguti	D	*acl-sto*	Erntestolz	D	*sto*	Pansta	NL	*vrn*
Amalfy	NL	*vrn*	Esta	D	*acl-spg-sto*	Pinki	D	*sto*
Amera	NL	*vrn*	Fanal	D	*acl-sto*	Pirola	D	*sto*
Amethyst	NL	*vrn*	Fatima	D	*chc*	Ponto	D	*acl-sto*
Amigo	NL	*vrn*	Fecuva	DK	*vrn*	Produzent	NL	*vrn*
Assia	D	*acl-sto-vrn*	Fiesta	D	*chc-vrn*	Promesse	NL	*vrn*
Astarte	NL	*vrn*	Fiona	UK	*vrn*	Proton	NL	*vrn*
Atrela	NL	*vrn*	Forelle	D	*acl-spg-sto*	Pruceres	NL	*vrn*
Aula	D	*vrn*	Fox	D	*acl-sto-vrn*	Puntila	D	*chc-vrn*
Baillie	UK	*S. microdontum*	Franzi	D	*acl-spg-sto*	Roeslau	D	*acl-sto-vrn*
			Fuga	D	*chc*	Ronea	D	*acl-spg-sto*
Barbara	D	*acl-spg-sto*	Gesa	D	*acl*	Roxane	B	*acl*
Benol	D	*vrn*	Hanne	D	*S. verrucosum*	Roxy	D	*chc-vrn*
Bison	D	*sto*	Heidrun	D	*acl-spg-sto*	San	PL	*acl-sto*
Bóbr	PL	*acl-sto*	Hilta	D	*acl-spg-sto*	Santé	NL	*sto-vrn*
Boubin	CS	*acl*	Hydra	D	*chc-vrn*	Saphir	D	*acl*
Brda	PL	*acl-sto*	Ida	D	*sto*	Semenic	R	*S. sparsipilum*
Bzura	PL	*acl-sto*	Jardinor	F	*vrn*			
Ceres	D	*sto*	Jessica	D	*acl*	Senator	NL	*vrn*
Christa	D	*sto*	Kero	D	*vrn*	Steffi	D	*acl*
Compagnon	NL	*vrn*	Klondyke	NL	*vrn*	Szignal	H	*acl-sto*
Corine	NL	*sto*	Krostar	NL	*vrn*	Tella	D	*acl-sto*
Craigs Defiance	UK	*S. commersonii* and *S. maglia*	Lajana	D	*sto*	Tempora	D	*acl-sto*
			Leander	D	*acl-sto*	Titana	D	*chc*
			Lotte	D	*sto*	Unisana	D	*acl*
Culpa	D	*chc-vrn*	Luna	D	*sto*	Ute	D	*acl-sto-vrn*
Danva	DK	*vrn*	Magyar Roza	H	*acl-sto*	Valetta	D	*vrn-sto*
Darwina	NL	*vrn*	Mara	NL	*vrn*	Vantage	NL	*vrn*
Domina	D	*S. verrucosum*	Miranda	D	*spg*	Veenster	NL	*vrn*
Dorado	D	*acl-sto*	Moni	D	*acl*	Viki	D	*acl-sto*
Dunja	D	*acl*	Morena	UK	*vrn*	Warinka	D	*sto*
Elin	S	*acl-sto*	Natalie	D	*acl-sto*	Wega	D	*acl-spg-sto*
Elkana	NL	*vrn*	Nausica	D	*chc-vrn*	Zenith	A	*acl*
Erntekrone	D	*chc*	Nordlicht	D	*acl-vrn*			

[1] country – *Land*
[2] wild species used in breeding – *Wildarten, die bei der Züchtung verwendet werden*

Hexaploid species such as the bivalent-forming *S. demissum*, give rise to many fertile pentaploid hybrids when crossed with *S. tuberosum*. These hybrids can be subsequently crossed with *S. tuberosum* without difficulty, with the chromosome number being gradually reduced until 2n = 48 is reached (VON WANGENHEIM et al. 1957). However, a more even transmission of *S. demissum* genes to *S. tuberosum* is possible when the hexaploid wild species is first combined with diploid species to give fertile tetraploid hybrids. Diploid bridge

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species which have been used in this way include *S. phureja, S. commersonii, S. fendleri* and *S. maglia* (REDDICK & PETERSON 1947) and *S. chacoense* and *S. verrucosum* (RUDORF & SCHAPER 1951). Embryo and endosperm culture is another possible way of establishing a hybrid in those cases where a zygote is formed but further development stops (CHAVEZ 1984). Generally it must be said, that in difficult cases, repeated pollinations may eventually lead to successful formation of at least one or several seeds.

Initial hybridization with *S. tuberosum* has to be followed by several back-cross generations in order to establish euploidy in appropriate cases and also to achieve general climatic adaptation and to eliminate unwanted wild properties, e. g. the formation of many small tubers (LAUER 1959). As outlined above, the genomes of the tetraploid and hexaploid wild species have followed a somewhat autonomous development. This moved them somewhat away from the basic genome of the diploid species and of *S. tuberosum*. Nevertheless, it has been possible to perform a nearly undisturbed combination breeding programme with an ordinary exchange of unwanted genes for wanted ones, so that after 4–6 generations of back-crossing a nearly complete »cultivated« genome is constructed. The potato in this respect, is certainly an exception among cultivated plants (VON WANGENHEIM et al. 1957). The question may arise as to whether it is best first combine the different wild species and thereafter start a single line of back-crossing or to build up separate back-cross lines and combine them at the end (convergence improvement, RUDORF 1958 b, column breeding, HERMSEN 1982). A comparative investigation is so far lacking, but the latter option has led to success (Section 2.5), since nearly all cvs. of *Table 4* have been bred by convergence improvement.

2.4 Origin of the cultivated potato

Radiocarbon dating has shown that the potato was taken into cultivation at least 8000 years ago. This happened on the altiplano of Southeast Peru and Northwest Bolivia (ENGEL 1970). No wild species is known which could be an ancestor of the diploid cvs. It could have disappeared by introgression and continuous hybridization with semicultivated and wild forms. Weed species and ruderal forms are often encountered (BRÜCHER 1966). Among the wild species the complex *S. canasense* / *S. leptophyes* is most closely related to the cultivated diploids (HAWKES 1978 b, CHAVEZ 1978). The complex *S. stenotomum* / *S. phureja* represents the most primitive of the diploid cultivated forms.

Attempts have been made by several means to elucidate the cytogenetic nature of *S. tuberosum*. The analysis of numerous major genes has left no doubt that their inheritance follows a tetrasomic pattern which contrasts with that in the tetraploid wild species. Moreover, a high percentage of quadrivalents is found, although some structural differences also occur.

S. tuberosum, therefore, is an autotetraploid or a segmental allotetraploid (VON WANGENHEIM et al. 1957, HAWKES 1958, HOWARD 1973, WOODCOCK & HOWARD 1975, RICKEMAN & DESBOROUGH 1978).

The transition from the diploid cultivated level to the tetraploid cultivated species could have happened by mitotic polyploidization or by the functioning of unreduced gametes (diplogametes) in both sexes of the same or of different parental species such as *S. sparsipilum (Fig. 4)*. The detection of such gametes and of the gene *ps* present in many tetraploid cvs. favours the assumption of the meiotic polyploidisation (Section 5.1.3.2; IWANAGA & PELOQUIN 1984). It is very likely that the heterotic nature of the tetraploid cvs. has resulted from the combination of diplogametes representing genetically divergent groups.

The evolution of the cultivated potato in its gene center is a steadily continuing process. UGENT (1970) has pointed to the permanent gene flow from wild species via hybrid swarms into cultivated forms. Farmers' fields in the Andes are usually planted with mixtures of cvs. Hybrids with different ploidy levels are formed therein (BRUSH et al. 1980, JACKSON et al. 1980).

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2.5 History and development of the cultivar assortments

The first introduction of tetraploid South American cultivars into Europe (Spain) probably took place about 1570. The tubers most likely come from Northern parts of Colombia (HAWKES 1978 a). Selection by geographic and climatic factors and by man converted these forms of the ssp. *andigena* into those of the ssp. *tuberosum*. The ssp. *andigena* with short-day characteristics acquired a tolerance to long days. Maturity occured earlier, the number of secondary sprouts was reduced, leaf dissection was increased, the stolons became less numerous and shorter, the tubers became larger and better shaped and yields increased. This process can be partially followed by means of contemporary herbarium specimens and woodcuts. It was almost completed in 1750 when LINNÉ described ssp. *tuberosum*. This development has been imitated in recent years by *Neo-Tuberosum* breeding programmes in Great Britain and the USA (Section 4.3). The forms of ssp. *andigena* which were brought by the Indians to the coast and islands of middle Chile underwent a similar change, but to a lesser extent (GLENDINNING 1975 c). These, too, evolved long-day genotypes.

The first introduction of ssp. *andigena* into Europe was followed during the 18th and 19th century by only a few further introductions (see VON RATHLEF 1932). In this way came Chilean forms of ssp. *tuberosum* (e. g. the cv. Rough Purple Chili and another Chilean introduction that became an ancestor of cv. Alte Daber) to Europe and the USA. Traits such as early maturity, resistance to scab, and hypersensitivity to PVX and PVA can be traced back to them (Ross 1958 a). GRUN (1979) assumes that the Chilean forms introduced »sensitive« plasmon into the cvs. (Section 3.1). Abrupt changes in the cv. assortment and composition by the loss of susceptible cvs. and breeding parents occured with the epidemics of late-blight (1845–1847) and wart (about 1910). A serious consequence of this was a reduction of genetic diversity (HOWARD 1978, GLENDINNING 1983). However, this situation improved considerably after the introduction of new Chilean cvs. of ssp. *tuberosum,* then *S. demissum* and *S. phureja* and finally of many other wild species.

Newly gathered informations has revealed that much more cvs. have primitive cultivated and wild species in their pedigree than was anticipated even some years ago (*Tab. 4;* Ross 1979). In 1912, a Chilean cv. of ssp. *tuberosum* was sent to the Biologische Reichsanstalt in Berlin (BROILI 1921, MÜLLER 1925, 1939). It gave rise in 1947 via a hybrid BRA 9089, to Hilla, the first cv. with resistance to pathotypes of wart and later on to many others. Another Chilean provenience (Villaroella) with extreme resistance to PVX was the progenitor via the hybrid USDA 41956, of cv. Saco, and many others.

The greatest influence on the composition of today's assortment of cvs. was exerted by the wild hexaploid species *S. demissum* and the cultivated diploid species *S. phureja* (syn. *S. rybinii*). The reason for the introduction and the success of *S. demissum* was its high resistance to late blight, and the high yield of subsequent back-cross generations (TOXOPEUS 1952). Breeding with *S. demissum* started in Germany when a seed packet named *S.* × *edinense* (a hybrid between *S. demissum* and *S. tuberosum*) was sent from the USDA to the Biologische Reichsanstalt, Berlin, in 1908. BROILI (1921) and MÜLLER (1925, 1951), used a back-cross programme to develop the so called »W-races« which, in 1934, gave rise to the first cv. with genes from *S. demissum,* called cv. Sandnudel. It was followed by very many others among them the cv. Aquila. At the Max-Planck-Institut in Köln, Germany, *S. demissum* was combined with ssp. *tuberosum* either directly or via the diploid hybrid, *S. chacoense* × *S. phureja.* Direct crosses were first made about 1933 and resulted among others in the valuable back-cross family, MPI 44.335. Their seedlings from this gave rise to more than 60 cvs. in the Federal Republic of Germany, The Netherlands (via MPI 19268) and Mexico, beginning in 1957 with cv. Delos. The backcross lines *S. chacoense – S. phureja – S. demissum* – ssp. *tuberosum* resulted among others in the clone 44.228/20. It became the parent of cv. Fatima (1970) and of many others. Combining the F_1 *S. demissum* × *S. verrucosum* (2x) with ssp. *tuberosum* (RUDORF & SCHAPER 1951), resulted in 1979 after several back-crosses in the cv. Hanne (M. SCHEIDT, pers. comm.). More than 83 % of cvs. of

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the Federal Republic of Germany carry genes from *S. demissum* and 26 % genes of other wild species, almost always in addition to those from *S. demissum (Tab. 4)*.

Not every parentage given for early bred cvs. can be taken at face value. Particularly controversial is the »wild« progenitor of cv. Majestic (GLENDINNING 1983) which was issued in 1911. SALAMAN (1941) detected resistance of *S.* × *edinense* to late blight in 1906 and crossed it with ssp. *tuberosum*. His hybrids probably went into some cvs. bred at the Plant Breeding Institute at Cambridge. In 1908 I. H. WILSON at the University of St. Andrews in Scotland combined *S. demissum* × *S. commersonii* (2x, 3x) with *S.* × *edinense* × *S. maglia* (2x, 3x). Back-cross lines of this material were used by the Scottish Plant Breeding Station to breed the cv. Craigs Snow-White which appeared in 1947 (BLACK 1947).

Further breeding with *S. demissum, S.* × *edinense* and *S. phureja* in Edinburgh began about 1930. Nearly all cvs. released there from cv. Craigs Bounty onwards are based on the two species *S. demissum* and *S. phureja* (BLACK 1945, 1952, HOLDEN 1976/77, GLENDINNING 1983). A private breeder raised the cv. Dr. McIntosh (1944) from a direct cross between *S. phureja* and cv. Herald. A hybrid, *S. microdontum* × *S. phureja,* was also employed in crosses and resulted in the cv. Kenya Akiba in about 1970 (BLACK 1972) and cv. Baillie in 1981. Finally, the line MPI 19268 mentioned above, occurs in the parentages of cvs. Sheriff and Kirsty (1983).

In the USA, *S. demissum,* first employed by REDDICK (1930), has also been combined with ssp. *tuberosum* in various ways. The first cv. with genes derived directly from S. demissum was released in 1945 (cv. Empire). The first cvs. from back-crossed hybrids *S. demissum* × *S. maglia* (2x, 3x) and *S. demissum* × *S. fendleri* (4x) was released in 1946 (cvs. Ashworth and Boone; HOUGAS & ROSS 1956). Another great impetus for the development of the cultivar assortment in the USA came from the introduction of W-races originating from S. demissum and described above (MÜLLER 1951). The cv. Kennebec arose from breeding with the W-race USDA 96–56 in 1948. It was followed by about 40 others (HOUGAS & ROSS 1956, THORNTON & SIECZKA 1980, SINDEN & SANFORD 1981).

It has already been mentioned that the breeding aim behind the introduction of S. demissum was improvement of late blight resistance. Most of this resistance faded away in the course of later back-crosses (Section 7.4), but the beneficial influence of the alien genes, remained to confer a heterotic benefit. Worth mentioning also is the influence of the primitive cultivated species *S. phureja (Fig. 2)* on the development of the European assortment of cvs. The main reasons for the wide utilization of *S. phureja* were firstly its ability to cross with *S. demissum* to give fertile tetraploid hybrids that combine easily with *S. tuberosum* and secondly its good combining ability with *S. tuberosum* genes. Other advantages are described in Section 2.5.

The following and present phase of breeding is characterized by an ever incrising introgression of further alien genes. Since 1954 known major genes and unspecified genes from additional wild species were introduced into cvs. as shown in *Table 4* (ROSS 1954, 1966, 1979 a). The first cv. with genes for resistance to Canadian wart pathotypes and the gene *Rx* for extreme resistance to PVX from *S. acaule* was cv. Saphir (1954). The first cvs. with unspecified genes from *S. acaule* were the USA – cvs. Nordak and Norgleam, released in 1958. The first cv. with unspecified genes ex *S. chacoense* was released 1969 (Hydra). Cvs. with the gene Ry for extreme resistance to PVY from *S. stoloniferum* appeared in 1968 (cv. Bison and Fanal), the first with resistance to *Globodera rostochiensis* from *S. vernei* in 1969 (cv. Hydra; Federal Republic of Germany) and to both *Globodera* species, in 1972 (cv. Mara; The Netherlands). The first cv. with genes from *S. spegazzinii* confering resistance to pathotype Ro 1 and 5 of *G. rostochiensis* was released in 1974 (cv. Cordia) and to pathotypes Ro 1–5. 1975 (cv. Miranda). Some cvs. harbour genes from up to six wild species (ROSS 1979 a, STEGEMANN & SCHNICK 1985). The often used breeding parents of the family MPI 55.957 *(S. acaule, S. chacoense, S. demissum, S. phureja, S. speggazzinii, S. stoloniferum)* carry major genes for resistance to PVX, PVY and *Globodera rostochiensis*. Genes from two cultivated species *(S. phureja* and *S. stenotomum)* and from five wild species *(S. acaule, S.*

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demissum, S. kurtzianum, S. microdontum (as *S. simplicifolium*) and *S. toralapanum)* are assembled in the Canadian cv. Conestoga.

A rather sudden change in genetic composition is taking place at the moment due to the threat of eelworm. The most commonly occurring pathotypes can be easily overcome by introducing the major gene H_1 of ssp. *andigena* CPC 1673. The first Ro-1 resistant cv. Antinema, appeared in 1961. Today 37 % of cvs. in the Netherlands, and 60 % of cvs. of the Fed. Rep. of Germany carry this gene. In Germany, it is already common to choose an H_1-parent in every cross.

The future development of cultivars assortment may be marked by a more rational construction of the genome. It may be possible to transfer distinct genes by asexual methods, such as protoplast fusion (asymmetric somatic hybrids, transfer of chromosome fragments) or by means of gene transfer (Section 5.2). Please note that in Section 2.5 the first named parent in crosses was not necessarily the seed parent.

2.6 Genetic resources and genebanks

Many collecting expeditions have been undertaken in South America and Mexico, initially stemming from purely botanical and taxonomic interests, but later on also for breeding purposes and to day to prevent erosion of genetic resources (HAWKES 1979).

The first living collection which still survives was built up in Leningrad starting in 1927, by VAVILOV, BUKASOV & JUZEPCZUK. In addition five large collections exist today (in order of their date of origin): Commonwealth Potato Collection (CPC) at the Scottish Crop Research Institute in Pentlandfield near Edinburgh, Collection of the Interregional Potato Project, Sturgeon Bay, Wis. (P. I.; HANNEMAN jr. 1981), World Potato Germ Plasm Collection of the International Potato Center (CIP; OCHOA 1962, 1981, HUAMÁN 1982), Argentine Potato Collection of the Instituto Nacional de Tecnologia Agropecuaria (INTA; OKADA 1976), Chilean Potato Genebank in Valdivia (CONTRERAS et al. 1980) and the Netherland-German Department of the Genebank of the Forschungsanstalt für Landwirtschaft in Braunschweig (Brunswick Genetic Resources Center, BGRC; VAN SOEST & SEIDEWITZ 1981, VAN SOEST 1983). The latter originated from the Erwin-Baur-Collection of the Max-Planck-Institut für Züchtungsforschung in Köln (EBS) and from the Wageningse Aardappelcollectie (WAC) in the Netherlands. It has been considerably augmented by exchange and by an expedition to Bolivia (VAN SOEST et al. 1983 a, b).

Several genebanks also possess in vitro collections of valuable pathogen-tested genotypes for international distribution (Anon. 1983 a) as basic materials for propagation, tuber and true seed production and long term storage (ESTRADA et al. 1983 b). In vitro germ plasm storage can be achieved by »minimal growth« (reduced carbon, temperature and light or addition of growth retardants) or by cryopreservation in liquid nitrogen at $-196\,°C$ (WHITHERS & WILLIAMS 1984). Care has to be taken to prevent somaclonal variation (Section 5.2.2; SCOWCROFT 1984). True seed can be stored at $+5\,°C$ for 25 years and more (HOWARD 1980). The BGRC has built up an in vitro collection of European cvs. (MIX 1981).

In the future, collection, maintenance and utilization of non-tuber bearing Solanum sections and species of related genera too may attract increased interest. These species, being at the border of the host range of many potato parasites, often exhibit higher resistances than those belonging to the mating group of *S. tuberosum*. Genes for resistance and other traits in these species may possibly be utilized by asexual gene transfer or asymmetric somatic hybridization (Section 5.2.3 and 5.2.4).

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3 Genetics

3.1 Fertility and sterility

Sterility is a very serious constraint in potato breeding. Many nuclear and cytoplasmic genetic systems are involved here to render combination breeding often difficult. GRUN & STAUB (1981) quote LIVERMORE: »Nine times out of ten one of the clones that you wish to cross will not flower but if they both flower, then nine time out of ten, they will both be male or both female sterile and so on.« After all, one third of the potato cvs. does not form berries.

One has to distinguish between pre-zygotic and post-zygotic inhibition mechanisms. Among the former is self-incompatibility, common among wild and cultivated diploid species. Exceptions are *S. bulbocastanum, S. polyadenium* and *S. verrucosum.* One- or two way-incompatibility (S-locus system) is characteristic of *Solanaceae.* Self-incompatibility can be overcome by doubling the chromosome number, but will be re-established in dihaploids extracted from the tetraploids. The relationships between species, on the other hand, are characterized by ready hybridisation to give fertile progenies (VON WANGENHEIM 1957 b). This behaviour has greatly promoted the utilization of genetic resources. However, in some cases, unilateral incompatibility occurs. This is the rule when diploid self-incompatible species serve as female parents in crosses with diploid self-compatible species used as pollen parents (ABDALLA & HERMSEN 1972 a).

HOGENBOHM (1973) assumes a sterility/fertility genetic system acts in more distantly related species in addition to the usual S-locus system. Genes for incongruity control the functioning of pollen (penetration capacity) and its acceptance or inhibition by the female parent (barrier capacity; HERMSEN & SAWICKA 1979).

Pre-zygotic crossability barriers are caused by a disruptive interaction between certain plasmon types and nuclear genes (cytoplasmatic male sterility, CMS; GRUN 1974, HANNEMAN Jr. & PELOQUIN 1981 and earlier, STAUB et al. 1982). Crosses to give F_1-progeny are possible in both directions, but the male organs of the flowers are only functional when the cross is made in one particular direction. When made in the reciprocal direction, a proportion of the progeny may have deformed flowers (gene *df*). Indehiscent anthers (gene *In*) or microspores are shrivelled and do not separate (tetrad sterility, *Tr;* ABDALLA & HERMSEN 1972 b). GRUN (1979) mentions certain cvs. respectively provenances of *S. tuberosum, S. stenotomum – S. phureja, S. maglia, S. vernei* and *S. chacoense.* But CMS was also found in *S. acaule* (LAMM 1953), *S. demissum* (DIONNE 1961), *S. stoloniferum* (ROSS 1966, BROWN 1984) and *S. verrucosum* (MAAG 1975). CMS *S. stoloniferum* hybrids are characterized by tetrad sterility (BROWN 1984). The PVY-immune *S. stoloniferum* back cross hybrids usually are functioning only as females. However, exceptions are found yielding male fertile progeny (Ross, unpubl.). Also PELOQUIN & IWANAGA (1981), provided evidence for the existence of restorer genes in ssp. *tuberosum* cvs.

According to GRUN (1974, 1979) the general differences in making crosses between ordinary breeding lines and cvs. are due to a sensitive plasmon introduced with Chilean forms of the ssp. *tuberosum.* This happened in the early 18th century. Indeed, the pollen

fertility is higher when the pollen is formed in plants with a non-sensitive plasmon from certain provenances of ssp. *andigena, S. phureja, S. acaule, S. spegazzinii* or *S. vernei* than in plants with a ssp. *tuberosum* plasmon (STAUB et al. 1982, Ross unpubl.). Recent research has shown that plasmatic male sterility is controlled by plasmonic elements, including mitochondria and plastids. It may possibly be cured in the future by fusion techniques (cybrids; *Tab. 10*) or by transfer of a responsible mitochondrial plasmid (SYBENGA 1983) or by treating protoplasts with chemical compounds (Section 5.2.3).

The post-zygotic blocking of seed development, on the other hand, usually results from the failure of the endosperm to differentiate. This leads to abortion of the embryo. The endosperm is formed by fertilization of the central nucleus (2x ♀) by a sperm (1x ♂) identical to the one which fertilizes the egg.

Normal differentiation of the endosperm depends on the ploidy level of both parents. It develops normally when the relationships between the ploidy levels of the mother plant, the endosperm and the embryo are 4:6:4 or 2:3:2 respectively. When the relationships deviate from these, differentiation of the endosperm begins too early or is too slow. However, there is a complication because the ploidy level is not always identical with the value necessary for endosperm development. E. g., both of the species *S. acaule* and *S. stoloniferum* are tetraploid with a ploidy level of »4«. But in relation to endosperm differentiation, they have the value »2«. A normal development of the endosperm and the embryo after crossing with ssp. tuberosum is accomplished when both wild species have converted to an octoploid level by mitotic doubling of the chromosomes (VON WANGENHEIM 1954, 1957 a, b; 1961, VON WANGENHEIM et al. 1957).

The production of triploid descendants in 2x · 4x – crosses, therefore, is inhibited by insufficient endosperm development, and the development of diploid unfertilized embryos with a hexaploid endosperm (4:6:2) is promoted instead. This is the case in the cross *S. tuberosum* (4x) × *S. phureja* (2x) which is of great importance for the production of dihaploids (VON WANGENHEIM et al. 1960, BENDER 1963, Section 5.1.2.1).

DEN NIJS & PELOQUIN (1977 c), JOHNSTON & HANNEMAN (1982) and EHLENFELD & HANNEMAN (1984) recognizing the hypothesis of VON WANGENHEIM, relate to the ploidy numbers of the female and the male which they attribute to the secondary endosperm nucleus. The authors assign an endosperm balance number (EBN) to each species according to its ability to form a functional endosperm. Thus, the ploidy level is equal to the EBN when two diploid species form endosperm and embryo in an ordinary way, i. e. 2x (2 EBN) *S. chacoense* × 2x (2 EBN) *S. phureja.* In contrast 2x *S. cardiophyllum* ssp. *cardiophyllum* is 1 EBN and its chromosome number has to be doubled in order to be crossed as female with 2x (2 EBN) species. The standard of a 4 EBN type is 4x ssp. andigena. Also 6x *S. demissum* is 4 EBN.

Often, an artificial polyploidization is superfluous in order to achieve hybrids with *S. tuberosum.* Unreduced gametes can be formed in the wild species, and the ratio is then correct when the tetraploid wild species is used as the seed parent. Moreover, some seeds occasionally develop although the ratios are unbalanced.

In spite of so many obstacles to sexual combination, it must be stated that, to date, the combination with *S. tuberosum* has been successful accomplished from nearly every wild species that has been worked with, not least due to the efforts of J. G. Th. HERMSEN and his group. No serious »wild« trait has yet been observed that has not disappeared after 4–6 back-crosses with ssp. *tuberosum.*

3.2 Genetics of tetraploids and diploids

Major or highly expressive genes segregate in clear cut categories and control mainly so-called qualitative traits as colour, hypersensitive resistance to parasites etc. *(Tab. 11).* They are present almost exclusively in the simplex state in breeding material.

The autotetraploid nature of *S. tuberosum* has important consequences for their transmis-

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sion to progenies since this confers a tetrasomic segregation pattern (SWAMINATHAN & HOWARD 1953, HOWARD 1970). The selfing or crossing of simplex autotetraploids with recessive parents leads to the same segregation figures as for diploid heterozygotes so long as the gene is situated close to the centromere (random chromosome segregation). Otherwise (chromatid segregation) a surplus of recessive segregants results, with ratios of 0.86 : 1 (dominant : recessive) when crossed and 2.48 : 1 when selfed. Duplex forms, occasionally resulting from double reduction, can be useful in subsequent crosses (TELLHEIM et al. 1982). Crossing duplex forms with recessives yield offspring with a high proportion of dominants (5 : 1 following chromosome segregation and 3.67 : 1 following chromatid segregation). Selection of major gene-inherited traits can then be postponed until a later vegetative field generation, where the seedling numbers are low. In population breeding (Section 4.3) monomer dominant traits as e. g. extreme resistance to PVY can be accumulated until 91 % of the seedlings because of duplex formation (BROWN et al. 1984 c). However, the production of multiplex parents often involves selfing which acts against tetraallelism (Section 3.3).

As already mentioned, the wild species *S. acaule* and *S. stoloniferum* are allotetraploids. Major genes such as *Rx* and *Ry* giving extreme resistance to PVX and PVY respectively *(Tab. 11)* segregate in these species in a disomic manner. They occur only in one of the two genomes. In back-cross generations with *S. tuberosum,* allosyndesis occurs with pairing between the genome of the wild species and that of *S. tuberosum* (Ross 1954, 1958 a, b; 1961 a, b, COCKERHAM 1970). The back-cross hybrids show normal fertility and a 1 : 1 segregation for the major gene character. In some cases a surplus of *Ry* gametes is observed, due perhaps to duplex forms arising by double reduction. The segregation of the gene *Fb* for nematode resistance in back-cross generations of *S. spegazzinii* (colchicine doubled, 2n = 48) with *S. tuberosum* follows the tetrasomic pattern as is to be expected (Ross 1962). The difficulty of interpreting segregation figures in polyploids was one of the reasons which led to breeding with forms whose chromosome number was halved (dihaploids, 2n = 2x = 24). A warning must be given against taking all published segregation figure at face value. Often the figures seem to be drawn from progenies already preselected by breeding.

Minor genes or polygenes (genes with low phenotypic expressivity) control quantitave characters such as yield, starch and protein content, photoperiodic reaction, resistance to infection by PLRV, PVY, late blight etc. A distinct phenotypic effect appears only when they are accumulated. Although the traits they control are called quantitative, it would be naive to believe that they only push forward a single reaction chain in small quantities and additive steps. They are involved in enhancing a property by qualitatively interacting with other reaction chains. The existence of specific combining ability in parents points to their complementary interaction in many cases. It should be possible, therefore, to improve a minor gene-inherited trait by a planned combination of its components (Section 7).

Several important traits in the potato, such as resistance to nematodes etc. are controlled by dominant major genes in combination with supporting minor genes (genetic background). It has often been observed that this complex breaks down during subsequent back-cross generations. Only a residual resistance then remains.

Phenotypic expression is greatly affected by the environment. The genetic component of total variance is the heritability. Its value differs with the methods of assessment. However, higher heritabilities can be attributed to dry matter, starch and protein content, tuber form, maturity and photoperiodic reaction than to tuber yield, tuber number, eye depth, shoot height and time of emergence (MARIS 1969, HUNNIUS et al. 1973, VON KAMEKE 1978, RUTTENCUTTER et al. 1979). High heritability, of course, allows more effective selection than a low heritability.

Reciprocal differences in the inheritance of character have often been observed (FRANDSEN 1967 a, HUNNIUS & SCHEIDT 1974, SANFORD & HANNEMAN Jr. 1982 b, HOOPES et al. 1980, GOREA et al. 1981, STAUB et al. 1982). In most cases these disappeared during subsequent vegetative propagation, or did not stand careful statistical analysis (ENGEL 1956). However, in some cases they may be real.

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3.3 Heterosis

Heterosis in the potato is when the progeny surpasses the value of the best parent or the parents' mean in vigour and tuber yield. The exploitation of heterosis is by far the most important goal in practical potato breeding. The inheritance of heterosis is by minor genes or by the side effects of major genes. Their cooperation can proceed in an additive (general combining ability) or in a non-additive manner (specific combining ability). In most cases both operate. PLAISTED et al. (1962 b) and KILLICK (1978) found the specific component to be dominant in yield experiments within ssp. *tuberosum*. In contrast, general combining ability, was more important within ssp. *andigena,* where the genotypes were more diverse (MUÑOZ & PLAISTED 1981). TARN & TAI (1983) reported a high value for general and for specific combining ability for total yield and its two components, tuber number and average tuber weight, their interaction being multiplicative. LANDEO & HANNEMAN (1982) observed a predominance of the general combining ability in breeding material comprising ssp. *andigena, S. phureja* and *S. stenotomum.*

A parent with high general combining ability often seems preferable because of its general usefulness as a good crossing partner. However, as mentioned earlier, when a trait needs to be improved by non-additive gene interaction, i. e. by complementation, the search and use of parents with good specific combining ability is essential.

The general theory of heterosis was first developed with diploid species. It says that the heterotic effect increases with heterozygosity, i. e. is highest when parents homozygous at as many loci as possible are crossed. In the past, attempts were made in potato breeding to obtain homozygous parents through several generations of inbreeding. Such a »simple« hybrid breeding strategy has not been successful, and no cv. is definitely known for have arisen from the crossing of inbreds.

The reason for the failure of »simple« hybrid breeding is to be found in the genetic structure of the potato which as an autotetraploid differs from that of the diploid. The crossing of completely homozygous diploids leads to a progeny with a coefficient of inbreeding of $F = 0$. The coefficient is $F = 0.333$ when potato inbreds are crossed: $a_i a_i a_i a_i \times a_j a_j a_j a_j = a_i a_i a_j a_j$. Maximum heterosis is to be expected only when all four alleles at a locus differ from one another (heteroallelism, tetraallelism; MENDOZA & HAYNES 1973, SKIEBE 1977, BINGHAM 1983 and earlier). Heterosis is based mainly on non-additive interaction of genes *(Fig. 6).* It comprises intralocus (overdominance) as well as interlocus (epistasis) interactions between genes and alleles.

Experimental confirmation in the potato resulted from the investigations of ROWE (1967 a, b, 1969), later interpreted also by MENDOZA & HAYNES (1974) and MENDIBURU et al. (1974). Inbreeding depression in the potato which occurs after the move from 4x to 2x by

Tab. 5: Number of interactions in an original tetrasomic locus
Anzahl der Interaktionen an einem ursprünglich tetrasomen Locus (MENDIBURU et al. 1974)

constitution		number of heteroallelic interactions of first / *Anzahl der heteroallelen Interaktionen der ersten*	second / *zweiten*	third order / *dritten Ordnung*	sum / *Summe*
$a_i a_j a_k a_l$	tetraallelic	6	4	1	11
$a_i a_i a_j a_k$	triallelic	3	1	–	4
$a_i a_i a_j a_j$	balanced diallelic	1	–	–	1
$a_i a_i a_i a_j$	unbalanced diallelic	1	–	–	1
$a_i a_i a_i a_i$	monoallelic	–	–	–	–

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extraction of dihaploids, cannot be due only to the loss of diallelic interactions. The loss of tri- and tetraallelic interactions must be taken into account as well *(Tab. 5)*. Moreover, MENDIBURU & PELOQUIN (1977) found clear indications of a non-additive variance in the inheritance of yield. Therefore, the segregation of heterotic seedlings in a population is likely to be greatest when three conditions are fulfilled:

1. the parents possess an as low a coefficient of inbreeding as possible;
2. as many loci as possible have different alleles;
3. the parents belong to different genepools which improves the chances of allelic diversity (wide hybridisation).

These requirements are in agreement with what is found in practical breeding. Crosses between genetically diverse parents, e. g. among ssp. *tuberosum* forms of different descendances (VEILLEUX & LAUER 1981), or between ssp. *tuberosum* and ssp. *andigena,* have often led to heterotic progeny (GLENDINNING 1969, 1979 b, MENDOZA & HAYNES 1974, CUBILLOS & PLAISTED 1976, TARN & TAI 1977, 1983, AMOROS & MENDOZA 1979, LANDEO & HANNEMAN 1982, STAUB et al. 1982). The same experience has been made in progenies of wild species with dihaploids *(Fig. 6)*. Different provenances of wild species parents as well as different dihaploid parents differ widely for tuber yield. Very remarkable also is the high yield of progenies of *S. acaule* with *S. stenotomum* (= *S. juzepczukii;* SCHMIEDICHE et al. 1982) and of the progeny *(S. acaule* × *S. phureja)* × ssp. *tuberosum* (ESTRADA 1984). Heteroallelic tetraploids can be constructed in an ideal way when they are synthesized out of monohaploids *(Fig. 14)*. This way, however, has the wealth of double monohaploids (2x = 24) as a pre-requisite as well as a functioning intraspecific protoplast fusion. Both are difficult to achieve for the time being.

Breeding for heterosis covers combination breeding at the same time. Statistics say that the more good traits one tries to combine the more rarely such seedlings turn out. Crucial in most cases is the low yield. A heterotic push would help a lot of good combinations jump over the yield barrier.

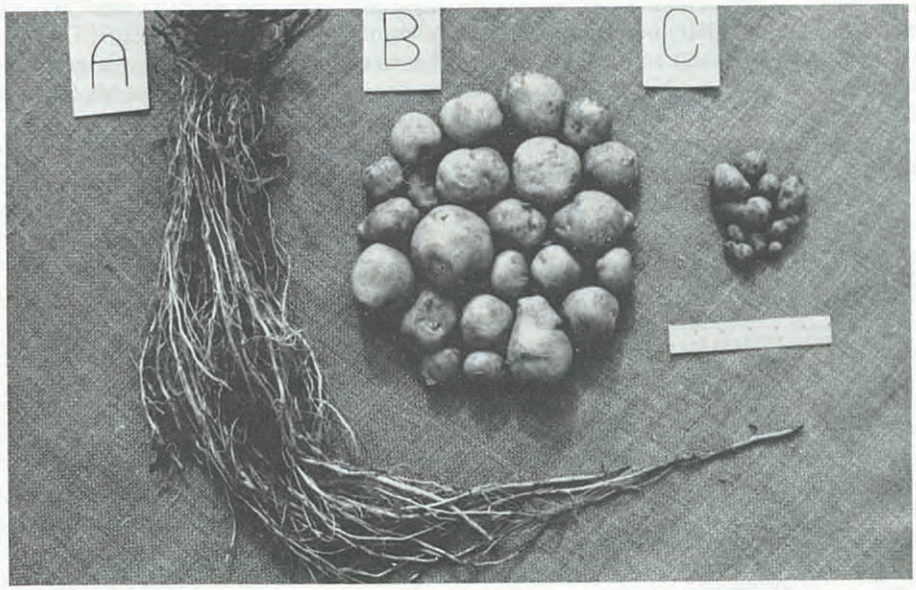


Fig. 6: Hybrid vigour in yield: *S. tarijense* (A), dihaploid ex cv. Merrimack (C) and their hybrid (B) *Heterosis im Ertrag* (by courtesy of SHELLEY HERMUNDSTAD)

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4 Breeding aims and basic breeding procedures

More than 50 traits should combine in a modern variety. They can be grouped as follows:

1. Yield, including all morphological, physiological and ontogenetical factors controlling its enhancement. Also special properties for adaptation to modern agricultural techniques in field growth, harvest and storage are comprised herein (VAN DER ZAAG & BURTON 1978);
2. Resistance to environmental factors, pests and diseases;
3. Quality with respect to different utilization purposes.

A logical procedure requires information about the genetics of the different traits. Relatively simple to handle are traits that are transmitted by major genes. A high percentage of the wanted segregants can be expected among the progeny. Major-gene-inherited traits are often suited to a pre-selection among the seedlings *(Tab. 11)*. That allows the complishment of the subsequent screening and selection with seedlings firmly possessing at least one of the wanted traits.

Most of the properties, however, are inherited by polygenes and only few seedlings express the trait to the same degree as the parent, let alone surpass it. Therefore, in order to breed a new improved variety, an enormous number of seedlings must be raised each year. It then takes 6–8 years to check the seedlings and to single out the best genotypes. Logically, the selected genotype will have a medium expression in most traits and a more than average expression in only very few.

4.1 Selection of parents and crossing techniques

Successful breeding chiefly results from choosing the right parents, yielding seedlings with complementary valuable traits. Most seedlings are discarded because of insufficient yield. It is, therefore, very advisable to combine parents from which a heterotic progeny may be expected. The chance of this is the greater the less genetically related the parents are (Section 3.3). Information about cvs. harbouring alien genes is given by STEGEMANN & SCHNICK (1985). Before a genotype is used as a parent, it must be screened in test crosses (100–200 seedlings; MÖLLER 1965). TAI & YOUNG (1984) could retain 4 times more seedlings from a parent with good general combining ability than from a bad parent. Analyses of progenies from selfings must be interpreted with caution because inbreeding effects are likely to pervert the results. The same is true for the dihaploid family of a breeding parent.

Crossing can be performed in the greenhouse as well as outdoors. The temperature is a crucial factor in both cases. It must always remain below 20–22 °C. Continuous light in the greenhouse is strongly recommended. Most satisfactory is the decapitation technique (Section 5.1.2.1). THIJN (1954), in order to improve the poor flowering of some cvs., introduced the method of planting tubers on stones. The roots are guided into the soil and the appearing tubers are cut off. Better flowering can also be attained by grafting shoots on tomato stock. Early cvs., moreover, can be stimulated to start flowering by late planting in the field.

A crucial point is the handling of the pollen. Several devices have been developed to collect the pollen by mechanical vibration of the anthers (TELLHEIM & BÜRCK 1979). Pollen can be stored for at least 19 months when dried before being frozen. 48 % survived at − 5 °C, but 87 % at − 20 °C (GOREA & ROTHACKER 1968). Possibilities for the exchange of pollen between countries are studied by TÄNDLER (1978).

4.2 Selection among seedlings

The optimal number of seedlings to be raised each year is a matter of controversy. Genetical statistics say that the more seedlings are raised, the more useful combinations are to be expected. On the other hand, several factors are limiting the number. It is, above all, the capacity of the economic and scientific unit. A stricter limitation of the number of seedlings is favoured by those who believe that the satisfactory handling of a smaller number is better than a necessarily less careful handling of a larger number. Nowadays, a breeding unit sows out 70,000–150,000 seeds each year, derived from 150–300 families. During their propagation over their first six years, the cloned seedlings must undergo intensive observation in the field and many screening tests in the laboratory and greenhouse *(Tab. 6)*.

Selection among first year seedlings can be performed only regarding those traits which differ genetically and phenotypically clear cut in wanted and unwanted types. This is the case mainly with major gene inherited traits (PLAISTED et al. 1984, SWIEZYNSKI 1984). With trials inherited by minor gene effects, the risk to discard good types is high when the heritability of the trait is only low (Section 3.2). Rejection in field trials results mostly from poor yield, from tubers which are too large, too small, or irregularly shaped, deep-eyed, whitefleshed or because the seedlings are late maturing, or highly susceptible to leafroll virus and Y-virus. More clones are discarded after special tests for susceptibility to common scab, nematodes, storage diseases and to mechanical damage, or following cooking and palatability trials. Exact measuring often cannot be replaced by visual inspection (TAI 1975). DAVIES & JOHNSTON (1974) and ANDERSON & HOWARD (1981) showed that in most cases, different people having to select visually from a given group of genotypes will differ in their choice of best types.

MARIS (1969) investigated the modificability of different traits over several years. The lowest variability was offered by flesh colour, tuber shape and maturity, whilst eye depth, length of stolones and underwater weight (starch content) showed a high, and type of stalks, number of stolons, date of emergence and incidence of scab, a very high modificability. Partly different results were obtained by KRUG et al. (1974 b). Generally, the risk of discarding valuable clones is very great when the selection in the first years is intensive (TAI & YOUNG 1984).

PFEFFER (1963) compared selection among seedling clones grown in pots with those grown in the field. He found conformity only in the case of negative traits, such as bad tuber shape, depth of tuber eyes, white tuber flesh and too many small tubers. NIOPEK (1967), working with 13 cm pots, came to similar conclusions. TELLHEIM (1975) found a good conformity in maturity and tuber yield of pot seedlings compared with field results, but poor conformity with tuber number and tuber size.

SWIEZYŃSKI (1979, 1984), trying to make a selection among pot seedlings less time-consuming, selected the largest and best-shaped from the bulk of tubers of each family for further field propagation. He achieved reliable results compared with those got by numbering single seedlings, growing them separately and selecting them the next season.

In all, a seedling will have been under test for at least 10 years before it can be included in the official variety list. Nowadays, a variety is not on average likely to remain for more than 7.5 years on the official list (HÖPPNER 1976), and only some 10 % remain on it for more than 20 years (HUNNIUS 1975, HOWARD 1978).

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Tab. 6: Breeding scheme of new varieties
Schema der Neuzüchtung (FITSCHEN 1984, modified)

year *Jahr*	number of clones *Zahl der Klone*	number of tubers per clone *Zahl der Knollen pro Klon*	Procedures *Verfahren*
1	production of 400–500 families, at least one parent with Ro-1 resistance *Erstellung von 400–500 Kreuzungsfamilien, wenigstens ein Elter mit Resistenz gegen Ro-1*		
2	140 000		Mass sowing in pots or seed boxes. Early progenies are sown in the form of single seed grain and negatively selected for 1–3 cm large and horizontally growing stolons *Massenaussaat in Töpfen oder Saatschalen.* *Frühe Nachkommenschaften werden als Einzelkörner ausgelegt und negativ auf 1–3 cm lange und horizontal wachsende Stolonen selektiert*
3	100 000	1	Strict selection among pot plants for virus infections and among the tubers for yield, form, eye deepness and flesh colour. Winter test for resistance to eelworm Ro-1 and screening of the sprouts for presence of viruses. Chemical weed killers are applied at the beginning of August for recognizing and discarding of late genotypes *Strenge Selektion unter den Topfpflanzen auf Virusinfektionen und unter den Knollen auf Ertrag, Form, Augentiefe und Fleischfarbe. Winterteste auf Resistenz gegen den Nematodenpathotypen Ro-1 und Prüfung des Krautes auf Virusgegenwart. Krautabtötung Anfang August, um die zu späten Genotypen erkennen und ausscheiden zu können*
4	7 000	7	Numbering of the clones, observations of growing type, maturity class, diseases and in winter time tests for taste, cooking type, starch content, raw discolouration, suitability for crisps and french fries, repetition of the eelworm resistance test *Numerierung der Klone, Beobachtungen des Wuchstyps, der Reifezeit und von Krankheiten, im Winter Koch- und Geschmacksprüfungen, Stärkegehalt, Rohverfärbung, Eignung für Chips und Pommes frites, Wiederholung der Nematodenprüfung*
5	1 400	50	Same as in the fourth year *Dasselbe wie im vierten Jahr*
6 7 7	500 (100)[1] 10	250 250 1 250	Micro yield trials. Tests for resistance to scab, wart, viruses and pathotypes of both nematode species and, in Tunisia, for adaptation to short day and warm climate conditions. At the end of the seventh year decision about registering at the responsible official institutions *Mikroleistungsprüfungen, Teste für Resistenz gegen Krebs, Schorf, Viren und Nematodenpathotypen und für Anpassung an Bedingungen eines warmen Kurztagklimas in Tunis. Am Ende des siebten Jahres Entscheidung über die Anmeldung von Stämmen bei den amtlichen Stellen*
8	4	5 000	Official tests *Amtliche Prüfungen*
9	4	0.75 ha	Official tests. Seed production starts *Amtliche Prüfungen. Klonenaufbau beginnt*
10	1	5 ha	Official tests. Seed production *Amtliche Prüfungen. Saatgutproduktion*

[1] Doubtful clones from the sixth year are subjected to a further year's screening
Zweifelhafte Klone des Vorjahres werden ein weiteres Jahr geprüft

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4.3 Pedigree breeding and population breeding *(Neo-Tuberosum)*

Potato breeding is usually done by pedigree breeding, i. e. by the intentional crossing of distinct parents whose properties and their modes of inheritance have been as completely established as possible. The method allows estimations of the combining ability, a planned combination of traits and their segregation, and a foreseeable combination of traits in the progenies. Its disadvantage is that it is labour-intensive and time-consuming. In contrast, population breeding is less laborious, but – as it is usually carried out – the exact parentage of a selected clone remains unknown, and precise segregation figures from a berry progeny cannot be expected. However, several variants are possible which may link both methods. A genotype selected from population breeding can be identified in its properties and its genetics and, thereafter, introduced into normal pedigree breeding.

Population breeding started in Edinburgh (Scotland) and Ithaca (New York) with clones of ssp. *andigena* (SIMMONDS 1966, GLENDINNING, 1975 a, b, c, d, 1976, 1979 a, b, PLAISTED et al. 1975, MUÑOZ & PLAISTED 1981). By this means it was possible to recapitulate the evolution from the ssp. *andigena* to the ssp. *tuberosum (Neo-Tuberosum)* and, by using groups of appropriate ssp. *andigena*-forms as a pre-adapted genepool, the resulting genotypes could then be used in pedigree breeding.

The principal method for obtaining *Neo-Tuberosum* types is as follows. A group of suitable ssp. *andigena* clones is selected over a period of 3 years for its tolerance to long days, the late and lower yielding clones being discarded. Thereafter, seeds obtained from open pollinations are mixed, sown, and visual selections made from the progeny. Hand pollination, using mixed pollen, was introduced when it was realized that the seeds obtained from open pollinations were, in fact, mostly self-pollinated. Several sequential cycles of sowing and selection follow until long day adaptation is evident. The resultant plant characteristics come close to those of ssp. *tuberosum;* the stems and tubers are less numerous, the tubers larger, the yields higher and maturity is earlier. The total yields of four of 71 *Neo-Tuberosum* clones were higher than those of the best of the 10 control cultivars (GLENDINNING 1975 d). Morever, genotypes were identified with resistance to late blight in haulm and tubers, to scab, wart, the golden nematode, as well as a gene for extreme resistance to virus Y.

It is doubtful if many necessary properties can be combined by population breeding. Nevertheless this method has the great advantage of giving access to traits of the ssp. *andigena* genepool which can then be introduced into the more usual pedigree breeding. Certainly, it can contribute much to increasing heterozygosity. CUBILLOS & PLAISTED (1975) and MUÑOZ & PLAISTED (1981) noted that when progenies of the 5th and 6th cycle of *Neo-Tuberosum* were crossed with ssp. *tuberosum,* the progeny yielded 15 % more than those from crossings of ssp. *tuberosum* forms with one another; the combining ability for tuber number, average and total tuber weight was significantly increased. WISSAR & MENDOZA (1978) carried out population breeding at the diploid level with *S. phureja* × *S. stenotomum* and found that after several cycles the average yield of selected clones was *ca.* 26 % higher than that of their controls.

Population breeding may be of particular value to those developing countries that do not already have high yielding varieties adapted to their day length. The International Potato Center (CIP) has developed a procedure for supplying developing countries with either preselected seed or small tubers from selected seedlings variously representative of several genepools of ssp. *tuberosum,* ssp. *andigena,* diploid cultivated species, and wild species (Section 2.6; MENDOZA 1983). Seedling populations are then generated by fertilization with either individual or mixed pollen and undergo natural selection by the particular climatic conditions of the country or region concerned. In addition, selected progenies can be tested for resistance to the principal diseases of that country or region (see also MARTIN 1979, RASCO et al. 1980, BROWN et al. 1984 c). In the USA, one new variety, cv. Rosa, has already been released with one parent derived from *Neo-Tuberosum* breeding and in Europe, a Scottish line has been submitted for official testing as a potential cultivar (MACKAY 1984).

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5 New breeding methods

Three main trends characterize the modern development in potato breeding (Ross 1984): the recognition of tetraallelism as a main source of heterosis (Section 3.3), the production of dihaploids and monohaploids as a way to construct tetraallelic genotypes systematically and, thirdly, by-passing of the meiosis.

Production of dihaploids ex tetraploid cvs. and breeding lines by pseudogamy has become a routine procedure. This has made it possible to perform a pre-breeding at the diploid level with all its advantages. A second reduction in the chromosome number through androgenesis (anther culture) can be performed in dihaploids, which leads to monohaploids. A systemic synthesis of heteroallelic tetraploids with high heterozygosity by means of protoplast fusion can be envisaged *(Fig. 7)*.

Although the meiosis is necessary in order to produce a new, and possibly better array of genotypes, there are cases, where it is aimed to attribute single lacking traits to a cultivar-genotype, selected as the best one among several 100,000s of seedlings. Ways for such by-

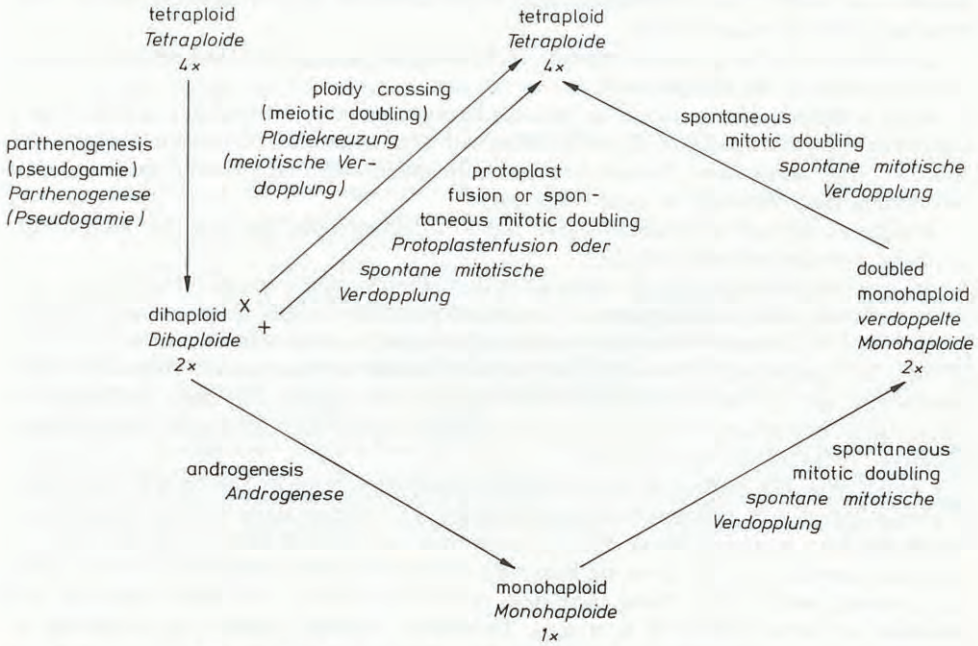


Fig. 7: Changes in ploidy levels from 4x via 2x to 1x and vice versa
Poloidieabbau und -aufbau (WENZEL (1981), slightly modified – *leicht verändert*)

passing of the meiosis are 1. selection among tissue and protoplast derived somaclones, 2. (perhaps possible in the future) asymmetric hybridisation and 3. (also a distant goal), gene-transfer, direct or by means of plasmids. However, these new methods are successful only when integrated in the ordinary potato breeding process. Dihaploids and monohaploids have to be extracted from, and gene transfer has to be applied to top combinations or varieties bred by classical breeding work. Classical breeding, therefore, is a precondition and must be performed not only at the tetraploid, but also at the diploid, level.

5.1 Breeding procedures with potatoes reduced in their ploidy level once and twice

5.1.1 Introductory remarks

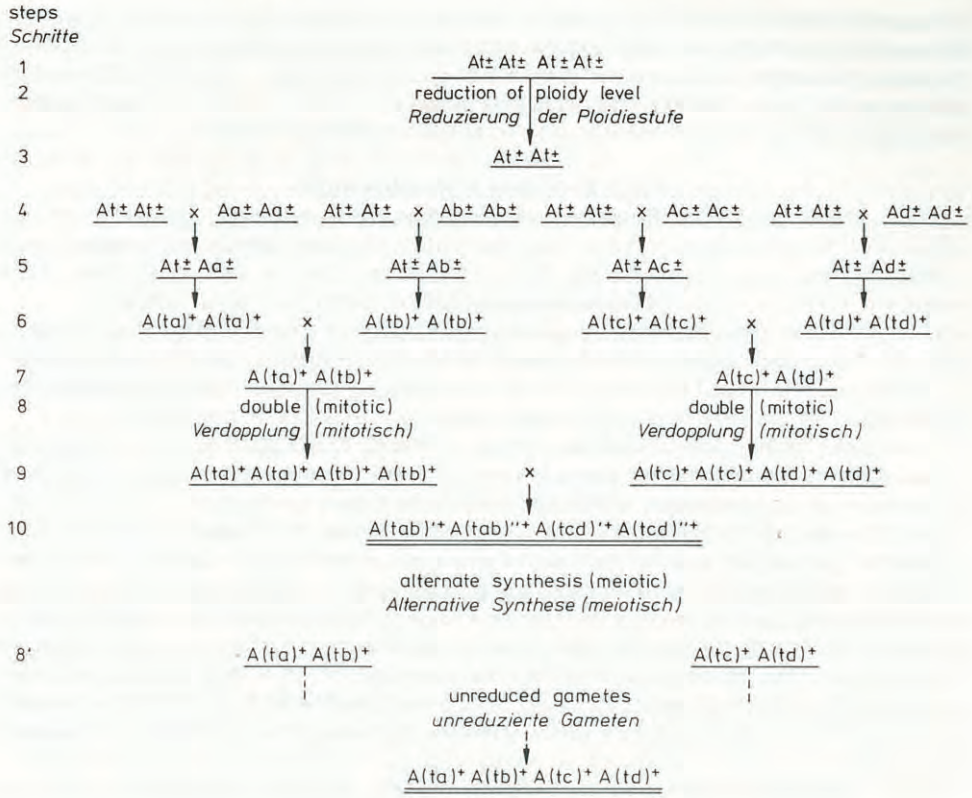
It was pioneer work when HOUGAS & PELOQUIN (1958) and HOUGAS et al. (1958) started to produce the first dihaploid potatoes by pseudogamy on a larger scale.

Breeding at the diploid level facilitates and improves the subsequent ordinary breeding work at the tetraploid level (HOUGAS & PELOQUIN 1958, VON WANGENHEIM 1962, MENDIBURU et al. 1974). The advantages are as follows:

1. evaluation of tetraploid genotypes by study of their extracted dihaploid families;
2. disomic segregation can be more accurately interpreted than polysomic segregation;
3. selection of polygenic characters is easier at the diploid level because of more easily interpreted segregation. The percentage of extreme segregants is higher in diploid progenies (STEBBINS 1956, ROWE 1967 b, HAYNES 1972);
4. reduction of the high number of seedlings. The number of positive traits demanded of a potato cultivar is continually rising, particularly since new research has shown that a trait for a distinct resistance is often composed of different components which must be completed by combination breeding. This can be accomplished at the diploid level with smaller seedling numbers and within fewer generations than at the tetraploid level (VON WANGENHEIM 1962, HOWARD 1978, IWANAGA 1982);
5. chromosome exchange during meiosis, facilitating the sorting out of undesirable genes, is more intensive at the diploid level;
6. when a diploid wild species is to be introduced in breeding, it is easier to start with a diploid hybrid (wild species × dihaploid) than with a tetraploid one (colchicine doubled wild species × ssp. *tuberosum*); hybrids between a dihaploid and a diploid wild species exhibit astonishing yield heterosis in some cases *(Fig. 6);*
7. dihaploids through androgenesis give rise to monohaploids, the base for an analytic-synthetic breeding scheme *(Fig. 14);*
8. the most important advantage arises when during return to the tetraploid level (4x ex 4x · 2x) unreduced gametes (diplogametes) come into play *(Fig. 7, Tab. 7)*. In consequence of a special kind of deviation from the normal cell division, i. e. by a first division restitution (FDR), 2n – gametes are formed, largely without meiotic rearrangement. The nearly intact genetic structure of a selected genotype is brought into the zygotes. The intra- and interlocus interactions, important for heterosis in yield and other polygenic characters, remain largely preserved (MENDIBURU et al. 1974).

CHASE (1963; *Fig. 8*) developed an analytical breeding scheme on the basis of dihaploids. It aims at tetraploids with maximal heterozygosity. The scheme starts with the extraction of dihaploids from varieties. These dihaploids are then crossed with different diploid wild and cultivated species in order to create lines with divergent genetic composition. After selfing and crossing within these lines separately (column breeding), and after selection, the products are intercrossed (*Fig. 8,* step 7). Thereafter, a mitotic doubling by colchicine or, alternative, a meiotic doubling by function of diplogametes in one of both parents is performed *(Tab. 7)*. The tetraploids then serve as parents in ordinary breeding or, exceptionally, can have variety rank.

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A = potato genome - *Kartoffelgenom*
t = gene system from S. tuberosum - *Genpool von S. tuberosum*
a - d = gene systems from diploid species or selection - *Genpoole ausgewählter diploider Kulturformen oder Wildarten*
\+ = favorable genes - *günstige Gene*
\- = unfavorable genes - *ungünstige Gene*
( ) = a recombination of genes - *Rekombination der Gene*
', '' = gene system altered by crossing-over *Änderungen des genetischen Systems durch crossing-over*

Fig. 8: A hypothetical analytic breeding scheme for the common potato. The scheme starts with extraction of dihaploids from cvs. After crossing with different diploid wild and cultivated species and upgrading within separate columns, interdihaploids with divergent genetical background are achieved. They then are doubled by colchizine treatment and thereafter crossed or, alternative, brought to the tetraploid level by action of unreduced gametes

Analytisches Zuchtschema. Das Schema beginnt mit der Extraktion von Dihaploiden aus Sorten. Nach Kreuzung mit verschiedenen Wild- und Kulturarten und Kreuzung von je zwei Stämmen miteinander erhält man Interdihaploide mit divergentem genetischen Hintergrund. Diese werden entweder mittels Colchizin verdoppelt und gekreuzt oder mittels unreduzierter Gameten auf das tetraploide Niveau gebracht (CHASE 1963)

MENDOZA & HAYNES (1974) criticized the selfing and the alternative of mitotic doubling and drew attention to the unfavourable effect of inbreeding (see also DE JONG & ROWE 1971). They confirmed CHASE in that a considerable heterotic effect could be expected from

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a tetraploid with maximal heterozygosity and optimal divergency in its origin. The basic ideas of CHASE, MENDOZA, and HAYNES, were confirmed experimentally by the work of PELOQUIN, HANNEMAN, MENDIBURU and others. In the meantime, the analytical breeding scheme with the unilaterally formed diplogametes has been adopted by many potato breeding institutes and performed in practical breeding with good success.

5.1.2 Breeding with dihaploids

5.1.2.1 Production of dihaploids and its cytology

Although it would be appropriate to name the parthenotes with 1n = 2x = 24 chromosomes »haploids«, the term dihaploids has been adopted in order to distinguish them from parthenotes with 1n = 1x = 12 chromosomes which are called here monohaploids.

IVANOVSKAJA (1939) obtained a dihaploid by pollination of a tetraploid potato cv. with *S. phureja*. The pollen induces the development of an unfertilized egg cell (pseudogamy, parthenogenesis). If used for pollination of dihaploids, on the other hand, it stimulates the development of monohaploid embryos and plants (VAN BREUKELEN et al. 1977).

Some superior inductors of diploids, such as *S. phureja* PI 225 682.1.1, 1.3, 1.22 and IVP 35 and 48 carry major genes for pigmentation and for distribution of the colour, enabling a recognition of the parthenotes whilst still seeds (PELOQUIN & HOUGAS 1959, HOUGAS et al. 1964, HOWARD 1970). The gene *P* controls the production of delphinidin. The allele B^d distributes the anthocyanin to the base of all organs homologous to leaves, such as the cotyledons of the embryo (seed spot visible through the testa), a violet band at the nodes and the eyebrows of the tubers. The anthocyanine even accumulates in cells of microspore callus (JACOBSEN & SOPORY 1978). The allele *d* does not occur in European cvs. HERMSEN & VERDENIUS (1973) were able to combine the genotype *PP* B^dB^d with a high pollinator efficiency. The dihaploid induction ability in *S. phureja* is inherited in a polygenic manner (IRIKURA 1975). VAN BREUKELEN (1981) assumes five loci with intermediate intra-locus interactions.

The cytological mechanism by which dihaploid embryos arise during meiosis is investigated in detail by VON WANGENHEIM et al. (1960), BENDER (1963), MONTELONGO-ESCOBEDO & ROWE (1969) and VAN BREUKELEN (1981). According to the most plausible explanation, the 12-chromosome sets of both male nuclei of a reduced pollen grain fuse with the central nucleus leading to a normal functioning hexaploid endosperm as a precondition for the subsequent embryo development. The nucleus of the reduced egg remains unfertilized but is stimulated to differentiate. In this way the unreduced (2n-) pollen gives rise to dihaploids. When a 1n-pollen fuses with the egg cell, triploid embryos are formed. Most of them die off.

There are several cytogenetical groups of seedlings arising from crosses of tetraploid genotype × *S. phureja PP* B^dB^d: diploid plants without colour marks are the desired dihaploids, diploid plants with colour marks may have been generated by androgenesis, tetraploids without colour marks may have originated as dihaploids and then spontaneously doubled or been generated from an unreduced egg cell and tetraploids with colour marks are hybrids (diplogametic tetraploids) generated by unreduced *S. phureja*-pollen. In addition triploids can occur, having overcome the triploid block, as well as aneuploids.

The frequencies of the different types vary considerably with the genotypes of the parents involved. As usual with cytological processes, they are very sensitive to environmental variation. For example, in four days in August 1975, VAN BREUKELEN (1981) extracted with *S. phureja* IVP 10 ex cv. Gineke 84, 115, 360 and 367 dihaploids per 100 berries. He obtained on average 160 dihaploids with IVP 35 (*PP* B^dB^d) out of seven varieties. The maximum (with IVP 48) was 998 dihaploids per 100 berries. FRANDSEN (1967 a) extracted 42 and 27 dihaploids per 100 berries with 1.1 (see above) and 0.22 respectively on average from 88 and 27 mother clones. WENZEL et al. (1982 a) obtained between 0 and 58.7 dihaploids per 100 fertilized flowers with an average of 20 %.

Measures improving flower production and seed and berry set, such as grafting onto tomato stocks, good manuring etc., will, of course, favour the dihaploid output. A special

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technique (decapitation) was used by PELOQUIN & HOUGAS (1959). Stalks with flowers and buds cut from vigorously growing tetraploid clones or varieties, put in bottles with a nutrient and sterilizing solution and placed in the greenhouse at a temperature between 20 and 25 °C.

For a provisional check of the chromosome number, the plastids in the guard cells of the stomata can be counted. The plastid number is strongly correlated with the chromosome number (ROTHACKER et al. 1966, FRANDSEN 1968).

Dihaploids can be obtained by androgenesis of pollen from tetraploids as well (WENZEL et al. 1982 a, b, MIX 1983, JOHANSSON 1984), but this method is perhaps more labour-intensive and needs equipment for sterile working.

5.1.2.2 Properties of dihaploids

The majority of the primary dihaploids has weak growth, although there are marked differences between the progenies of equally growing and yielding mother cvs. Very many do not flower and do not form tubers.

Their inbreeding coefficient is equivalent to that of a tetraploid progeny after three consecutive selfings (HOUGAS & PELOQUIN 1958, MENDIBURU et al. 1974). The dihaploids reflect the genetic composition and potency of the mother genotype (HANNEMAN 1980). Therefore, it was possible to predict which parent genotype transmits in 4x · 4x – crosses the highest and the lowest yield, and the highest specific gravity. Genetic analyses of dihaploid families as well as comparisons of their properties with those of the mother clone were performed for yield and several morphological and physiological traits by WÖHRMANN (1966) and DE, MAINE (1984 a). BAERECKE (1968) studied the pattern of variation in dihaploid families with infection resistance to leafroll virus. PEREZ-UGALDE & PELOQUIN (1967) and DE, MAINE (1982, 1984 a) investigated resistance to late blight and eelworm, MARIS (1973) to wart, CIPAR & LAWRENCE (1972) to scab and DE JONG (1981) the genetics of the russet tuber surface. A certain percentage of dihaploids exceeded in many cases the score of the tetraploid parent. Monomer dominant segregation figures known from tetraploid progenies for yellow flesh (MOK et al. 1976) and extreme resistance to the viruses X and Y (Ross unpubl.) could be corroborated.

LANDEO & HANNEMAN (1982) found in dihaploids an additive genetic variance in yield, but it was non-additive in the specific gravity of the tuber and in dry and fresh green matter.

MCHALE & LAUER (1981 a, b) found in *S. phureja* × dihaploid hybrids specific as well as general combining ability, but the tuber number was inherited in a dominant manner.

The very low fertility of the primary dihaploids (PEREZ-UGALDE et al. 1964, GOREA 1970, ABDALLA & HERMSEN 1972, CARROLL & LOW 1976) is the greatest handicap for their further use in breeding. It is due to the general weakness of the dihaploids and to their S-alleles which regain their activity at the diploid level. Ross et al. (1964) obtained 3 % male fertiles out of 665 dihaploids. Female fertility generally is better. WENZEL et al. (1982 c) found 5 % selffertiles among primary dihaploids and 9 % among inter-dihaploids (see also IWANAGA 1982, 1984 a).

5.1.2.3 Pre-breeding at the diploid level (Inter-dihaploids)

The primary dihaploids form the basis of many fields of modern potato breeding. In the first phase of their utilization it is necessary to inter-mate dihaploids of different sources in order to repair the inbreeding effect, to combine different traits and for general upgrading. CHASE proposed (1963; *Fig. 8*) the creation of inter-dihaploids with maximal heterozygosity by combining the primary dihaploids with diploid cultivated and wild species. These, after selection, should be brought back to the tetraploid level. PELOQUIN & HOUGAS (1960), SOSA & SOSA (1971, 1972), LANDEO & HANNEMAN (1979), MCHALE & LAUER (1981 a, b) and SANFORD & HANNEMAN (1982 c) choose *S. phureja, S. stenotomum* and *S. chacoense* and IWANAGA (1984) in addition *S. microdontum*, and *S. sparsipilum* and ROSS & JACOBSEN (1976), UHRIG & WENZEL (1981), WENZEL & UHRIG (1981) and UHRIG (1983) eelworm resistant wild species as *S. gourlayi, S. sparsipilum, S. spegazzinii* and *S. vernei* as

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crossing partners for primary dihaploids. Resistant F_1 – backcross progenies with sometimes remarkable increased vigour, tuber yield and fertility were obtained. The heterotic reaction varied considerably with the used dihaploid genotype and with the special provenance of the wild species (*Fig. 6;* HERMUNDSTAD & PELOQUIN 1984).

Another possibility to obtain highly heterozygous dihaploids with valuable genes for resistance is to intermate dihaploids derived from cvs. or breeding lines carrying genes from different wild species. In such sources unfavourable genes are already eliminated by selection during the several back-cross generations. In this way inter-dihaploids could be bred with genes from *S. demissum,* with genes from *S. acaule* including the gene *Rx* conferring resistance to PVX, with genes from *S. stoloniferum* including *Ry* and with genes from *S. oplocense,* coding for eelworm resistance (BAERECKE, FRANDSEN & ROSS unpubl. and LANGTON & ROSS 1982). A remarkable improvement of fertility, vigour and yield was observed already in F_1 when selected inter-dihaploids were used as parents. This tendency was enhanced in further generations of inter-mating the farther the diploid parents were related.

5.1.3 Resynthesis of tetraploids

Diploid forms of the cultivated potato cannot fulfil the requirements of a modern variety (Section 2.2). This is true for dihaploids as well. The upgraded dihaploids, i. e. the inter-dihaploids, have to be brought back to the tetraploid level (*Fig. 7, 8* and *14; Tab. 7*). This can be performed in different ways: 1. by mitotic polyploidization by means of colchicine or by spontaneous doubling of the chromosomes; 2. by meiotic polyploidization, i. e. by making use of diplogametes; 3. by somatic hybridization, i. e. protoplast fusion. The fusion method has the greatest advantages because all dominant inherited traits of both parents show up in the one hybrid. Of course, it cannot be expected to become a cultivar itself, but it could be a parent with high breeding potential. The first proven protoplast fusions between inter-dihaploids have been accomplished recently by HELGESON (1985, in press).

5.1.3.1 Mitotic chromosome doubling

FRANDSEN (1967 b) treated seed with a 0.25 aqueous solution of colchicine for 1–2 weeks. He obtained 38 and 80 seedlings from 152 and 225 seeds respectively. 50 % of the plants grown from tubers proved to be doubled in all three tissue sheets, i. e. 39.5 and 38.7 % of the seedlings respectively. In order to double the chromosome number of distinct genotypes, shoots were grafted onto tomatoes (Dionne method; Ross et al. 1967, FRANDSEN 1967 c, LANGTON 1974, DE, MAINE & FANTES 1983). The top and the axillary buds were removed and a cotton pad soaked with colchicine solution put onto the subaxillary meristem. The probability of entirely doubled plants grown from tubers of these plants is 11 %.

A simple method is the regeneration from dihaploid callus in vitro. Several plants of the same dihaploid genotype were regenerated from cell clusters and small leaf pieces (BEHNKE 1975). They differed in vigour, and the larger plants turned out to be entirely tetraploid (JACOBSEN 1978 a). JACOBSEN (1978 c) found that by starting with leaf-piece callus 26–90 % of the plants of 5 dihaploid genotypes had doubled their chromosome number, but the remaining three did not react. It is remarkable that no sign of chimaerism was found. Tetraploids are often recognizable by a more conspicuous rounding of the end-leaflet.

HERMSEN et al. (1981 a) confirmed these results by growing *S. phureja*-dihaploid hybrids from leaf-piece callus as well (explants). They obtained 83.9 % entirely tetraploid plants. There seems to exist in dihaploid as well as in monohaploid calli and tissue a general tendency for the chromosomes to double.

The use of mitotically induced tetraploids in breeding is often hampered by their decreased pollen fertility. In the potato, most of the doubled genotypes show a reduced male fertility as well. There are, however, cases where the doubled derivatives show the same, or even a higher, pollen fertility (FRANDSEN 1967 b, LANGTON 1973, LANGTON & ROSS 1982).

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Tab. 7: Resynthesis of tetraploids from dihaploids (terminology)
Resynthese von Tetraploiden aus Dihaploiden (Terminologie)

A asexual		gene loci – *Genloci*
1 by mitotic chromosome doubling[1] *durch mitotische Chromosomenverdopplung*		
a) treatment of 2x-seed with colchicine *Colchizinbehandlung von 2x-Samen*	formation of chimeras *Bildung von Chimären*	partly duplex heterozygote, partly homozygote *teils duplex heterozygot, teils homozygot*
b) treatment of 2x-meristems with colchicine *Colchizinbehandlung von Meristemen*		
c) regeneration of 2x-callus (endomitosis) *Regeneration von 2x-Kallus (Endomitose)*	little or no formation of chimeras *keine oder geringe Chimärenbildung*	
2 by somatic cell fusion *durch somatische Zellfusion*		highly heterozygote *hoch heterozygot*

B sexual, by meiotic chromosome doubling *sexuell, durch meiotische Chromosomenverdopplung*[1]			
1 unilateral[3], by ploidycrosses *unilateral, durch Ploidiekreuzungen*			
cross *Kreuzung*	unreduced gametes *unreduzierte Gameten*	4x-progeny *4x-Nachkommen*	
	2n-gametes = diplogametes[2] *2n-Gameten = Diplogameten*[2]	diplogametic tetraploids[2] *diplogametische Tetraploide*[2]	in the case of FDR highly heterozygote *mit FDR hoch-heterozygot*
4x · 2x = 4x	diplandroids[3] *Diplandroide*[3]	diplandroid tetraploids[3] *diplandroide Tetraploide*[3]	
2x · 4x = 4x	diplogynoids[3] *Diplogynoide*[3]	diplogynoid tetraploids[3] *diplogynoide Tetraploide*[3]	in the case of SDR less heterozygote *mit SDR weniger heterozygot*
2 bilateral[3], by 2x–2x-crosses *bilateral, durch 2x–2x-Kreuzungen*			
2x · 2x = 4x	as above *wie oben*	quadrigametic tetraploids *quadrigametische Tetraploide*	

[1] Skiebe et al. (1963), [2] Ross und Jacobsen (1976), [3] Mendiburu and Peloquin (1976)
FDR = First division restitution
Restitution nach der ersten Kernteilung
SDR = Second division restitution
Restitution nach der zweiten Kernteilung

This applies mainly to the less fertile dihaploids, so that chromosome doubling of such genotypes could be a means to improve the crossability of valuable combinations.

Dihaploids and their mitotic tetraploid derivatives are comparatively investigated in some traits. Rowe (1967 a) compared the growth, the traits and yield of 15 pairs of a F_1 *S. phureja*

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× dihaploid. The tetraploids showed a decrease in yield of 31 % on average. The yields of single 2x – plants were 1193–1588 g, those of the 4x – derivatives 761–1157 g. The tuber number of the tetraploids was halved compared with that of the diploids: flowering time began 4.3 days later, and the stalks were several cm longer. Crossings among the tetraploids produced only half as much seed as those from inter-diploid crossings with equal number of fertilizations. De, Maine (1984 b) found no difference or only a slight increase in yield and rate of photosynthesis in leaves of tetraploids compared with their chromosome doubled derivatives. Langton & Ross (1982) compared the eelworm resistance of the diploid vs. the tetraploid genotype in 12 pairs. All but one were F_1-hybrids from crosses between the resistant wild species *S. spegazzinii, S. vernei* and an undescribed Peruvian species related to *S. sparsipilum* on the one side and susceptible dihaploids on the other. The last genotype was a hybrid *S. vernei* × dihaploid × dihaploid. The tests were performed with two pathotypes of *G. rostochiensis* and two of *G. pallida.* All in all, 19 comparisons were made. 12 showed no difference in resistance, but in 7 comparisons a significant decrease in the resistance of the tetraploid partner was observed. The reason is unknown. The resistance is inherited by a few major genes of medium or low expressivity, while minor genes vary the degree of the resistance. In experiments with major genes it must be noted that a simplex condition turns into a duplex one by mitotic doubling.

5.1.3.2 Meiotic chromosome doubling

Diplogametes and their induction

2n-gametes (unreduced gametes, diplogametes) result from deviations of the normal meiosis mechanism. They can be formed unilaterally in 4x-2x-crosses or bilaterally in 2x-2x-crosses *(Tab. 7)*. Bleier (1931) was the first to observe 2n-gametes in the potato (see further Swaminathan & Howard 1953). Unreduced gametes, particularly those of *S. phureja,* were used occasionally and unconsciously in breeding varieties (Section 2.5), but their true value was first stressed by Chase (1963). He pointed out the importance of diplogametes for the breeding of highly heterozygous genotypes *(Fig. 8)*. The 2n-gametes function in the last phase of his scheme when the diploid lines are brought to the tetraploid level, as an alternative to the treatment of the dihaploids with colchicine. The first ploidy crosses caused a distinct increase in yield (Mendiburu & Peloquin 1971), as confirmed in later investigations. These results have awakened general interest in breeding with unilaterally formed diplogametes.

Hanneman & Peloquin (1967, 1968) studied the occurrence of diplogametes in 107 not preselected *S. phureja* – dihaploid hybrids crossed with 21 tetraploid varieties and breeding lines. They counted 6863 seeds in 3525 berries. 1161 berries were seedless. 2.10 seeds per berry were found in 2x-4x-crosses and 1.76 reciprocally. Nearly all of these seeds were tetraploid, because of the triploid block (Section 5.1.2.1). The most productive *S. phureja* × dihaploid hybrids were the clones W 5295.7 (*S. phureja* × dihaploid ex cv. Katahdin) and W 5337.3 (*S. phureja* × dihaploid ex cv. Chippewa). The former yielded a maximum of 56.3 seeds in 4x · 2x-crosses and 6.8 when reciprocally crossed. Both hybrids have since been used as dihaploid parents in many institutes. Ross & Jacobsen (1976) investigated 76 unselected dihaploids (primary dihaploids, inter-dihaploids, diploid hybrids of dihaploids with *S. megistacrolobum, S. phureja, S. sparsipilum, S. spegazzinii* and *S. vernei* among their parentage) in their ability to produce diplogametes. 1.74 seeds per berry were found on average in the direction 2x · 4x, but 1.11 in 4x-2x-crosses. Progeny seedlings of tetraploids with unselected dihaploids not harbouring *S. phureja* – genes, delivered a maximum of 63 seeds per berry in 2x · 4x-crosses, while dihaploids with *S. phureja* – genes, selected for high seed-productivity, delivered 100 seeds under the same conditions (see also Hanneman Jr. & Peloquin 1967, and Landeo et al. 1981).

The abnormal meiosis of the microspore is followed by the formation of dyads and triads and lateron of big pollen with four instead of three pores.

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The inheritance of seed productivity, i. e. of the production of diplogametes, is rather complex (Ross & Jacobsen 1976, Den Nijs & Peloquin 1977 b, Jacobsen 1978 b, 1980 a, Veilleux & Lauer 1981). The influence of the environment is obvious. Jacobsen (1976) observed a variation between 0.4 and 31 % of big pollen of a diploid genotype grown at different places in one greenhouse! The correlation between big pollen frequency and seed number per berry is low in most cases. It can be concluded that the inheritance generally follows a polygenic pattern with the consequence that some progeny seedlings turn out with a diplogamete production surpassing that of the parents' mean. The highest seed number per berry (188.8) was reached with die dihaploid W 5337.3. Conditions connected with the formation of unilateral diplogametes were furthermore studied by Quinn & Peloquin (1973), Quinn et al. (1974), Sudheer (1978), Den Nijs & Peloquin (1977 a), Jacobsen (1978 c, 1980 b) and McHale (1982).

Ross & Jacobsen (1976) found 5.5 % triploids in 2x · 4x-families and 19.8 % in the opposite direction. The higher the seed number per berry the higher the percentages of triploids. Sudheer (1975) observed 8 % and 62 % triploids resp. in the same 4x · 2x progenies resulting from crosses in two consecutive years. Some are male fertile (Mok et al. 1975) and can be crossed with tetraploids to give a possibly heterotic tetraploid progeny.

Bilateral meiotic doubling

When 2n-gametes are formed in both dihaploid parents, tetraploid seedlings can originate (quadrigametic tetraploids, *Tab. 7*). This happens in unpreselected parents comparatively seldom. 108 diplogametic tetraploids were formed among 793 seedlings out of 13 families, with a maximum of 58 % in one progeny (Mendiburu 1971, Mendiburu & Peloquin 1976, 1977, Mendiburu et al. 1974, Okwuagwu & Peloquin 1981, Ramanna 1983). Quadrigametic tetraploids exhibit a certain heterosis, as is to be expected (see next section), and are often believed to be »wrong« plants when standing in the field between the less vigorous inter-dihaploids. Diplogametes can be formed in both sexes of the same genotype rendering the plant self-fertile and berry forming. The seeds can then be entirely tetraploid. Such selfed tetraploid progeny from a dihaploid genotype is usually only weak growing.

Cytology of diplogamete formation

Diplogametes can originate by two main deviating meiotic processes. One is equivalent to a first division restitution (FDR). It occurs in some genotypes together with genes for desynapsis. The other is equivalent to a second division restitution (SDR). A new general survey of the mechanisms is given by Hermsen (1983).

Cytological investigations have shown that often the spindle after M II is not, as is usual, at an angle of 60°, but parallel oriented (parallel spindles, ps, *Figs. 9* and *10*). Only two poles with two groups of chromosomes are formed, where the male and the female chromatid join again to form two restitution nuclei. The meiotic process ends with the formation of one cell wall only. This mechanism was detected by Höglund (1970). The process was further analyzed in its cytology and its genetical consequences by Mendiburu (1971), Mok & Peloquin (1975 a, b), Lam (1974), Mendiburu et al. (1974), Quinn et al. (1974), Ramanna (1974, 1979) and Veilleux et al. (1982). A monomere recessive gene *ps* is assumed by Den Nijs & Peloquin (1977 a, b).

The genetic advantages of the ps-mechanism are as follows. All chromatids of the pollen parent are rejoined in the restitution nucleus which is then genetically equivalent to a first division restitution (FDR) gamete. Its chromosomes are distinguished from the original ones only by exchanges having occured between the first crossing over and the end. 80–83 % of the heterozygosity of the parent will be transmitted to the progeny. The diplogametes, therefore, are nearly identical to each other and to the parental genotype. The intra- and interlocus interrelationships with their additive and non-additive (epistasis) gene actions are transfered largely intact to the progeny. Combinations of heterozygote major gene inherited traits of a dihaploid parent do not segregate (Jacobsen 1980 a). 2n-gametes, therefore,

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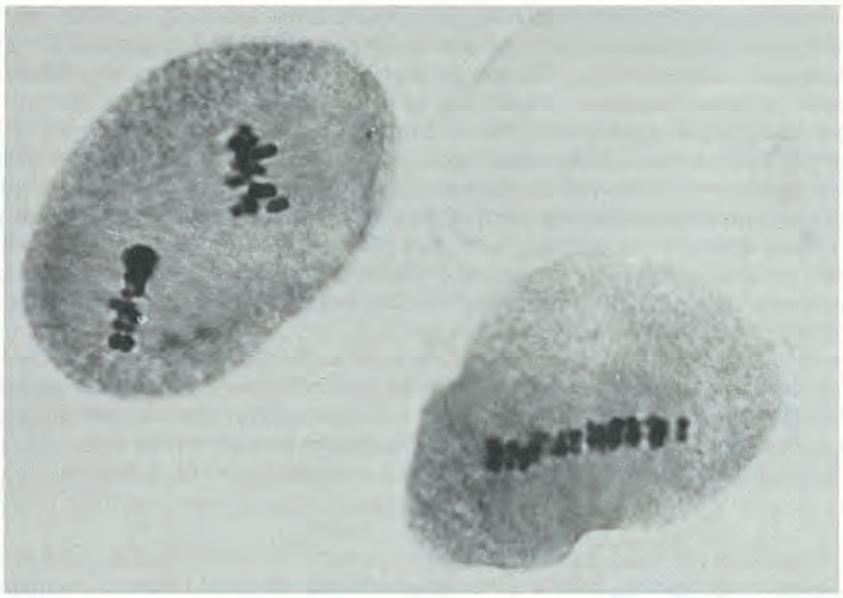


Fig. 9: Arrangement of chromosomes in cells with normal (l) and fused (r) spindles at metaphase II stage in a dihaploid ex cv. Gineke

Gruppierung der Chromosomen in Zellen mit normalen (li.) und fusionierten (re.) Spindeln im Metaphase-II-Stadium einer Dihaploiden ex cv. Gineke (RAMANNA 1974)

particularly when they carry the genotype of a selected parent (inter-dihaploid), are a powerful instrument in the breeder's hands.

Another meiotic deviation, a certain form of desynapsis, leads to a further improvement (IWANAGA & PELOQUIN 1979, OQWUAGWU & PELOQUIN 1981, RAMANNA 1983, JONGEDIJK 1983, IWANAGA 1984 b). It was observed that in some dihaploid genotypes the synapted homologues in MI do not fall apart. This leads to a random distribution of the univalent chromosomes and non-function of the pollen *(Fig. 10)*. The deviation is inherited by a recessive dominant major gene (*ds,* RAMANNA 1983; *sy*^{4}, IWANAGA 1984 b). If, as in the case of USW 5295.7, USW 5337.3 and dihaploids ex cv. Atzimba, the gene *ps* is likewise present, an equational division of the complement before AI results in the formation of 2n-pollen transmitting the entire intact parental genotype. The same mechanism functions in the megasporogenesis as well and is perhaps controlled by the same genetic constitution. This leads to tetraploid progenies when the diploid FDR-genotype is selfed. Sexual hybrids of parents carrying *ds (sy*^{4}*)* and *ps* would be equivalent to their somatic hybrids, because the entire unaltered somatic genotypes of both parents pass unchanged to the zygotes; with the exception of the plasmon which is supplied to the somatic hybrid by both parents.

A second deviating mechanism is equivalent to SDR. It happens either in anaphase I (pc-1) or prophase II (pc-2). The second meiotic division is missing. This leads also to dyads and triads.

The genetic consequences of SDR are characterized by the fact that all primarily heterozygote loci between the centromere and the first chiasma are homozygote, and those between the first and the second, heterozygote. A part of the intra- and interlocus interrelationships may then be destroyed and their heterotic potential may be lower compared with FDR-diplogametes. However, they are still superior to the products of the normal meiosis (MENDIBURU et al. 1974). They are highly homozygous but heterogeneous.

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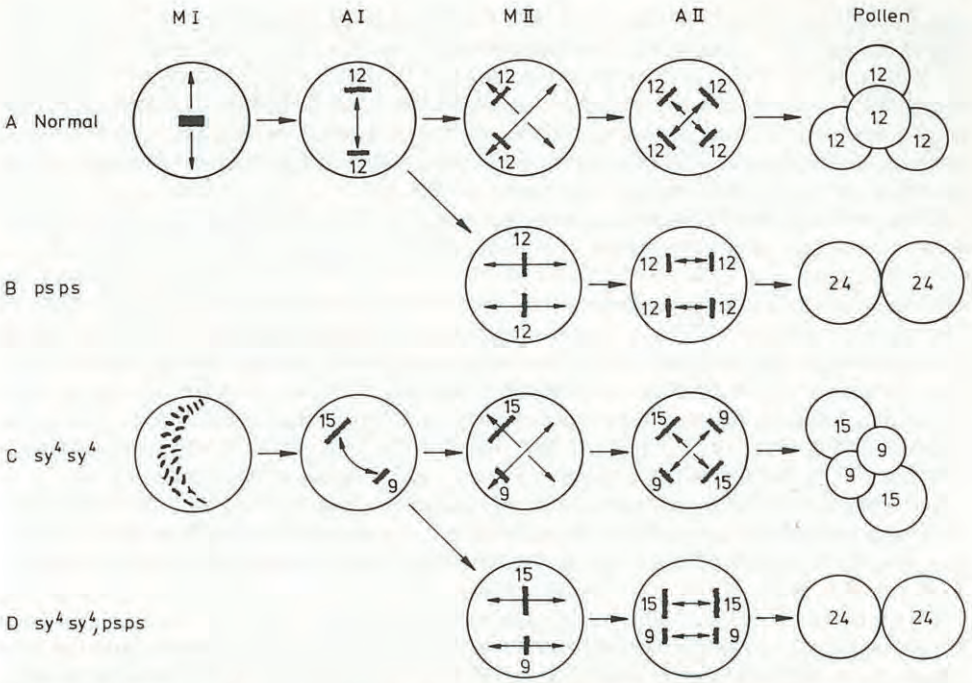


Fig. 10: Sequences of microsporogenesis in four different genotypes: A normal resulting in tetrads; B in presence of the gene ps for parallel spindles resulting in dyads; C in presence of the gene sy 4 for a synaptic mutant resulting in non-functional univalents; D in presence of both genes ps and sy 4 resulting in dyads

Ablauf der Mikrosporogenese in vier verschiedenen Genotypen: A normal mit Bildung von Tetraden; B in Gegenwart des Gens ps für parallele Spindeln, führt zu Dyaden; C in Gegenwart des Gens sy 4 für eine Synapsismutante, führt zu nicht funktionsfähigen Univalenten; D in Gegenwart beider Gene ps und sy 4, führt zur Bildung von Dyaden (IWANAGA 1984 b)

SIMON & PELOQUIN (1976) observed a higher vitality of the ps-pollen compared with pc-pollen. Ps-pollen grows faster, lives longer and forms more seed.

MOK et al. (1976) followed the segregation of a cross of white-fleshed mothers (y^4) with two yellow-fleshed pollen parents (Yy^3). The FDR-pollen parent gave rise to 7 % and the SDR-pollen to 38 % white-fleshed, i. e. nulliplex progeny. This corresponds to a distance of 12–14 units between the locus *Y* and the centromere.

ROSS & LANGTON (1974), by analyzing the segregation of the gene Rx_{acl} for extreme resistance to PVX in crosses 2x · 4x considered an FDR equivalent mechanism also the probable origin of diplogynoids during megasporogenesis. Three dihaploid genotypes *Rxrx* were crossed with two nulliplex tetraploid pollen parents. The progeny (16 seedlings) segregated in 87 % *Rx*-seedlings. Six of these tetraploid seedlings were crossed with nulliplex tetraploids (16 families). The seedlings proved to be simplex: a further argument for FDR-equivalent formation of the original egg-cell. With SDR, duplex heterozygotes would have resulted. The data favour a chromosome segregation rather than a chromatide one.

MOK et al. (1976) also stated that in 2x (Yy) · 4x (y^4) crosses some dihaploids formed the majority of their egg-cells according to FDR (86, 91, 92 and 93 % yellow-flesh progeny) and one to SDR (62 %).

Investigation of the mode of origin of 2n-eggs in tetraploids was performed by TAYLOR (1978). He analyzed the segregation figures of several continuously and discontinuously

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segregating properties in a cross of a tetraploid genotype of ssp. *andigena* with *S. phureja BBPP*. It resulted in a mixed progeny of tetraploid parthenotes without embryo spot (3.9 per berry), tetraploid hybrids with embryo spot by function of diploandroids, and thirdly dihaploids. A tetraploid selfed progeny was raised in addition. When the segregation figures were compared, it turned out that those of the tetraploid parthenotes more closely resembled the dihaploids than the tetraploid selfed progeny. This means that the tetraploid mother must have formed its egg-cells by the SDR.

Connected with the FDR-mechanism is the formation of parallel spindles and dyads, and in most cases big pollen. While MOK & PELOQUIN (1975 a) found a high correlation between both, VEILLEUX and LAUER (1981) and MCHALE (1982) observed different reactions to environmental factors such as temperature. MOK & PELOQUIN (1975 a, b), MOK et al. (1976), DEN NIJS & PELOQUIN (1977 a, b) assume a monomere recessive inheritance for each of the three anomalies: *ps, pc-1* and *pc-2*. The often used clone *S. phureja* P. I. 225.682.1.22 is *Psps*. JACOBSEN (1978 c, 1980 a) concludes from his studies of FDR using the formation of big pollen as a criterion, that recessive inheritance by major and minor genes occurs. VEILLEUX & LAUER (1981) on the contrary, after studying the same criteria, find a monomere dominant inheritance with incomplete penetrance and variable expressivity.

The formation of parallel spindles is not restricted to *S. phureja.* They have been observed in many wild species, was well as in most USA cvs. (QUINN et al. 1974, MOK et al. 1975 b, DEN NIJS & PELOQUIN 1977 a, CAMADRO & PELOQUIN 1980, IWANAGA & PELOQUIN 1984).

Breeding value of diplogametes

The first trials to investigate the yield potency of diplogametes were undertaken with the two *S. phureja* × dihaploid clones W 5295.7 and W 5337.3 as pollen parents. These two clones were proven to be outstanding in yield among similarly raised progenies and form their pollen according to FDR (HANNEMAN & PELOQUIN 1969, MENDIBURU & PELOQUIN 1971).

Tab. 8: Mean yields of diplogametic tetraploids originating from 4x · 2x-families. Two localities
Ertragsmittel von diplogametischen Tetraploiden, die 4x · 2x-Familien entstammen. Zwei Versuchsorte (MENDIBURU 1971)

2x-parent	W 5295.7	W 5337.3
4x-parent	3.75[1]	1.94
Wis 231	5.00**[3]	4.38**
3.67[2]	3.71	2.81
Wis 639	4.30*	3.80**
3.87	3.81	2.91
Wis 643	5.01**	4.54**
4.84	4.30	3.39
Kennebec	4.07	4.16**
4.78	4.26	3.36
Platte	5.23**	4.73**
3.86	3.81	2.90
Merrimack	4.79**	3.69**
3.08	3.41	2.51

[1] mean yields of the 2x-parents in lbs per plant
Ertragsmittel der 2x-Eltern in lbs pro Staude
[2] same for the 4x-parent
Dasselbe für die 4x-Eltern
[3] mean yields of the diplogametic tetraploids, below: mean yields of both parents
Ertragsmittel der diplogametischen Tetraploiden, darunter Ertragsmittel beider Eltern

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MENDIBURU (1971) compared systematically the yields of two 4x · 2x- and six 2x · 4x-progenies with those of their parents at two localities. The progeny with the 2n-pollen outyielded in most cases to an unexpected extent not only the parents' mean but also the better parent *(Tab. 8)*. The yields of the progenies with diploid mothers were not as high.

MOK & PELOQUIN (1975 b) compared in their yield trials 4x · 2x-progenies originating from FDR- (group A) with those from SDR-pollen parents (group B). The progenies of group A yielded at the one locality 7.4–7.8 lbs per hill and at the other 5.9–6.2. But the yields of progenies from group B originating from crosses with the same 4x-parents were 5.0–5.3 and 4.0–4.3, respectively. The yields of a group C of nine 4x-parents inter-mated were 4.2–4.8 and 2.7–3.4, resp.

Yield increases of this amount could only rarely be repeated even with the same FDR-dihaploids MENDIBURU had used in his crossings.

ERJEFÄLT (1978, 1984) used the FDR-dihaploid W 5295.2 and MPJ $H^2$408 as pollen parent in crossings with varieties in Sweden and found no increase in yield.

DE JONG & TAI (1977) and DE JONG et al. (1981) crossed in two years 12 and 8 varieties and breeding lines respectively with W 5295.7 and W 5337.3. The mean yield per hill of 278 4x · 2x-progeny seedlings was higher only in 1975: 4.99 kg (1.99–10.75) compared with 4.56 (2.63–5.80) ($P < 0.001$). Also significantly higher was the number of tubers and stems. The single tuber weight was not higher. But remarkably, the extremes of variability (transgressions) in the 4x-progenies ex 4x · 2x often exceed those of 4x · 4x-families. A comparison of marketable yield between the three best diplogametic tetraploid and six high yielding Canadian varieties brought a positive result for the former, but the difference was small.

The investigations of MCHALE & LAUER (1981 b) and of VEILLEUX & LAUER (1981) showed neither with W 5295.7 and W 5337.3 as pollen parent nor with FDR – *S. phureja* genotypes adapted to the Minnesota climate any increase in the yield of the diplogametic tetraploids over that of the progenies of variety inter-matings.

SANFORD & HANNEMAN (1982 a) compared in extensive studies the yields of three groups of 4x · 2x-progenies where different tetraploid and dihaploid parents were involved. The constitution is given below. The groups comprise 9 and 10 progenies respectively.

TTTT = 4x – ssp. *tuberosum* genotype × dihaploid ssp. *tuberosum* genotype
TTTP = 4x – ssp. *tuberosum* genotype × W 5295.7
TATP = (4x – ssp. *tuberosum* genotype × 4x – ssp. *andigena* genotype) × W 5295.7
W 5295.7 is *S. phureja* × dihaploid ex cv. Katahdin.

The three groups of progenies differed in the divergency of the subgenome constitution. The less related the subgenomes are, the higher the heterotic potency to be expected. *Figure 11* shows that, indeed, the TTTP and TATP groups yielded significantly more than the TTTT group, but there was no difference between the TTTP and the TATP group. The authors assume a threshold has stopped a further rise.

LANDEO (1983) in extensive studies estimated the yield of diplogametic tetraploids at a lowland (San Ramon) and a highland (Huancayo) site in the Andes. The average tuber weight in g per plant in Huancayo was 1259 g with TTAA (4x · 2x), 1373 g with TTAS and 1452 g with TTAP, where S is *S. stenotomum* instead of *S. phureja*. The weights of the crosses 2x · 4x were 2120, 2256 and 1797 respectively. Similar results were obtained at San Ramon. The yield of most of the hybrids was as good as that of the best advanced clones. LANDEO points to the importance of using upgraded inter-dihaploids from different gene-pools in these experiments. SCHROEDER & PELOQUIN (1983) using upgraded dihaploids in 2x–4x crosses found a significant correlation between 2x parent yield and 4x hybrid progeny yields, but not between those of the 4x parent and the progeny.

As described above, a 4x-progeny can arise from 2x · 2x crosses when 2n-gametes are formed in both sexes. The value of these quadrigametic tetraploids was investigated by MENDIBURU & PELOQUIN (1977). They compared the mean tuber weights of the quadrigametic tetraploids (4x ex 2x · 2x) with those of the diploid seedlings of the same families. The

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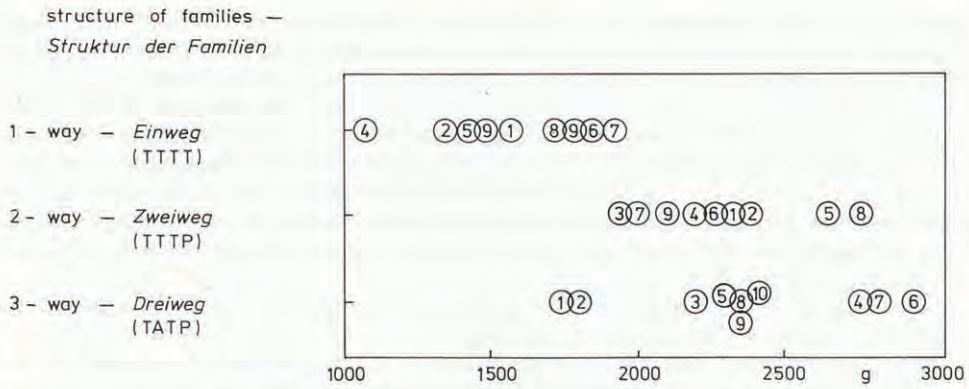


Fig. 11: Distribution of family mean yields of about one to nine families of three groups of crossings: 1-way (control, 4x · 4x), 2-way (4x with genes ex ssp. *tuberosum* × dihaploid) and 3-way (4x with genes ex ssp. *tuberosum* and ssp. *andigena* × dihaploid). The dihaploid parent is a F_1-hybrid *S. phureja* × dihaploid ex cv. Katahdin. Average of a three years trial at Hancock, Wisconsin.

Gemittelte Staudenerträge von rd. 1–9 Nachkommenschaften dreier Kreuzungsgruppen: Einwegkreuzungen (Kontrolle 4x · 4x), Zweiwegkreuzungen (4x mit Genen von ssp. tuberosum × Dihaploide) und Dreiwegkreuzungen (4x mit Genen von ssp. tuberosum und ssp. andigena × Dihaploide. Der dihaploide Elter ist ein F_1-Bastard S. phureja × Dihaploide ex Katahdin. Mittelwerte aus einem Dreijahresversuch in Hancock (Wisconsin) (SANFORD & HANNEMAN Jr. 1982 a)

parents originated from a F_1 *S. phureja* × dihaploid. The results are the means of two field trials. The weights surpass those of the diploid progenies by 0, 19, 23, 29, 32, 33 and 41 %. The last three weights differ significantly at $P < 0.005$. The yields did not exceed those of varieties in comparable trials.

SANFORD & HANNEMAN (1982 b) fertilized about 120 seedlings from dihaploid intercrosses with a pollen mixture of selected dihaploids. Surprisingly, about 50 % of the progeny were tetraploid (quadrigametic tetraploids), the rest diploids and some triploids. The mean tuber yield per hill was 1934 g in the control with conventional tetraploid families but 1150 g in the group of the quadrigametic tetraploids and 576 g in the group of the inter-dihaploid progenies.

Although the number of quadrigametic tetraploids may be high under favorable circumstances, they will presumably not play an important role in breeding, because the yields seem to be insufficient.

Remaining on the diplogametic breeding pathway, it is important to know the usefulness of the (unilateral) diplogametic tetraploid as a parent. As described in Section 5.1.2.1 only relatively few tetraploid seeds are formed in 4x · 2x-crosses, while the diplogametic tetraploids as parents deliver as much seed as the usual parents or even more.

Do the diplogametic (secondary) tetraploids transmit their heterotic potency to the progeny? QUINN & PELOQUIN (1973) studied this question by inter-mating diplogametic tetraploids and by crossing them with varieties and breeding lines. The families were distinguished by different pedigrees: the same dihaploid and tetraploid genotype appeared in one progeny once or in another progeny twice among the grandparents. The less often the same ancestor appeared as a grand parent, the higher was the yield. The four best yielding seedlings of four randomly selected families produced 8.36, 8.90 and 9.30 lbs per hill, while the four highest yielding varieties reached a weight of 4.8 lbs. Generally, however, it would be wrong to attribute to those progenies of the diplogametic tetraploids the same heterotic potential which characterizes the diplogametic tetraploids themselves.

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The transfer of traits other than yield by means of diplogametes to a tetraploid progeny was studied by De, Maine (1982). He crossed dihaploids resistant to *Phytophthora infestans* and others with resistance to *Globodera pallida* as seed parents with tetraploid pollen parents possessing only an intermediate resistance to *P. i.* and no resistance to *G. p.* The level of resistance of the tetraploid progeny did not differ from that of the parents' mean.

It is an important question as to whether the genotypic diversity and the number of available diplogametic tetraploids is sufficient to combine all the properties necessary for a variety. There are several bottle-necks: not every variety can produce dihaploids and only 5 % of them are fertile. Not all dihaploids form their 2n-gametes with FDR. The formation of diplogametic tetraploids is restricted, too. Only in special crosses could the seed number per berry be raised to 188. This reduction in the diversity of genotypes is counteracted 1) by the elimination of lethal and sublethal alleles and by the selection of vitality operating from the extraction of dihaploids as far as the formation of FDR-diplogametes, and 2) by the possibility of utilizing at the steps $4x \cdot 2x$ and $(4x \cdot 2x) \cdot 4x$ the wealth of 4x-genotypes presented by the breeding clones and the world's cultivars as parents.

These last steps obviate the drawbacks considerably. In conclusion it can be stated from practical experience that breeding nurseries can be planted with diplogametic tetraploids and their consecutive tetraploid generations without risk. This is evident from the work at the Max-Planck-Institute (Baerecke, Frandsen, Hemme & Ross unpubl.). The output of best breeding lines for delivery to private breeders for variety breeding was about 10–20 % higher from seedlings built up with dihaploids than from those bred in the ordinary way. *Figure 12* shows a leaf outline of a progeny $(4x \cdot 2x) \cdot 4x$ compared with that of cv. Nicola of the same breeding garden. It must be taken into account that a large proportion of the dihaploids used was entirely unselected, and that cytological investigations as to whether *ps* was present was not made. Secondly the majority of the seedlings were not descended from

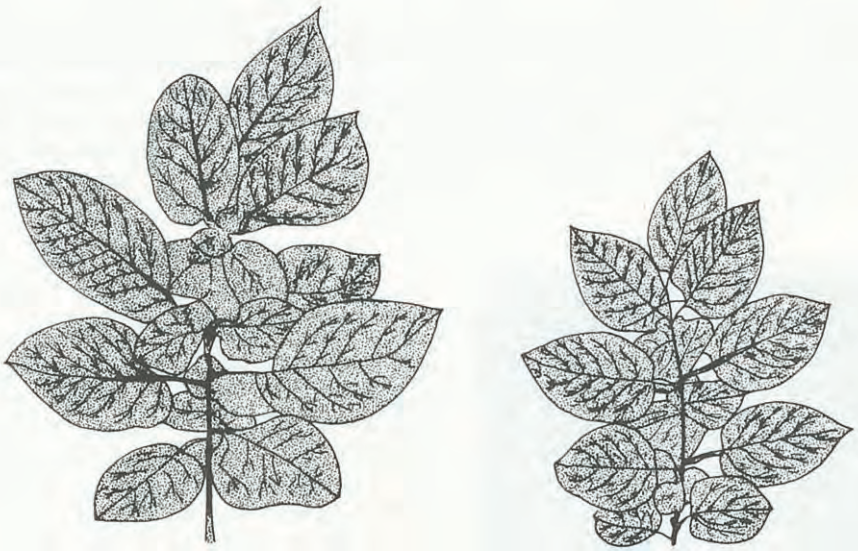


Fig. 12: Heterotic potency of a diplogametic tetraploid (4x ex $4x \cdot 2x$); l. a basal leaf's outline of a selection of a progeny (diplogametic tetraploid) × (tetraploid breeding line); r. cv. Nicola, ¼ of natural size

Heterotische Potenz einer diplogametischen Tetraploiden (4x ex $4x \cdot 2x$); l. Umriß eines Basalblattes eines ausgewählten Klons einer Kreuzung (diplogametische Tetraploide) × (tetraploider Zuchtklon); r. Sorte Nicola, ¼ natürlicher Größe

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4x · 2x crosses, but from the next generation 4x · 4x. It should be mentioned that the first variety bred via dihaploids was released in North America: cv. Yukon Gold (= cv. Golden Bake) = cv. Norgleam × W 5279.4 (*S. phureja* × dihaploid ex Katahdin). Two others, cvs. Ica and Sirena, have since been released in Columbia.

The other way of resynthesising tetraploids, the mitotic method proposed by CHASE (1963), is of comparable interest and needs further study. Sterility problems and the low seed production in 4x–2x-crosses can eventually be more easily overcome. A scheme for comparison of both methods is proposed by JACOBSEN (1978 c, 1980 a).

Before a final judgement about the value of meiotic chromosome doubling can be made, the theoretical preconditions must be fulfilled. These are the extraction of primary dihaploids ex genepools as divergent as possible, wide crosses in order to maximize the heterozygosity of inter-dihaploids and again wide crosses of FDR-inter-dihaploids with the tetraploid seed- or pollen parents. These provide diplogametes and quadrigametic tetraploids to be built into the ordinary breeding work.

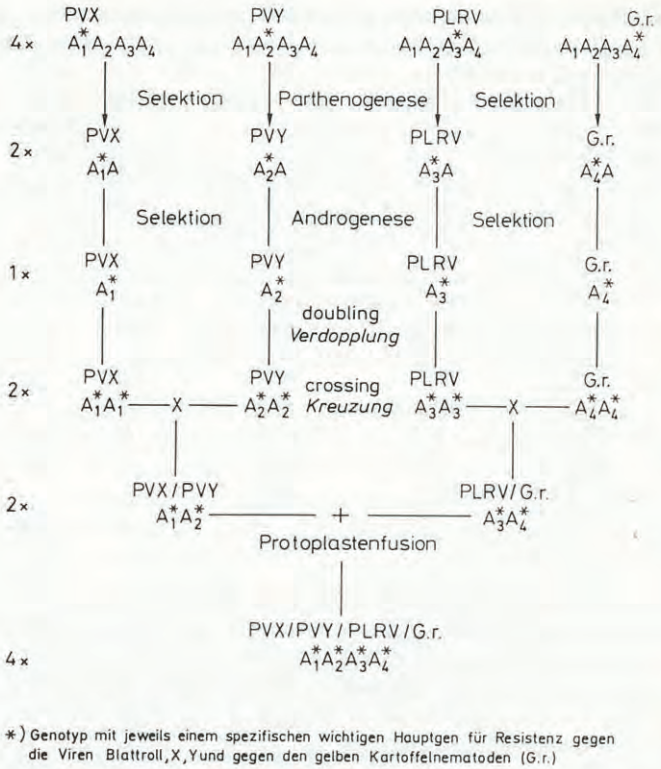
5.1.4 Monohaploids, Anther culture

Further halving of the chromosome number leads from dihaploids (2x = 24) to monohaploids (1x = 12). There are two ways of producing monohaploids from dihaploids: induction of the development of the megaspore by pollination with pollen of a dihaploid (parthenogenesis or gynogenesis), and regeneration of microspores (androgenesis; *Fig. 7, 8, 13, 14, 15*). Monohaploids should be valuable instruments in several fields of breeding. They present a wealth of genetically different genotypes which can be submitted to selection and, when homozygous, they could serve as parents for the production of the true seed potato. Utilization closest to breeding practice would be intercrossing of homodiploids in order to get completely heterozygous diploids. Protoplast fusion of heterozygous diploids of divergent origin is an indispensable step in the building up of a tetraploid with a maximum of heterozygosity (tetraallelism). In this way the analytic scheme of CHASE (1963; *Fig. 8*) could become an analytic-synthetic one (WENZEL et al. 1979; *Fig. 14*). The somatic tetraploid hybrids would combine the selected genotypes of inter-dihaploids with their intra- and interlocus relationships preserved intact. Moreover, when built up systematically from monohaploids they would combine major genes for resistance, for example, to viruses *(Rx, Ry, Ns* and *Nl)* and both eelworm species (H_1 etc.), as planned. There are, however, preconditions which must be fulfilled before the analytic-synthetic scheme can be followed, and these are: regeneration ability of a broad variety of genotypes, the availability of homozygous diploids



Fig. 13: Microspores regenerating within an anther *Mikrosporen, in einer Anthere regenerierend* (SOPORY et al. 1978)

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*) Genotyp mit jeweils einem spezifischen wichtigen Hauptgen für Resistenz gegen die Viren Blattroll, X, Y und gegen den gelben Kartoffelnematoden (G.r.)

Fig. 14: Analytic-synthetic breeding scheme. Monohaploids of divergent origin and carrying major genes for resistance to four important pathogens are bred until to tetraploids by polyploidisation, crossing and somatic hybridisation

Analytisch-synthetisches Zuchtschema. Monohaploide divergenter Herkunft und mit Hauptgenen für Resistenz gegen vier wichtige Pathogene werden durch Polyploidisierung, Kreuzung und somatische Hybridisierung bis zu Tetraploiden gezüchtet (WENZEL et al. 1979, slightly modified – *leicht verändert*)

and the ability for protoplast fusion of various genotypes. These preconditions have as yet not been fully met.

The first monohaploid of *S. tuberosum* was obtained by BAERECKE & FRANDSEN (cit. FRANDSEN 1968). They fertilized a dihaploid with *S. phureja*. Subsequent trials (VAN BREUKELEN et al. 1977, JACOBSEN 1978 b, WENZEL et al. 1982 b, c) showed that gynogenesis, as compared with androgenesis, is much less effective. Anther culture in axenic media was developed by MURASHIGE & SKOOG (1962, see also NITZSCHE & WENZEL 1977). IRIKURA & SAKAGUCHI (1972) and IRIKURA (1975) obtained 350 monoploids, diploids, tetraploids and aneuploids ex diploid wild and cultivated species. FOROUGHI-WEHR et al. (1977), SOPORY (1977), SOPORY et al. (1978), JACOBSEN & SOPORY (1978), JACOBSEN (1978 a, b) and BINDING et al. (1978) were the first to produce monohaploids ex dihaploids through androgenesis. They were followed by SOPORY & TAN (1979), WENZEL (1979, 1985), WENZEL et al. (1979, 1982 a, b, c, 1984 b, 1985), WENZEL & UHRIG (1981) and UHRIG (1983).

Flower buds of 4–5 mm length containing uninucleate pollen are plated on a Murashige-Skoog medium for induction. After about four weeks, macroscopic structures (embryoids) appear and are transferred to a differentiating MS-medium with 10 % coconut milk, 0.3 mgl^{-1} zeatin and 3 % sucrose. After morphogenesis has started a second transfer to

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Tab. 9: Anther culture from well reacting dihaploid genotypes carrying different traits for resistance
Ergebnisse der Antherenkultur von gut reagierenden Klonen mit verschiedenen Resistenzeigenschaften (WENZEL et al. 1982 c)

anther delivering clone *Antherenspender-Klon*	resistent against	number of *Zahl der* plated anthers *plattierten Antheren*	macroscopic structures *makroskopischen Strukturen*	total *gesamt*	number of plants *Zahl der Pflanzen* 1x	2x	4x
H^3 703	–	17910	11185	1441	–	1021	120
H 79.1123/3	–	3150	1620	289	2	163	24
H 78.01/10	PLRV	2452	1483	323	63	260	10
H 78.01/27	PLRV	3765	2215	1624	401	1088	30
H 79.1045/11	PVX	324	2	2	–	2	–
H 79.1038/18	PVY	831	1009	48	12	28	3
H 79.1038/27	PVY	833	5049	31	–	29	2
H 78.2022/14	PCN; Pa 1, 3	6031	492	–	–	–	–
H 78.2020/26	PCN; Pa 3	1303	89	27	–	27	–

another varied MS-medium is necessary. Only 10 % of the structures survive the transfer. A summary of media for regeneration from anthers, cells, and tissues is given by WENZEL et al. (1982 b), see also FOXE & PRAKASH (1983), CARLBERG et al. (1983), KIKUTA et al. (1983) and MILLER & LIPSCHITZ (1984). The response to anther culture differed greatly between the parental genotypes. In the experiments of WENZEL et al. (1979) and WENZEL & FOROUGHI-WEHR (1984) with inter-dihaploid parental genotypes, preselected for vigour and fertility, about 75 % developed structures *(Fig. 13, Tab. 9)*. The rate of low responding genotypes can

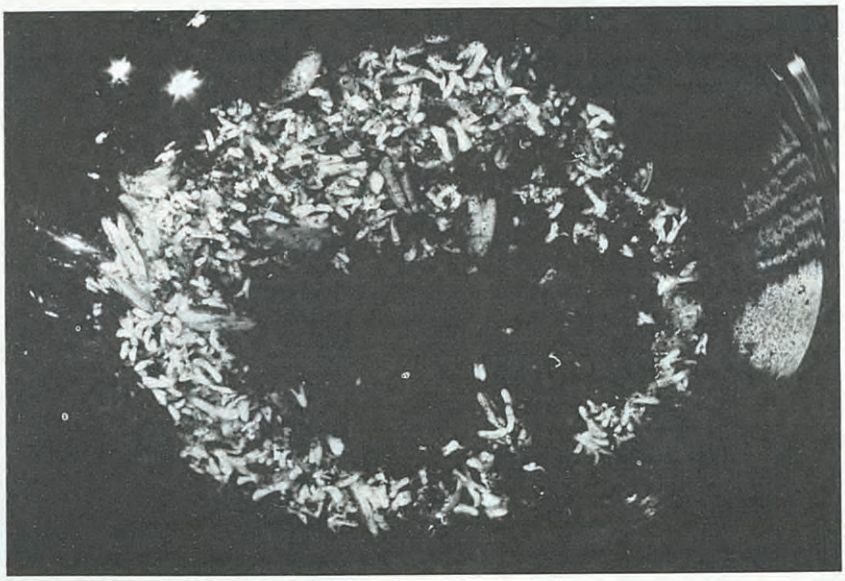


Fig. 15: Group of dihaploid anthers with regenerating monohaploid microspores in a liquid medium
Gruppe von dihaploiden Antheren mit regenerierenden Mikrosporen in einem flüssigen Medium (by courtesy of H. UHRIG)

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be increased by intermating with well responding ones. Good response could be combined with resistance to PVX, PVY, PLRV and to nematodes (WENZEL & UHRIG 1981, UHRIG & WENZEL 1981, UHRIG 1983). UHRIG (1985) found a surprisingly high regeneration rate and response when using a rotating liquid medium *(Fig. 15)*. HEBERLE-BORS (1985) and JOHANSSON (1984) assume that the anthers contain predetermined embryogenic pollen grains (P-pollen) beside the normal ones. The frequency of the P-pollen is high in male sterile plants and can be raised experimentally by pretreatment of the donor plant that shifts the sex balance to femaleness, as e. g. by application of NAA. Embryogenesis in tetraploids potatoes could be increased fourfold by application of Alar 85, an anti-gibberellin.

Very few of the anther-derived plants had the monohaploid chromosome number of 12. They were weak and sterile even after colchicining. The rest of the plants were $2n = 24$ and few $2n = 48$. All the diploids could not have been derived unaltered from unreduced microspores because they generally were identical neither among each other nor with the mother phenotype.

Some selfed progenies of the 3 % self-fertile anther derived (A_1-)-plants could be rised, but all were segregating (WENZEL 1984). The same phenomenon was observed in A_1-plants from tobacco (DE PAEPE et al. 1981, 1982/83). SUNDERLAND (1980) holds an exchange of DNA between the vegetative and the generative pollen nucleus during the first phase of pollen development possible. DE PAEPE et al. base their explanation on cytological events involving both pollen nuclei and regarding the symptom of crompled leaves on gene amplification. WENZEL et al. (1982 b, c, 1984 b) assume that the diploids originated from unreduced microspores and were submitted later to a high cross over and mutation rate.

Moreover, a relationship to the uncleared phenomena of somaclonal variation (Section 5.2.2.1) cannot be denied. In many cases regeneration in anther culture passes a callus phase. Therefore, those causal factors for somaclonal variation may be responsible, at least partly, for variation of anther derived plants too.

The realization of the analytic-synthetic breeding scheme *(Fig. 14)* requires homozygous diploid parents to be crossed. Because true homozygotes seem not to exist, the available heterozygote doubled monohaploids would be a second choice. WENZEL et al. (1984 b) detected hybrid vigour in progenies of crosses between A_1-plants *(Fig. 16)*. UIJTEWAAL & MATTHEIJ (1986) produced monohaploids from different *Solanum* species and their hybrids by prickle-pollination and anther culture.

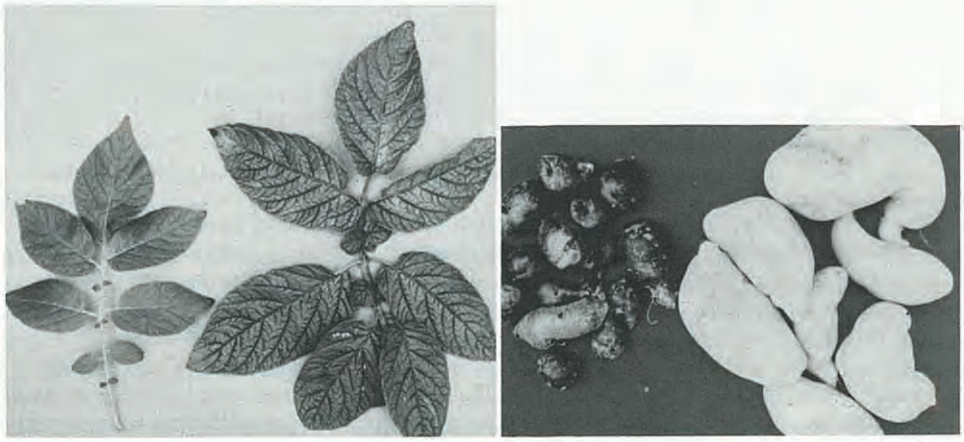


Fig. 16: Doubled monohaploid (2x; l.) and F_1-hybrid of doubled monohaploid parents (r.)
Verdoppelte Monohaploide (2x; l.) und F_1-Bastard (r.) aus verdoppelten monohaploiden Eltern (WENZEL et al. 1984 b)

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5.2 Tissue-, cell- and protoplast culture

Potato plants can be regenerated from organized tissue, cell aggregates, and from cells deprived of their cell walls, i. e. protoplasts. A rough relationship between the origin and degree of the variability of the regenerated plants can be stated. The more organized the donor tissue the higher the stability in the chromosome number and the lower the frequency of variation (DENTON et al. 1977, AHLOOWALIA 1982, KARP et al. 1982, WRIGHT 1983, JONES et al. 1983). The extremes are regenerants from tip meristems on the one hand and those originating directly from generative cells on the other.

Plants regenerated from protoplasts, and in many cases those from cells and tissues as well are characterized by an outburst of variations (SHEPARD & TOTTEN 1977: protoclones, LARKIN & SCOWCROFT 1981: somaclones, ROEST & BOKELMANN 1980, VAN HARTEN et al. 1981, AUSTIN & CASSELS 1983, CASSELS et al. 1983). The amount of variation is strongly depending from the genotype, from the regenerating plant part and from the length of time, the regenerate remains in callus state (D'AMATO 1984).

5.2.1 Regeneration of plants from organized tissue

Axenic culture of organized tissue is aimed to produce plants which do not deviate from the mother genotype. They serve different purposes: 1. to produce virus-free plants from cvs. totally invaded by viruses (Section 9.2); 2. for conservation of plantlets for international transfer of genebank material (Section 2.6); 3. for production of sterile plants and, particularly, tubers for resistance screening (Section 7.8); 4. for sterile micropropagation in foundation seed programmes (Section 10). Only for the creation of virus-free plants must the culture start with meristems of stem apices. With other tissues as axillary buds, leaf discs (WEBB et al. 1983), leaf explants (ROEST & BOKELMANN (1980), VAN HARTEN et al. (1981) and tuber discs LAM (1977) a lower or higher amount of variation may happen.

It is an old established procedure in order to maintain the true type of a cultivar, that the production of seed tubers starts with single healthy tubers. The single tuber progenies are kept separately in the field. When grown in rows side by side aberrant types can be detected and discarded. Several kinds of morphological variants are occuring: grossly aberrant types and more or less conspicuous changes in growth habit, leaf shape and colour, tuber skin and colour, flower abundancy, vegetation time, etc. (SWANINATHAN & HOWARD 1953, RUDORF 1958, HOWARD 1970). HEIKEN (1958) observed a rate of morphological variants between $> 1.8 \times 10^{-3}$ to $> 4.6 \times 10^{-5}$ of single plants in nine varieties. Variants of the cvs. such as those with coloured or russet tuber surface could be isolated and became new varieties occasionally. The variability could be increased by mutagenic treatments (VAN HARTEN et al. 1981, SANFORD et al. 1985).

5.2.2 Regeneration of plants from single cells and protoplasts

Comprehensive report on the present state of research is given by DAVEY & KUMAR (1983), REISCH (1983), SCOWCROFT (1984) and LÖRZ (1985). DUNWELL & SUNDERLAND (1973) were the first to succeed in regenerating potato plants from protoplasts.

The procedure starts with sterile leaf pieces which undergo enzymatic digestion of their cell walls resulting in single protoplasts. The protoplasts, cells and calli are transferred subsequently to several media for division, callus growth and shoot regeneration. For media composition see Section 5.1.4. *Figure 17* shows the sequence of the different steps with the method of KIKUTA et al. (1983). HABERLACH et al. (1985) could regenerate protoplasts from 32 clones of 36 provenances to shoots. The provenances belonged to *S. tuberosum* (2x, 4x), *S. brevidens, S. demissum, S. etuberosum* and *S. pennelli.* Up to 69 % of the calli of the single lines, transferred to differentiating medium, formed shoots.

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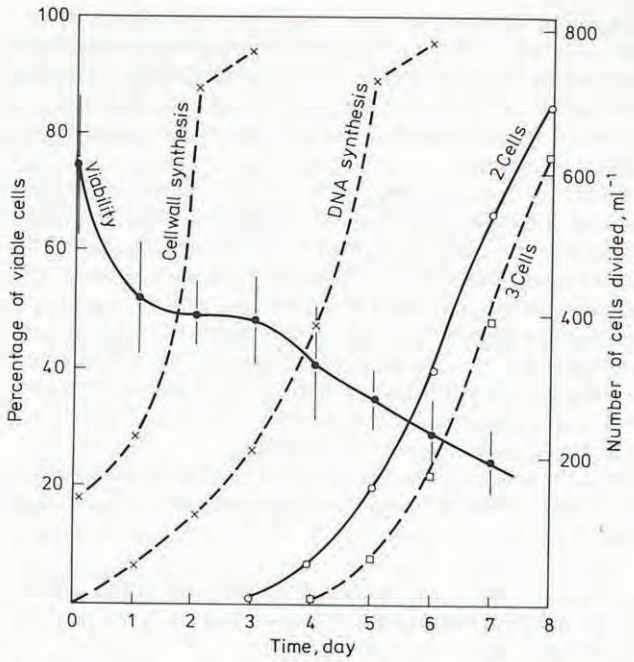


Fig. 17: First phases of regeneration of potato protoplasts
Erste Stadien bei der Regeneration von Kartoffelprotoplasten; l. lebende Zellen in Prozent; r. Zahl der Zellen im Zwei- und Dreizellstadium in ml⁻¹; m. Zellwandsynthese, DNA-Synthese; unten Zahl der Tage (KIKUTA et al. 1983)

5.2.2.1 Somaclonal variation

The first large group of plants from potato protoplasts was produced and investigated by SHEPARD & TOTTEN (1977). They cultured protoplasts from cv. Russet Burbank etc. and were confronted with an unexpected outburst of variation. Subsequent experiments by SHEPARD and his co-workers confirmed the observation (MATERN et al. 1978, SHEPARD et al. 1980, 1983, GUNN & SHEPARD 1981, SECOR & SHEPARD 1981, SHEPARD 1981, 1982 a, b, PAVEK & CORSINI 1982). Trials with other cvs., partly performed with regenerants from callus tissue had similar results, as was reported by THOMAS (1981) and THOMAS et al. (1982) with cv. Maris Bard, KARP et al. (1982) with cvs. Maris Bard and Fortyfold, JONES et al. (1983) with cvs. Desirée, Maris Bard, Fortyfold and Majestic, SREE RAMULU et al. 1983, 1984 a, b, BOKELMANN & ROEST (1983) and CARLBERG et al. (1983) with cv. Bintje, JELLIS et al. (1984) with cvs. Maris Piper, Foxton and Feltwell, BRIGHT et al. (1984) with cv. Record and MACKAY (1984) with cvs. Désirée and Pentland Crown. Still further studies with cvs. and dihaploids were published by TIEMANN et al. (1984), CASSELS (1986) and VAN SWAAIJ & JACOBSEN (1986). Similar experiments with German cvs. have not been made so far.

Somaclones were also raised by NELSON et al. (1983) from *S. brevidens* and by BARSBY et al. (1984) from *S. brevidens, S. etuberosum* and *S. fernandezianum,* three diploid leaf-roll resistant wild species.

JONES et al. (1983) obtained with his method colonies of cells from 35 % of the protoplasts of cvs. Maris Piper and Désirée and sprout regeneration from these calli in 50–80 %.

The variations comprise 1. grossly aberrant plants; 2. more or less slight morphological deviations in type of habit, leaf shape, leaf- and flower colour, tuber traits, such as shape, texture of the surface, flesh colour, uniformity of the tuber nest, and others; 3. physiological

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variations, such as time of flowering and length of vegetation period; 4. resistance to *Globodera pallida, Alternaria solani, Phytophthora infestans* in the leaves, common scab, PLRV, PVY frost and variations in the content of reducing sugars. The number of different deviating morphological traits in a single protoclone varied between one and 16! The frequency is influenced by the length of time the genotype remained in the callus stage. Subcalli differed in their variants.

Variants with resistance to fungal parasites are of special interest. For tests with *Alternaria solani,* MATERN et al. (1978) isolated two phytotoxins similar to lipids from the culture filtrates. Mixed together and transferred with a needle onto the leaves, they caused typical concentric rings. However, five among 500 somaclones showed lesser and smaller lesions. This resistance could also be recognized with infection with spores of *Alternaria.*

In the studies with *Phytophthora infestans,* young plants 6–8 cm high from 500 clones were sprayed with a zoospore suspension of the pathotype. 0.2 % of the clones remained nearly undamaged, while the rest died off partly or completely. Some of the resistant clones proved to be resistant to the pathotype 1.2.3.4 as well. The resistance is said to have been maintained during the several years of observation.

Very interesting is the somaclonal variation in cyst formation with eelworm pathotype Pa-3. It ranged in cv. Désirée from 261 and 373 respectively and in its protoclones from 129 to 469 (MACKAY 1984).

5.2.2.2 In vitro selection among somaclones at the cell level

A review about the theme treating many species including potato is given by WENZEL (1985).

In selection trials for resistance to *Phytophthora infestans,* BEHNKE (1979, 1980 a) placed pieces of calli cultivated from leafcell clusters of three dihaploid genotypes of intermediate resistance in a medium containing culture filtrates of the pathotypes 1.2.3.4, 1.2.3.4.5.7 and »Köln«. Several subcultures proved resistant. The process was repeated twice. Of 42.440 calli, only 45 survived. 32 plants were grown from resistant calli. After infection with the fungus itself, 31 of the plants exhibited lesions being on the average 20 % smaller than those of the mother genotype. Whether or not the selected plants retain their resistance under field conditions could not be fully explored as yet (WENZEL 1985). BEHNKE (1980 b) also performed similar selection trials with culture filtrates of *Fusarium oxysporum.* 107 plants selected for leaf resistance maintained their resistance even when inoculated with a spore suspension of the Fungus.

REHBEIN (1983) treated callus pieces grown from cell clusters of dihaploids with culture filtrates of *Erwinia carotovora* var. *carotovora.* The calli decayed, but after some weeks, some cells from other calli, having withstood the toxin, started a new growth. The plants, regenerated after some selection cycles, were infected with the bacterium itself. Some had preserved their resistence. However tubers of only few of the plants reacted hypersensitively, while the controls decayed. It is certainly true, as WENZEL (1985) stated, that more knowledge about host-pathogen relationship is needed before observations at the cell level can be interpreted correctly and selection trials be planned and performed accordingly. A problem is the often observed incongruity between screening results of cells and tissues on the one side and those of intact plants on the other. Some of these discrepancies may be due to stress responses overcoming the wanted reactions of resistance genes (compare the case of *Phytophthora infestans,* HELGESON 1983).

5.2.2.3 Nature of somaclonal variation

The possible causes of the variations observed in protoplast-derived plants have been discussed extensively by several authors (LARKIN & SCOWCROFT 1981, SHEPARD 1981. SCOWCROFT & LARKIN 1983, EARLE & DEMARLY 1982, MEINS 1983, BERLYN 1983, LARKIN et al. 1984, EVANS 1984, COCKING 1985, KARP & BRIGHT (1985). SCOWCROFT & LARKIN (1982) classify the possible genetic events as epigenetic variations, karyotypic changes, factor mutations in the

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genome and plasmon, translocations, deletions, inversions, cryptic chromosome changes, gene rearrangements, and non-conventional mutations comprising gene amplifications and diminution, transposable elements, etc. Particularly non-conventional mutations at the molecular level are discussed by ORTON (1983).

Epigenetic changes do not survive the meiotic process. Their true nature is unknown. They can be valuable in a vegetatively propagated crop, such as the potato, although such a protoclone, of course, cannot serve as a breeding parent.

As known from other crops karyotypic changes, particularly aneuploidy, are the most widespread genetic events in tissue cultures. Chromosome counts of KARP et al. (1982) and JONES et al. (1983) revealed 96 % of cv. Maris Bard somaclones to be aneuploid and polyploid with 2n = 46–93. 30 % of the variants are correlated with aneuploidy, 20 % of the normal looking plants are also aneuploid, and even the rest show »somaclonal« variation.

Studies by SREE RAMULU et al. (1983, 1984 a, b) present the following picture of the cytological events in the calli of cv. Bintje. The variation begins with the first division of the protoplasts. The cell colonies and calli contain a mixture of cells with from 24 to 200 chromosomes. In further growth stages of the calli there is a trend to increasing numbers of cells with the tetraploid chromosome number and aneuploids around 48 chromosomes. Other cells are continuously sorted out. Regenerants of the same callus, being segregants of chimeras, deliver different phenotypes. 63.2 % of the regenerated plants are normal looking and euploid tetraploids. 20 % vary in few characters with the optimum chromosome numbers between 39 and 47. The rest of the somaclones show variation in several characters and have chromosome numbers about 40–96. The calli differ in giving rise to only normal or only variant plants or mixtures. See also CREISSEN & KARP (1985) and WHEELER et al. (1985). For practical purposes it seems of interest to note that the cvs. differ considerably in quality and quantity of somaclones they produce.

For an autotetraploid, such as the potato, a surplus of chromosomes need not necessarily imply detrimental consequences for the phenotype. 20 % of the normal looking Maris Bard protoclones were aneuploid (see above). Previous work in the Max-Planck-Institute of crosses of octoploid forms of *S. acaule* and *S. stoloniferum,* as well as of the hexaploid *S. demissum,* with ssp. *tuberosum* and back crosses has shown that useful hybrids can be selected with chromosome numbers up to at least 2n = 60.

Somaclonal variation among plants derived from dihaploid protoplasts is comparatively minor. Only 67 % of a group of inter-dihaploids responded to protoplast culture at all, and only few of these profusely (WENZEL et al. 1979). Nearly all regenerants had doubled their chromosome number. Of the Petri plate cultures only 4 % were aneuploid, being abnormal and sterile, of the suspension cultures, 10 %. A group of 30 rather uniform looking somaclones were screened by polyacrylamid gel-electrophoresis of the total proteins. The pherograms were considered as identical (WENZEL et al. 1984 b). Similarly BINDING et al. (1978) found less karyotic and morphological variability in protoplast cultures of dihaploids, but compare TIEMANN et al. (1984).

Factor mutations in the heterozygous potato are not as easy to detect as ploidy mutations and investigations with potato protoclones have not been done so far. But PRAT (1983) and LÖRZ & SCOWCROFT (1983) showed with somaclones of *Nicotiana silvestris* and *Nicotiana tabacum* respectively and SACRISTAN (1985) with *Phoma lingam* resistant/tolerant somaclones of rape that factor mutations and mitotic segregation can account for a large part of the somaclonal variation. Well known is the case of maize and *Helminthosporium maydis* race T (UMBECK & GENGENBACH 1983).

Here mutations in the chondriom are responsible for somaclonal variation. Resistant regenerates could be isolated with and without addition of the fungal toxin to the cell cultures. LARKIN et al. (1984) suggest transposable elements as responsible for wheat somaclonal variation. See also SIBI et al. (1984).

For the cv. Russet Burbank variants, SHEPARD (1982 c) draws attention to chromosome rearrangements as possible causative factors, and Gill, according to SREE RAMULU et al.

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(1983), found translocations and other chromosomal events. LANDSMANN & UHRIG (1985) observed gene deamplification in tetraploid potato somaclones and DE PAEPE et al. (1982/83) heritable quantitative and qualitative changes in the nuclear DNA of doubled-haploids obtained by pollen culture of *Nicotiana silvestris*. MEINS (1983) believes that in protoplast culture a »single primary destabilizing event« could have generated an outburst of diverse heritable changes. But LARKIN et al. (1984) and SANFORD et al. (1985) deny a principal difference between the variability of plants from protoplast and non-protoplast sources. ORTON (1984) presented the hypothesis that cells during mitotic reproduction have a finite mistake rate. These mistakes come to light, when the tissue-context is dissolved. This could explain at least the tremendous rise in variation observed in protoplast regenerates in contrast to regenerates from meristems, germs, buds etc.

Apart from investigations on the nature of somaclonal variation, it is the task of the variety breeder to find out whether the production of somaclones is a new recommendable method in potato breeding. For this purpose phenotypically normal somaclones in greater number must be submitted (over many years) to the ordinary method of breeding new varieties with all the usual screening and discarding (Section 4). The advantage compared with ordinary seedlings is the maintenance of the basic gene assortment of the mother cv. The same somaclone can be varied in several traits and in positive as well as negative direction. It is, therefore, not surprising that in PAVEK & CORSINI's (1982) preliminary trials no observed of 341 outyielded the controls. The percentage of such outyielders may be comparable with that of ordinary breeding populations, i. e. low. But it is to be expected that some somaclones after several years of screening, will end up with a positive balance in the changed properties.

5.2.3 Parasexual hybridisation

Comprehensive reports about techniques and results, elaborated in most cases with *Solanaceae* other than potato, are given by EVANS et al. (1983) and SCHIEDER (1982, 1986). Relatively few experiments have been performed with the potato.

Fusion experiments start with isolation of leaf-cells of sterile plants followed by digestion of the cell-wall as described in Section 5.2.2. The protoplasts are charged negatively which prevents their fusion. The charge is suspended in a medium with high p^H. Addition of calcium ions and also polyethylenglykol promotes the fusion. After first having formed a dikaryon, the nuclei fuse to a synkaryon. Many protoplasts die off and only about 3 % are obtained fused.

The initiation of the second phase, i. e. the organogenesis, is more difficult to achieve. The plantlets grown from embryoids represent a variety of different products: diploids, tetraploids and aneuploids. Only some of the tetraploids are true heterofusions. Other tetraploids originate either from homokaryotic fusions (homofusions) or they result from chromosome doubling that may have happened within the parent protoplasts. Aneuploids arise often either by incomplete transfer of the chromosomes or by elimination afterwards.

Several methods of recognition have been developed (SCHIEDER 1983). The common method, i. e. the use of recessive chlorophyll deficient mutants as one of the parents, is not possible when breeding goals are aimed at. The genotypes of both parents have to present top combinations of valuable genes. The cell content can be modified phenotypically: e. g. cell suspension cultures contribute cells which produce a dense plasma and pale plastids, while plantlets grown in vitro give protoplasts with green plastids. The visually recognized heterofusions can be picked up with a microcapillary pipette in combination with a micromanipulator (*Fig. 18;* HEIN et al. 1983). The heterofusions then have to be cultivated together with nurse protoplasts, i. e. chlorophyll or nitrate reductase deficient tobacco mutants, in order to establish a certain density of protoplasts in the culture medium necessary for regeneration. Single picked protoplasts grow also in microdroplets to callus (HEIN, pers. comm.). Heterofusions often show hybrid vigour. Confirmation of the hybrid

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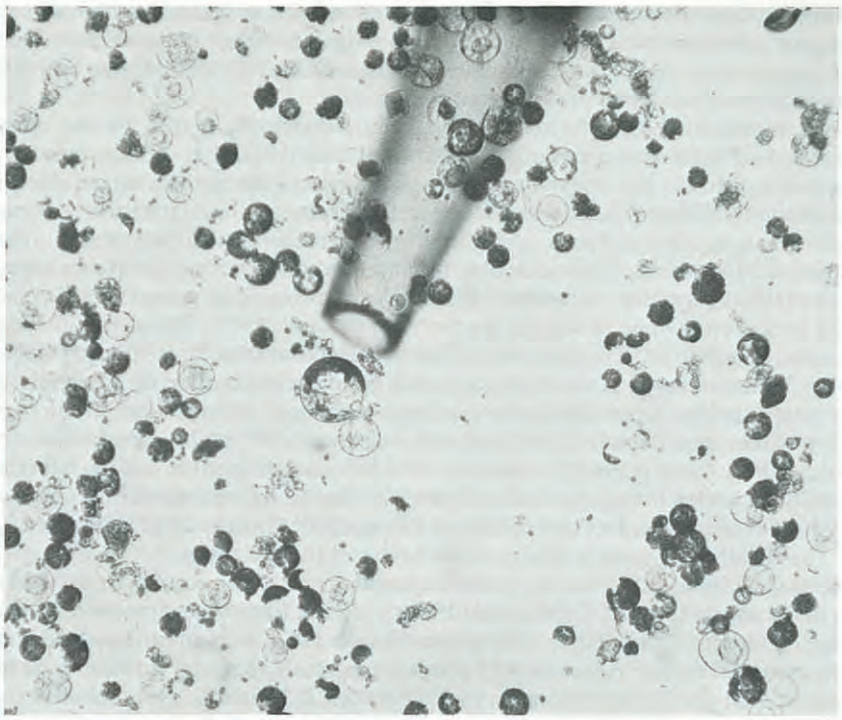


Fig. 18: Isolation of a fused protoplast by a micropipette combined with a micromanipulator. The dihaploid protoplast population consists of a light coloured genotype originating from a suspension culture and a dark coloured one from a greenhouse plant

Absaugen einer Heterofusion mit einer Mikropipette, kombiniert mit einem Mikromanipulator, aus einer Mischpopulation von zwei dihaploiden Genotypen. Der eine Genotyp ist hellgrün gefärbt und stammt aus einer Suspensionskultur, der andere, dunkelgrüne stammt von einer Gewächshauspflanze (by courtesy of T. HEIN)

nature is performed by enzyme electrophoresis, by DNA-hybridization, or by a technique involving plasmids (SAUL & POTRYKUS 1983).

In protoplast fusion, the DNAs of the nucleus (genome), the plastids (plastom) and the mitochondria (chondriome) are involved, each in a different manner. In the simplest case the nucleus of a fusion represents a summation of the parental chromosomes. In other cases chromosomes of one of the genomes, after some division, may be partly eliminated (SCHIEDER 1983). This asymmetric hybridisation (GUPTA et al. 1984) happens particularly when the partners are sexually incompatible and/or belong to distantly related species. The chromosome elimination can extend over several subsequent vegetative generations and leads to sorting out of different aneuploids. This process includes also the incorporation of fragments of a donor chromosome into a recipient genome. Irradiation of the donor genome favors the transfer of fragments. Fusion ability remains intact thereby (SCHIEDER et al. 1983).

GUPTA et al. (1984), in fusion experiments with *Physalis minima* and *Datura innoxia,* which are sexually incompatible and in normal fusion trials do not survive the callus stage, was able to transfer one single chromosome from irradiated *Physalis* protoplasts to recipient *Datura* protoplasts. Some fusion products regenerated into an adult plant and contained biochemical traits of *Physalis.* This remarkable investigation proved that it is possible to transfer single chromosomes from genus to genus! Sometimes, particularly in symmetric and

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asymmetric fusions between very distantly related species or genera, a hybrid callus is formed, but morphogenesis is blocked. SCHIEDER (pers. comm.) believes that in case of refused regeneration the transfer of mutated Ti-plasmid or T-DNA could be helpful since they may have a tendency to promote morphogenesis.

A very important role in fusion characteristics is evidently played by the cytoplasm (GALUN & AVIV 1983, LEAVER et al. 1985). It should be noted that somatic hybrids differ from sexual hybrids in that they contain the plasmons of both parents mixed. Reciprocal crosses show that differences between both parents' plasmons are relatively rare. However, they affect male sterility and some resistance traits. Already in 1975 POWER et al. observed that in fusion experiments a genome could be combined with an alien cytoplasm to produce a cybrid plant. In this way a »sterile« plasmon of the recipient parent (CMS) could be replaced by a »fertile« one in several species (GALUN et al. 1983). The plasmon comprises plastom and chondriome. The plastom, as shown by MELCHERS et al. (1978) and GRESSEL et al. (1984), in fusions rarely is a mixture of plastids of both parents often the entire plastom of the one parent is eliminated and the heterofusion contains a plastom either of the one or of the other parent (see below). Latest research has revealed that the chondriome behaves differently. It is now generally assumed that the chondriome in fusion hybrids is a combination of both parents' chondriomes and that chondriome-encoded traits, mostly male sterility, can segregate and recombine (EVANS et al. 1983, GRESSEL et al. 1984, FLICK et al. 1985). The details of mitochondrial genetics have still to be clarified.

Compared with other *Solanaceae,* fusion experiments with the potato are rare. MELCHERS et al. (1978) and MELCHERS (1980, 1982, 1984) were the first to generate somatic hybrids involving the potato. They combined a potato dihaploid with a chlorophyll deficient mutant of *S. lycopersicum* ssp. *cerasiforme.* All hybrids were grossly aberrant, formed no tubers and were sterile. The chromosome number varied between ≧ 50 and 72. Not one possessed the amphidiploid number 2n = 48, with one exception. Electrophoretic enzyme studies as well as restriction DNA-fragment patterns (SCHILLER et al. 1982) revealed that some hybrids contained potato plastids (pomatoes) and others tomato plastids (topatoes). SHEPARD et al. (1983) combined chlorophyll-deficient protoplasts of a variegating protoclone of cv. Russet Burbank (2n = 48) with the tomato (2n = 24). Abcisic acid was added to the medium in order to suppress regeneration of the pure potato calli. Similarly, divisions of the tomato protoplasts were suppressed at a temperature of 24° and under light. The chromosome number of the four plants obtained was always 2n = 72. The growth habit was grossly disturbed and potato-like. Later on, in shoots from cuttings, numbers of 2n = 60–72 were observed with a tendency to lose tomato chromosomes. These cuttings flowered profusely but produced no viable pollen. Extranuclear DNA elements turned out to be only those of the potato, as could be concluded from the restriction DNA fragment pattern. A second somatic generation was raised from protoplasts of the cuttings. Plants with more similarity either to the potato or to the tomato turned out to have changed chromosome numbers.

BINDING et al. (1982) were able to fuse dihaploid potato genotypes with *S. nigrum* (2n = 72) which is resistant to the herbicide atrazin. This resistance is maternally inherited. Both species are sexually incompatible. Nine clones showed several traits of both parent species, among them resistance to atrazin. Their chromosome number was 2n = 90 and more. It could be demonstrated by DNA restriction analysis (GRESSEL et al. 1984) that the atrazin-sensitive plants contained potato plastom and all atrazin-resistant plants the *S. nigrum* plastom. The dihaploid plants derived from unfused protoplasts gave rise to many morphological variants in contrast to those created partly from the same dihaploid genotypes but cultured in slightly different media (Section 5.2.2.3).

BUTENKO & KUCHKO (1980) mixed protoplasts of the cv. Priekulski Rannii with the wild species *S. chacoense* (2n = 24) in a selective medium in which only heterofusions could grow. They obtained 0.5–1.0 % heterofusions. Two of these plantlets were free from chlorophyll. One other grew into a normal plant with 2n = about 60 (instead of 2n = 72), nearly indistinguishable from a sexual hybrid, but it showed a distinct heterosis. The somatic

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hybrids had electrophoretic peroxidase patterns different from those of the sexual hybrid. This can be taken as an argument for the involvement of the plasmon which in case of the sexual hybrid originated only from one parent.

Fusion of an albino mutant of cv. Russet Burbank and the non-tuber bearing species *S. brevidens* (Section 2.3) resulted in vigorous green plants with morphological traits intermediate between both parents (BARSBY et al. 1984). Isoelectric focusing of polypeptides in polyacrylamide gel confirmed the hybrid nature, but only the *S. brevidens* chloroplast genome was present. Chromosome number varied between 72 and 96. Pollen did not function and only parthenocarpic fruits were produced.

AUSTIN et al. (1985) succeeded in raising normal looking fusion plants of PLRV-resistant *S. brevidens* and *S. phureja/S. stenotomum*. The chromosome numbers were at or near 48 (Section 7.2.1). (See also FOULGER et al. 1986).

Another important objective was pursued by WENZEL et al. (1979, 1982 a, b, c) with the analytic-synthetic breeding scheme (*Fig. 14;* Section 5.1.4). Somatic hybridization of selected inter-dihaploids would provide unique opportunities for maximizing heterozygosity in the resulting tetraploids. The more distantly the dihaploid genotypes are related, the greater is the chance of the production of heteroallelic and possibly heterotic tetraploids as fusion products. The first 4x hybrid via 2x protoplast fusion has been produced by AUSTIN, BAER & HELGESON 1985). For electrofusion of 1x and 2x potatoes see DE VRIES et al. (1986) and PUITE & ROEST (1986).

Not only heterofusions would be of interest. Most of the regenerants resulting from these fusion experiments are homofusions, e. g. doubled dihaploids of both parents of the constitution $a_i a_i a_j a_j$ and $a_k a_k a_l a_l$, i. e. diallelic tetraploids. These diallelic tetraploid genotypes, when crossed, produce 44 % tetraallelic progeny $(a_i a_j a_k a_l)$ to which heterotic potency can be attributed.

What perspectives for further potato breeding can be inferred from the results obtained until now? In early theoretical considerations of fusion, its applicability to the potato seemed to be restricted to intraspecific fusions of dihaploids in order to reach the tetraploid level, and these investigations certainly need further study. Moreover, intraspecific hybrids at the tetraploid level by which a »sterile« plasmon can be replaced by a »fertile« one, would, without doubt, be a great help in potato breeding where plasmatic male sterility is often a serious problem. The technique is the more ideal in that it leaves the nuclear genotype unaltered by the meiosis which otherwise follows and rearranges the chromosomes. The reciprocal replacement would facilitate the creation of male sterile parents in TPS-work (Section 6).

Thirdly, potato breeding could make use of the augmented variability the fusion hybrids show as compared with sexual hybrids, by recombination and segregation of the organelle DNA, particularly the chondriome (FLICK et al. 1985).

Asymmetric nuclear fusion of cvs. with closely as well as with distantly related species has a special aspect in breeding for resistance. The host range of every parasite has a natural limit. Species beyond this border are non-hosts. Whilst the hosts of a pathogen are mostly sexually compatible with each other, many non-host species are sexually incompatible with the former group or at least with the central species of this group. The causative factors of being non-host are many and only insufficiently known. Some non-host species react hypersensitively (e. g. bean with *Phytophthora infestans*). But there are other groups of non-hosts which do not admit any multiplication at all (e. g. tomato with PVS and *Datura metel* with PLRV!). Such types of non-host resistance are entirely beyond the scope of conventional breeding because of the sexual incompatibility. Is there not a possibility of transferring non-host resistance to cvs. by means of asymmetric nuclear fusion in order to form somatic addition hybrids? It should be mentioned in this connection that the addition of single alien chromosomes or chromosome fragments to a polyploid genome of potato or wheat need not impair the agronomic value. Examples worthy of mention are the many wheat cvs. where chromosome fragments of *Agropyrum elongatum* or *Secale cereale* have

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Tab. 10: Possibilities of application of parasexual hybridisation in potato breeding
Nutzungsmöglichkeiten der parasexuellen Hybridisierung in der Kartoffelzüchtung

technique *Technik*	crossability *Kreuzbarkeit*	ploidy level *Ploidiestufe*	purpose *Ziel*
symmetrical *symmetrisch*	compatible *kompatibel*	2x + 2x	transfer of two upgraded dihaploid or doubled monohaploid genotypes to the tetraploid level *Transfer von zwei selektierten interdihaploiden oder verdoppelten monohaploiden Genotypen auf das tetraploide Niveau*
asymmetrical *asymmetrisch*	compatible *kompatibel*	cv. or breeding line (4x) + wild species (2x or 4x) *Sorte oder Zuchtklon (4x) + Wildart (2x oder 4x)*	to reduce the number of back cross generations necessary for sorting out of unwanted wild species genes *Um die Zahl der zum Austausch der Wildartgene notwendigen Rückkreuzungen zu reduzieren*
	incompatible *inkompatibel*	as above *wie oben*	to make use of the stronger resistance of non-hosts *Um die stärkere Resistenz von Nichtwirten zu nutzen*
cybridisation *Cybridisierung*	compatible or incompatible *kompatibel oder inkompatibel*	CMS-cv. or breeding line + ♂ fertile genotype *CMS-Sorte oder Zuchtklon + ♂ fertiler Genotyp*	replacement of a CMS mediating cytoplasm by a fertile one *Ersatz eines CMS-Plasmons durch ein fertiles Plasmon*

been built into wheat chromosomes (translocation hybrids) via first formed sexual addition- or substitution-hybrids. *Table 10* summarises the possible applications of parasexual hybridisation in potato breeding.

5.2.4 Transfer of genes by means of plasmids and direct

Plasmids can be used as vehicles for the transport of foreign genes from a donor genotype to an acceptant one (transformation; SCHELL et al. 1984, HOOYKAAS & SCHILPEROORT 1984). Many bacterias contain plasmids that are mobile, circular, extranuclear DNA. Most of the dicotyledonous species, and in a certain way monocotyledonous too, are hosts of *Agrobacterium tumefaciens* and its tumour inducing Ti-plasmid. The T-DNA region of the Ti-plasmid is transferred to the host plant. It encodes the enzyme lycopindehydrogenase (LpDH) which induces the formation of deviating aminoacids as opines etc., responsible for tumor formation (crown gall). Most important, the T-DNA can accept foreign DNA-sequences and transfer them into the host plant. Of even great importance was the detection that the T-DNA region, after mutative deletion of some genes induced not further undifferentiated tumour callus but sprouts *(Fig. 19)* which can grow into flowering sprouts but contained LpDH and opines. This could be shown with tetraploid potato (HH 258) and with tobacco (OTTEN et al. 1981). The LpDH formation segregates as a dominant Mendelian trait. The T-DNA therefore is integrated in the nuclear DNA. This could be proved with transformed potato and tobacco plants in crosses with non transformants. The F'_2 with another dihaploid potato and with *S. phureja* as well segregated according to Mendelian rules (WOESTEMEYER 1982, WOESTEMEYER et al. 1983, OTTEN, pers. comm.). – Also, protoplasts of potato and tobacco can be infected in co-culture with mutated *A. t.* yielding transformed shoots.

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Fig. 19: Tobacco stem infected with a mutant T-DNA of the Ti-plasmid of *Agrobacterium tumefaciens* producing a variety of normal shoots instead of a crown gall. Foreign DNA will be incorporated into the nuclear DNA of the host and encode for changed traits

Tabakstengel, der mit einer mutierten T-DNA aus dem Ti-Plasmid von Agrobacterium tumefaciens infiziert wurde. Statt der Wurzelhalsgalle entstehen an der Infektionsstelle normale Sprosse. Fremde DNA wird in die Kern-DNA inkorporiert und codiert Eigenschaftsänderungen (by courtesy of J. SCHELL)

Transfer and expression of a gene from one genome to a foreign one is possible only by constructed chimaeric genes where the T-DNA and the sequences to be transformed are joined to sequences of the plasmid promoter which initiates the expression (HERRERA-ESTRELLA et al. 1983). By this method the integration of a gene for resistance against the bacterial aminoglycosid kanamycin from bacteria in the genome of tobacco could be performed (BEVAN et al. 1983). DE BLOCK et al. (1984) observed in crosses with the resulting intact plants a meiotic segregation of the kanamycin resistance in tobacco crosses. Kanamycin is a herbicide that kills protoplasts, calli and plants. Slicing a bacterial DNA-region that encodes resistance to kanamycin into the chimeric gene allows the selection of the relatively few transformed protoplasts and calli already in the Petri disc after kanamycin has been added to the culture.

The first transfer of a gene from one angiosperm species to another by means of a plasmid was accomplished by MURAI et al. (1983). The authors transferred the gene for the formation of phaseolin, a storage protein from bean, to sunflower. Rootless and weak plantlets with traces of phaseolin developed. Similar experiments were successful with the gene Z4 for zein synthesis from maize and its transfer to sunflower. Only callus developed from protoplasts with traces of zein in this case (MATZKE et al. 1984). Moreover it is possible to transfer foreign genes direct into protoplasts as was shown with tobacco (PASZKOWSKI et al. 1984 and HAIN et al. 1985) and *Triticum monococcum* (LÖRZ et al. 1985) by incubation with naked chimaeric DNA. While transformed tobacco protoplasts did grow into normal plants, in the case of Triticum only transformed cells could be gained at present.

For practical purposes in potato breeding, the transfer of major genes, i. e. genes of high phenotypic expressivity, is a priority. Many valuable properties are each controlled by one single dominant major gene, such as the genes *Rx, Ry, Nl, NS, Fb,* H_1 etc. for the type of extreme resistance or localized hypersensitivity to the main virus diseases and of resistance to the golden eelworm (Section 7.2 and 7.3). Many of these genes, most originating from

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wild species, have already been incorporated in varieties in the course of up to seven backcrosses. That took 20–30 years of classical combination and selection breeding. Nevertheless, the outsourting of undesired wild genes and the combination of the resistance genes with so many indispensable genes for quality, yield etc. was only occasionally satisfactory. The direct transfer of genes for resistance into »top«-varieties, therefore, would be of utmost importance. It should also be mentioned that NITZSCHE (1983) proposed the transfer of genes for chitinase and WILLMITZER (1986) for lysozym formation in order to prevent infections with *Erwinia* and other fungi. Attention should be paid as well to the beetle killing beta-endotoxin of *Bacillus thuringienses* (CANTWELL & CANTELO 1984). The search for a bacterial toxin killing aphids and its transfer would be even if not more important. A recent transfer project, particularly important for the potato, deals with immunisation against specific viruses (BEVAN et al. 1985). Virus inoculations fail, if a related virus has alread occupied a plant (cross protection). This is due to the presence of the protein coat of the virus which by itself does not cause a disease. It is aimed to transfer the responsible viral RNA-gene as a complementary (c) DNA to the host genome.

Plasmids, moreover, can initiate regeneration in otherwise not responding species (STEFFEN et al. 1986) and can help to identify asymmetric hybrids by markers (SCHIEDER 1986).

Perhaps promising for gene-transfer seems to be the electroporation. Here by, genes are brought into protoplasts by electric forces (LANGRIDGE et al. 1986, SHILLITO 1986).

Microinjection of DNA into protoplasts by a special built apparature was successfully performed by MORIKAWA & YAMADA (1985).

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6 True potato seed (TPS)

Sowing true seed instead of planting tubers is advantageous in many respects. Above all, viruses would disappear from the list of the most harmful parasites, because they are – with few exceptions (JONES 1982 b) – not transmissible by seed. A seed potato crop would make the application of the whole expensive technology of tuber seed production, virus testing and supervision superfluous. Moreover, no costs would arise for the storing and transport of the tubers, and fields for seed potato production would be used for growing ware potatoes.

The auspices for TPS are different for developed and developing countries. The introduction of TPS in developed countries is crucial because of the incomplete homogeneity of the tubers. The parents available at present yield progenies segregating in many tuber traits. Another handicap in developed countries is the lower yield compared with that of certified tuber seed. The third handicap is the high costs for raising the seedlings in heated greenhouses and their transplanting in the field. However, the use of pillated seed may reduce these costs a little. All these factors are of no or only minor importance in developing countries. The non-homogeneity of the harvest does not matter so much, since many farmers in the Andes and elsewhere plant their fields with a mixture of varieties. Moreover, some crosses deliver a less inhomogeneous crop.

Potato cultivation from tubers requires seed tubers free or nearly free from viruses. The transfer of the necessary technology to developing countries often fails due to the sociological and economic realities. The import of seed tubers from overseas is very costly.

The costs of imported seed potatoes are estimated at 40–70 % of the whole production input (SADIK 1983). ACCATINO & MALAGAMBA (1983) stressed that the high price of seed potatoes is the main obstacle for the expansion of the potato in developing countries.

There are various ways of bringing out TPS to the field: 1. direct sowing in the field (MALAGAMBA et al. 1984). The yield, however, is often lower compared with the other procedures (MARTIN 1983); 2. sowing in nursery beds and planting the seedlings (transplants) in the field; 3. planting tuber harvest from nursery beds in the field (WIERSEMA 1985); 4. selection among first year field seedlings and planting the selected clones next year (LI 1983).

Also favorable for TPS in developing countries is the fact that the technology for transplanting the seedlings from nursery beds to the field has been in use in Asian countries, for example, in rice cultivation. It seems not difficult to apply this technique to the potato.

THOMPSON & MENDOZA (1984) studied variance, heritability and correlation coefficients of several traits in TPS populations of ssp. *andigena* under short day conditions.

In order to grow 1 ha with 40,000 plants, five seedlings per hill are necessary with the expectation that three will survive. That means 200,000 seeds or 110 g. For their production 80–100 profusely flowering, very fertile parent plants are required which must be raised to their optimal growth. Profusely flowering parents are required with ample fertile pollen, high seed set and berries containing as many well sized seeds as possible. Also, the type of the embryo seems to be important for the performance of the crop. Seeds must germinate simultaneously and deliver vigorous and uniformly growing seedlings (ACCATINO &

MALAGAMBA 1982, 1983, UPADHYA 1983). Tuber yield was highly correlated with 1000-TPS weight in trials of DAYAL et al. (1984).

The ultimate aim of TPS-breeding is the production of a complete homogeneous progeny. This aim can be attained only step by step. At present endeavours are directed at the study of the potency of varieties available to produce increased high yielding and as far as possible uniform tuber progeny. It has been demonstrated that selfed and open pollinated seed gives a lower yield. Comparisons of hand pollinated progenies of different combinations of varieties and breeding lines, conducted in several countries, showed considerable differences in homogeneity (grading 1–4) and yield. The yield generally is lower but some combinations give yields close to that of varieties. It is expected that the yield will be enhanced in the future when more experience has been gained (ACCATINO 1982, UPADHYA 1983). A TPS cv. Waitangyi is sold in New Zealand (Anon. 1981 b) and said to be accepted there in ordinary dishes by consumers in the towns. Moreover, another seed from a US cv. is offered under the name Explorer. Its yield, however, is far below that of ordinary cvs. (VEILLEUX & RELF 1983).

An interesting way to reduce the non-homogeneity after harvest is practised in China (LI 1983). The harvested tubers are selected with respect to diseases and uniformity and used as tuber-seed for the next vegetation period. The second harvest then is used for consumption. The first generation of tubers can be raised in nursery beds as well (WIERSEMA 1985).

As mentioned above, only seed of exclusively hybrid origin guarantees satisfactory yield. This means that pollinations usually must be performed by hand which is very costly and laborious. An alternative could be the use of self-incompatible pollen parent genotypes grown along side or mixed with the seed parent. Whilst, as PELOQUIN (1983) mentions, bumblebees do not visit and fertilize the usual self-sterile plants, this is not true for *S. stoloniferum* based breeding lines and cvs. possessing like cv. Pirola a plasmatically, i. e. by mitochondrias inherited tetrad sterility (BROWN 1984 and pers. comm.). It should be mentioned here that it may be possible to confer pollen sterility to a cv. or a clone by somatic transfer with responsable mitochondrial plasmids (SYBENGA 1983).

Resistance traits also are indispensable for a TPS crop and its parents (SADIK 1983). Although viruses generally loose their importance when the potato is propagated by true seed, there remains the danger from the seed-transmissible viroid PSTV. TPS, moreover, must possess resistance to many seedlings' diseases, a problem not so much encountered when the crop is grown from tubers. Many other diseases retain their importance, such as *Rhizoctonia* (damping off), *Phytophthora infestans,* nematodes and larvae and insects feeding on leaves and tubers. On the other hand *Fusarium, Corynebacterium, Erwinia* a. o., being predominantly tuber borne, seem to be of less danger.

As outlined already, the perfect TPS has to deliver a completely uniform tuber progeny. This is only to be achieved from crossings of homozygote parents. However, preliminary trials have shown that homozygosity minimizes vigour and vitality in the potato to such an extent that it seems impossible to proceed with true inbred lines.

The versatile potato, however, offers other possibilities for a rather uniform seed progeny. Partially homozygote parents with a corresponding reduction of variation among the progeny are diallelic tetraploids, such as $a_i a_i a_j a_j$ that should be combined with $a_k a_k a_l a_l$ in order to initiate heterosis. These tetraploid parents are obtained from dihaploids through spontaneous doubling of the chromosomes or through treatment with colchicine.

The most promising method is the production of a tetraploid progeny from ploidy crosses (Section 5.1.3; PELOQUIN 1983, MARTIN 1983, KIDANE-MARIAM et al. 1985). Preliminary trials with presently available inter-dihaploid and tetraploid parents have demonstrated a high uniformity and about twice the tuber yield of the progeny from open pollinated seed. A drawback still is the relatively poor seed set which can eventually be increased by the breeding of suitable parents.

For the future, the production of uniform seed by way of (autonomic) apomixis, i. e. without pollination, is envisaged. The possibilities are extensively discussed by HERMSEN

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(1980, 1983) and IWANAGA (1983). The seed progeny then would be like a vegetatively propagated one. At the moment, any proof for the existence of apomixis among *Solanaceae* is lacking. There are three possible ways for this very important trait to be introduced into the potato: 1. application of mutagens; 2. artificial induction of apomictic development of the unfertilized and unreduced egg cell by chemical compounds; 3. molecular transfer of responsible genes from taxonomic groups other than the *Solanaceae*.

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7 Breeding for resistance

7.1 General remarks

Resistance means a relationship between the host and the parasite where the host is striving to keep the damage as low as possible or finally to exclude the parasite completely. The host has developed many different ways for this purpose.

The yield loss caused by diseases and pests in the potato is estimated at 22 % per year. Propagation by tubers favors the transfer of parasites and germs from one vegetative generation to the next. For excellent pictures of the main diseases see HOOKER (1981). Viruses are the most dangerous of all parasites as they spread through the whole plant with the exception of the apex meristems. A vegetative progeny remains infected for ever. The high number of different parasites makes it necessary to set priorities. They are ranged according to incidence, damage, possibility of chemical protection, transmission to the next generation, importance for economic management and expenses of a breeding programme to introduce the respective resistance.

The group of highest priority comprises in Europe and North America: golden and white cyst nematode, leafroll virus (PLRV), virus Y (PVY), PSTV, late blight, *Fusarium* dry rot (in Great Britain *Phoma* dry rot), black leg and virus M (PVM). Of highest priority in the subtropics and tropics are leafroll virus, Virus Y, late blight, *Bacterium solanacearum* as well as leaf and tuber damaging insects.

Breeding for resistance to parasites requires at least one resistant parent in case of the resistance being monomer or oligomer inherited. But both parents must carry a certain degree of resistance when a polygenic field resistance prevails. Infection trials are performed among the progeny as early as possible. In the case of field resistance, which in most cases is a less pronounced resistance, field trials over several years are needed for assessment, whereby a negative selection is perfórmed. Even then, the resistance score of both parents must not be below average or the progeny is likely to become susceptible very suddenly. In this way, an increase in the resistance can be expected in consecutive generations (transgression). The rest of the diseases are managed by the negative selection method as well.

Susceptible lines are discarded in the course of the usual 5 to 7 years of propagation and observation in the field. A variety is in the last analysis a compromise between yield, quality and a certain level of resistance to the most important diseases. One distinguishes between two main types of resistance: infection or field resistance (horizontal resistance) on the one hand, and hypersensitivity (vertical resistance) including extreme resistance, on the other (RUSSELL 1978). Types of the first category are controlled nearly exclusively by minor genes (less expressive genes), those of the second, by major genes (highly expressive genes). The infection resistance generally acts independently of the different pathotypes of the respective parasite (race unspecific), but its effects in most diseases are relatively low.

Field resistance is a complex composed of obstacles to attack, adherence, entrance, growth and propagation (multiplication) of the parasite. Breeding, when trying to improve field resistance, aims to accumulate minor genes. They are then often treated as quantitative units. An enhancement of resistance is commonly expected from »blindly« intercrossing

parents with an above-average resistance. This concept is eroded by the results of modern investigations. There is not just one morphological trait or one biochemical pathway leading to field resistance, but several qualitatively different ones. Minor gene effects do not push forward just one single reaction or pathway in small portions, but act on qualitatively different ones. Consequently, in order to intensify field resistance, those resistant parents have to be recognized and combined whose resistance-directed actions are complementary. The same must be considered when minor genes are required to intensify the effect of major gene conditioned resistance. A precondition, of course, is knowledge of the resistance components which has hitherto been insufficient.

Major gene inherited hypersensitivity, including extreme resistance, is characterized by a necrotic reaction. The more intensive the reaction, the more localized remains the infect. In certain potato diseases it is possible to distinguish genetically and by phenotypes between a systemic hypersensitivity, where the plants often die off after field infection, localized hypersensitivity, which limits the infect to the point of entry and the surrounding cells, and extreme resistance with no visible reaction after field infection.

Tab. 11: Hypersensitive types of resistance acting against important potato viruses and their inheritance
Typen der Überempfindlichkeitsresistenz bei wichtigen Kartoffelviren und ihre Vererbung

virus [1]	type of resistance *Resistenztyp*	[2]	breeding progressed up to *Züchtung fortgeschritten bis zu*
PLRV +++	intolerance *Intoleranz*	*Nl*	cultivars *Sorten*
PVY +++	localized hypersensitivity *lokalisierte Überempfindlichkeit*	*Ny*	breeding lines with genes ex *S. phureja* and wild species *Zuchtklone mit Genen ex S. phureja und Wildarten*
	extreme resistance *extreme Resistenz*	Ry_{sto}	cvs. ex *S. stoloniferum* *Sorten ex S. stoloniferum*
		Ry_{adg}	breeding lines ex ssp. *andigena* *Zuchtklone ex ssp. andigena*
PVX ++	localized hypersensitivity *lokalisierte Überempfindlichkeit*	*Nx, Nb*	cultivars *Sorten*
	extreme resistance *extreme Resistenz*	Rx_{acl}	cultivars ex *S. acaule* *Sorten ex S. acaule*
		Rx	cultivars ex »Villaroela« *Sorten ex »Villaroela«*
		Rx_{adg}	cultivars ex ssp. *andigena* CPC 1673 *Sorten ex ssp. andigena CPC 1673*
		Rx_{scr}	breeding lines ex *S. sucrense* *Zuchtklone ex S. sucrense*
PVS +	localized hypersensitivity *lokalisierte Überempfindlichkeit*	*Ns*	cv. Szignal and breeding ex ssp. *andigena* *Sorte Szignal und Zuchtklone ex ssp. andigena*
PVM +	localized hypersensitivity *lokalisierte Überempfindlichkeit*	*Gm*	breeding lines ex *S. gourlayi* *Zuchtklone ex S. gourlayi*
PVA +	localized hypersensitivity *lokalisierte Überempfindlichkeit*	*Na*	cultivars *Sorten*
	extreme resistance *extreme Resistenz*	Ry_{sto}	cultivars ex *S. stoloniferum* *Sorten ex S. stoloniferum*
TRV +	intolerance *Intoleranz*	*Nt*	cultivars? *Sorten?*

[1] present economic importance
Gegenwärtige wirtschaftliche Bedeutung

[2] major gene or genes
Hauptgen oder -gene

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It is often assumed, that major genes are generally of less importance since they are all race specific and could be overcome by new pathotypes of the parasite in a short time. An example is offered by the late blight fungus *Phytophthora infestans* where a gene-for-gene system of several major host genes for breeding corresponds to recessive fungal genes for virulence. But in fact many major genes for necrogenic reactions in the potato and its gene resources exist, which are practically non-specific and durable. They control hypersensitive reactions to the viruses PLRV *(Nl)*, PVY *(Ry)*, PVX (*Rx*, with certain reservation, see Section 6.1.3), PVA *(Na)*, PVS *(Ns)*, PVM *(Gm)*, and to the golden eelworm *(H_1, Fb) (Tab. 11)*.

It is of additional advantage that some of the major genes for hypersensitivity are very highly expressive ones *(Ry, Rx, Na, Ns)* and act sufficiently without co-operating minor genes, while other less expressive, ones (e. g. *Nl* and *Fb*) confer a satisfactory repulse only in combination with minor genes. Another advantage of major gene inherited resistance is – as a prospect for the future – its accessibility to gene manipulation, e. g. to gene transfer by means of plasmids or segment transfer in the case of somatic hybrids.

Tolerance does not rank among types of resistance in the narrow sense in the potato. It denominates an equilibrium in the symbiotic pathosystem where the host shows no or only weak symptoms and injuries despite complete or nearly complete infection. Tolerant varieties soon become infected and threaten the non-tolerant ones in the environment. However, tolerance to virus diseases can be an interim solution in developing countries, and tolerance to eelworm infection is a desirable additional quality to resistance.

7.2 Viruses

A survey of the main properties of the potato viruses, the transmitting aphids and their importance and combat is given by Beemster & Rozendaal (1972), Hille Ris Lambers (1972), Weidemann (1979) and Bode (1983). Test methods for the presence of viruses are described in Section 10.

Viruses, because of their tuber-perpetuation and the heavy yield losses they cause, are the most dangerous parasites of the potato. In the Rhineland of the Federal Republic of Germany the first harvest from uncertified seed yielded 77 %, and the second only 44 % compared with the harvest from a potato field grown from certified seed (Bode 1983, Hunnius 1977 a).

Natural infection, specific for each virus, happens either exclusively through wound contact (wind, man, animal, machine) in the case of PVX (here, too, by soil fungi), or exclusively by aphids as vectors in the case of PLRV or through both (PVY, PVA, PVM, PVS), or through wound contact and nematodes (TRV).

Infection methods in experiments are: inoculation with virus containing plant sap, grafting of shoots *(Fig. 20)* and tubers, or infection with aphids (de Bokx 1972 b, Ross 1978). After the inoculation the virus particles are transported via the conductive system and plasmodesmata into the cells of all organs. They replicate in the cells and the infection becomes systemic. The ripe seed and the apical meristems remain free of the virus. By axenic cultivation of the tips, totally infected cvs. can be freed from viruses (Section 10). The symptoms are typical for each distinct virus, but vary with the host cv. and its age. Common to the mosaic viruses PVA, PVM, PVS, PVY and TRV is a ± clearly expressed light-dark green mosaic.

Every virus exists in nature in different genetic variants or strains. They together form the population of a certain region. The strains are distinguished by their symptoms, physical and chemical characteristics, their concentration in the hosts and by their serological reactions. The composition of a virus population changes when new successfully competing strains enter the region from abroad, when the spectrum of cvs. changes through environmental influences (replacement of cvs.) and when competing mutants arise. Strains producing only weak symptoms in widely spread cvs. are very dangerous, since diseased plants of these

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cultivars can be recognized and eradicated only with difficulty. It is difficult even to produce virus-free healthy seed from such cvs. (Section 7.2.2).

After invasion, virus genes activate resistance genes in the host and initiate different types of resistance reactions. The »resistance to infection« or »field resistance« type as a general defence reaction occurs with each virus and is to be found in every cv. but in different degrees. BARKER (1984) observed field resistant cvs. to contain less virus than susceptible ones. They receive less inoculations when settled with infectious aphids.

The effect of field resistance is minor compared with the necrogenic types of resistance. A satisfactory protection is rendered only when infection pressure is relatively low (Ross 1983). The degree of infection resistance is assessed by counting the number of plants infected throughout one season. Potato viruses, such as PVX (FOXE & PRAKASH 1983), PVY and PLRV (BARKER & HARRISON 1982, 1984), infect protoplasts. Virus multiplication proved to be related to cvs.' resistance. It may be possible, therefore, to perform selection for infection resistance at the cell level. Quantitative differences in the number of infected protoplasts determined by ELISA and fluorescent antibodies could reflect differences in infection resistance. This would save years of hitherto necessary field tests.

The inheritance of the infection resistance by minor genes requires that both parents possess at least an intermediate level of resistance, if seedlings of the same or higher degree of resistance should show up in the progeny. A parent with pronouncedly low resistance considerably lowers the level in the progeny.

By contrast, the necrogenic types of resistance, such as localized hypersensitivity and extreme resistance, confer an absolute protection. No sign of infection is to be seen in field



Fig. 20: Potato genotypes grafted onto PVY-infected tomatoes: l. Ry-genotype, reacting with extreme resistance; r. type reacting with systemic hypersensitivity

Kartoffelgenotypen, gepfropft auf PVY-infizierten Tomaten: l. Ry-Genotype, mit extremer Resistenz reagierend; r. Genotyp, der mit systemischer Überempfindlichkeit reagiert

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plants. Extreme resistance is an intensified localized hypersensitivity. Both can be distinguished by grafting onto a virus-infected stock *(Fig. 20)*. The first type shows a necrotization of the top of the scion (genes *Na, Ns, Ny* and *Nl*). The gene *Nx* as well is expressed in protoplasts also where is causes an impressive reduction in virus multiplication compared with nx – cvs. In the case of extreme resistance either no symptoms appear (genes *Rx, Ry*) or needle point necroses occur on the upper leaves, and in older grafts necroses at the leaf margins on the lower leaves become visible (gene *Ry*). The main aim shoot often stops growing, while the side buds grow out to become symptom free shoots. Back tests to test plants as well as serological tests are negative in both types with very few exceptions, perhaps due to an initial short phase of replication of the virus, or due to virus particles transported passively to the scion (DELHEY 1974, 1975). Antiviral protein compounds such as the AVF (SELA 1980) or the IVR (LOEBENSTEIN & STEIN 1985) are involved in the reactions. They act nonspecifically.

The elucidation of the resistance pathway is important for the identification of the relevant viral gene which then could possibly then be transferred to a cv. genome (Section 5.2.4).

The third of the necrogenic types of resistance, i. e. the systemic hypersensitivity or intolerance, is characterized by necrotization of large parts of the plants when infected in the field. Often the whole plant together with the tubers or their eyes dies off. This type in combination with infection resistance is important in the case of PLRV and the *Nl*-gene.

The major genes for necrogenic reactions occur mostly in simplex condition in cvs. and breeding lines, giving a progeny of about 50 % seedlings of the same complete resistance when crossed with another parent, be it a resistant or susceptible one, in contrast to the minor gene-inherited field resistance. Selection among seedlings is performed in a simple way by means of a spray gun (Section 7.2.2).

Most of the major genes are race non-specific in a broad sense (COCKERHAM 1970, ROSS 1983). No breakdown of the resistance conditioned by major genes was observed. This characteristic is named very appropriately »frozen vertical resistance« by ROBINSON (1976). The rare occurrence of resistance-breaking mutants in virus pathosystems could be due to the relatively few information units a virus genome harbors. It seems to be more difficult for a virus than for a fungus to overcome host resistance. Occasionally, strains of PVX and PVY have been found breaking major gene controlled resistance in greenhouse experiments. However, they seem to occur only seldom.

A defense against aphid transmitted viruses may also be possible by means of a dense pubescence with glandular hairs carried by some wild species (MEHLENBACHER & PLAISTED 1983). Both four-lobed (type A) and simple (type B) trichomes trap the aphids physically and immobilize them. Type B trichomes, moreover, secrete an alarm pheromone (farnesene derivative) that affects aphid behaviour (GIBSON et al. 1984). The inheritance of the hairs' density and droplet size was followed in crossings with *S.* × *berthaultii* up to F_3 and found to be a quantitative one in each case. *S.* × *berthaultii* plants had 87 *Myzus persicae* per 10 leaves, three cvs. had between 335 and 431, and 32 *S.* × *berthaultii* × *S. tuberosum* F_3-plants between 47 and 366 aphids (RYAN et al. 1983). However, virus resistance tests in the field with one accession of *S.* × *berthaultii* carrying type A- and B-trichomes, and another with only A, showed that both became totally infected with PVY. There were however, only 22 % PLRV-infections with A- and B- *S.* × *berthaultii* and 84 % with A- *S.* × *berthaultii* (RIZVI & RAMAN 1983). It is also intended to breed for natural antibiotics which influence the development of the larvae of *Myzus persicae* and occur in wild species and cvs. in different concentrations (BINTCLIFFE & WRATTEN 1982).

7.2.1 Leafroll virus (PLRV)

The incorporation of a type of resistance to PLRV that excludes the virus from the potato crop is one of the greatest challenges in potato breeding. Since, as will be outlined below,

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»excluding« resistance types (localized hypersensitivity and extreme resistance) against PVA, PVM, PVS and PVY have already been conveyed to cultivars, »excluding« resistance to PLRV is the only one lacking. A cultivar that excluded PLRV in addition to PVY would make the application of aphidicides superfluous and would enable potato growers in the Third World to use their own harvest as seed. The high costs of chemical treatments and of certified seed are one of the main obstacles to a greater spread of potato cultivation among low-income farmers in developing countries (Section 10).

PLRV is transmitted by several aphid species (HILLE RIS LAMBERS 1972). By far the most important one is the peach-potato aphid *Myzus persicae*. Next in effectivity is perhaps *Aphis rhamni*. *M. p.* can carry PLRV to a crop from a distance of 40 km. PLRV is a circular virus with persistent transmission. The period between acquisition and inoculation feeding lasts several hours or even days. The aphid probably propagates the virus and continues to transmit it during its life time. The transmission is very effective. The majority of the cvs. attracts a virus infection in 20–40 % of the plant individuals per year in areas and years of medium aphid infestation. The relationships between the incidence of the aphids, depending mainly on the climate in May and June, and the incidence of infection with PLRV and PVY is represented in recent articles by ROBERT (1971), GABRIEL et al. (1975), HUNNIUS (1977 a), KLEIN & HUNNIUS (1979) and VAN HARTEN (1983).

There are two types of resistance: resistance to infection and intolerance. Unfortunately, the types localized hypersensitivity and extreme resistance are not encountered. This may have anatomical reasons. PLRV is exclusively restricted to the phloëm and seems to move through the plant's conducting system without transgressing into the parenchyma (WEIDEMANN & CASPER 1982).

The infection resistance is inherited by minor gene effects (BAERECKE 1958, 1961, DZIEWOŃSKA & POCHITONOW 1971, DAVIDSON 1973, HAMANN & MÖLLER 1979). The resistance level of the progeny depends largely on the degree of resistance of both parents and grandparents. A susceptible parent may reduce the resistance of the progeny drastically. In order to assess the degree of resistance, field trials are performed in a region of intermediate and, as far as possible, regular yearly aphid infestation. Ten or more plants of each clone are planted in rows with or without infection rows in between. One or several tubers of each plant are regrown in the next season or screened for the presence of the virus serologically by tuber indexing or in other ways (Section 10). In a well differentiating year, the percentage of infected plants varies between 10 and 70 according to the resistance of the respective cv. genotype. Several variants of this method are in use (BAERECKE 1958, 1961, HAMANN et al. 1968, DEMEL 1968, WIERSEMA 1972, DAVIDSON 1973). A method for preselection among seedlings was developed by CHUQUILLANQUI & JONES (1980). Leafroll infected aphids are shaken out over a tray planted with seedlings a few cm high. The aphids distribute themselves among the seedlings in an astonishingly regular manner. Diseased seedlings can be recognized after a few weeks. In order to differentiate the resistance degree, single stemmed plantlets are inoculated with 5, 25, and 50 PLRV-carrying aphids and the plants later on tested serologically for the presence of the virus. It is hoped that this very laborious method can be replaced in the future by assessment at the cell level, i. e. by infection of protoplast culture with PLRV. Such methods, possibly, could serve also for selection of resistant genotypes arisen from somaclonal variation.

Resistance in present day cvs. can be traced back in many cases to *S. demissum* – ssp. *andigena* – ssp. *tuberosum* hybrids. Well-known examples are the so called »W-races« bred by K. O. MUELLER (Section 2.3 and 2.5). Other resistant cvs. originated from the hybrid family MPI 44.335 which included the clone MPI 19268, an ancestor of many leafroll-resistant Duch cvs. A high resistance is also inherited by *S. acaule* × ssp. *tuberosum* – back-cross – hybrids, e. g. MPI 44.1016/10.

The degree of leafroll resistance in the European cvs. is listed by STEGEMANN & SCHNICK (1985). It increases from year to year (DZIEWOŃSKA & POCHITONOW 1971, WIERSEMA 1972, HAMANN & MOELLER 1979). However, cvs. with the highest leafroll resistance flower

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sparsely, their male fertility is low and culinary quality is inadequate in many cases (DAVIDSON 1979/80).

Other new germplasm for a possibly higher infection resistance in the future may come from species of the series *Etuberosa* as *S. etuberosum* and *S. brevidens* or from *S. acaule*. HAWKES (1947) observed no PLRV-infection in *S. brevidens* grown in the Commonwealth Potato Collection in an environment of high aphid infestation. JONES (1979) got negative results when trying to infect *S. brevidens* with PLRV by means of aphids. RIZVI (1983), studying the hybrid progeny of 4x *S. etuberosum* × *S. pinnatisectum,* was unable to infect resistant seedlings either by aphids or by means of grafting onto a PLRV infected stock. H. C. BROWN (pers. comm.) could detect PLRV with ELISA in the *verrucosum-etuberosum-pinnatisectum-tuberosum* hybrids (HERMSEN 1984) not before some months after grafting. AUSTIN et al. (1985) obtained fusion hybrids between *S. brevidens* and *S. phureja / S. stenotomum.* Shoots of the hybrids were inoculated with PLRV-carrying *Myzus persicae.* Plants were assayed six weeks later by ELISA-method. Most of the hybrids exhibited the same high resistance to multiplication of the virus as did *S. brevidens.* Another source of resistance is found in some provenances of *S. acaule.* Pentaploid hybrids ex colchinized 3x *(S. acaule* × *S. phureja / S. stenotomum)* × ssp. *tuberosum* could be received. The resistance segregated as a monomer dominant trait and because necrosis are absent, it seems to present a new type of resistance: non-necrotic resistance to multiplication. The virus cannot be refound in plants after graft-inoculation (Anon. 1984 b, 1985 b, BROWN et al. 1984 b).

Some leafroll resistant cvs. (Apta, Carla, Ida, Monza, Sedira of the Federal Republic of Germany and Kama of Poland) carry the type of systemic hypersensitivity or intolerance (ARENZ 1956, BAERECKE 1958, 1968). It seems to be a reliable type for breeding, since the crop of an intolerant cv. can be entirely free of infected plants if the cv. possesses a certain degree of infection resistance at the same time, as is true for cv. Apta (ARENZ 1956). BUTKIEWICZ (1978) and ZADINA & NOVAK (1983) found the inheritance of intolerance to be monomere dominant *(Nl)* and modified by minor genes. Among the not many wild species that react with hypersensitivity to PLRV, *S. raphanifolium* (2x) should be mentioned (BAERECKE, see Ross 1966).

Symptoms very similar to those of PLRV are also produced by the Beet Western Yellow virus (BWYV) (DUFFUS 1981). Both have several properties in common, e. g. transmission by *Myzus persicae,* and often occur in association. The viruses can be differentiated by serology and by transfer to *Capsella bursa pastoris* which is not infected by BWYV. BWYV has not yet been found in Germany (CASPER 1984).

7.2.2 Virus Y (leafdrop streak virus, PVY)

PVY-infected plants are often more severely damaged than PLRV infected ones. A further unfavorable attribute of PVY is the formation of weak strains causing mild symptoms. Such strains can even remain totally latent. The short time needed for acquisition and inoculation feeding is characteristic for the non-persistent transmission of this virus by *Myzus persicae* and other aphids (KOSTIV 1981). It makes a defence using many of the aphidicides (organophosphates) ineffective as is substantiated by many authors, e. g. SCHEPERS (1972) and MÜLLER & HETTICH (1983). Those aphidicides induce a relatively long phase of hyperactivity and thus offer the possibility of inoculation of many plants in a short time. Differently constituted aphidicides may present a better solution (GIBSON et al. 1982, GIBSON 1984 and earlier).

Three groups of PVY strains are distinguished. PVY^{o} comprises most of the strains. These react with the typical primary symptoms such as dark streaks on the underside of the leaves and the dropping off of older and, finally, of younger leaves, too. The secondary symptoms in the years to follow often change to severe mosaic.

PVY^{n} represents another group of strains differing from PVY^{o} in their symptoms in tobacco: PVY^{n} producing a distinctive browning of the leaf underside, which PVY^{o} does not. Potato cvs. can react to strains of both groups with typical symptoms or with ± complete

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latency. It seems that PVY^n strains cause latent infections in more cvs. than PVY^o strains (Weidemann & König 1979).

The group PVY^c is of less importance for the potato breeder. It comprises strains not transmissible by insect vectors and reacting with localized hypersensitivity in the presence of the gene *Nc*. This gene is harboured by most of the cvs. Certain progenies show pseudo-PVY symptoms not caused by a virus. These symptoms can be distinguished visually from true virus symptoms only with difficulty. The pseudo-PVY symptom is occasionally met with in the true seed potato.

Breeding for resistance is based mainly upon the types: »infection resistance« and hypersensitivity *(Tab. 11)*. In some cvs. as well as in some provenances of *S. phureja* and *S. stenotomum* a high resistance to infection is connected with necrotic reactions coming close to systemic hypersensitivity. The infection resistance is based on minor gene effects. Screening methods are the same as those described for PLRV. However, in the case of PVY it is possible to enhance the natural infection pressure in the field trials by spray infection with sap from PVY-infected plants (see below). The air temperature should be above 18 °C to ensure that the inoculation is sufficiently effective. It is, therefore, recommended that infection trials be carried out in quite late spring. Recent breeding work with hybrids of *S. phureja* and *S. stenotomum* carrying the infection resistance type is described by Chrzanowska (1976), Davidson (1978), Solomon (1978) and Fernandez-Northcote & Brown (1981).

A serious problem has been latency, i. e. the difficulty in recognizing an infection when some cvs. with weak strains of PVY^o or PVY^n become predominant. When special conditions prevail, such as early and high aphid infestation, a high number of infection sources, presence of special PVY^o- and PVY^n-strains and a broad distribution of cvs. reacting latently to these strains, a ± total contamination of such cvs. can occur (Rohloff 1979, Ross 1979 b). This happened in the years 1955/56. When it became nearly impossible to produce virus-free seed from some cvs. which were then discarded from the variety lists. A similar situation in the years 1975/76 could be kept under control in Germany. Complete sterile micropropagation and ELISA-serology hopefully will prevent such situations in the future (Section 10).

Major genes *N* and *R* are used in breeding for necrogenic resistance. *N*-genes govern a necrogenic reaction situated between localized and systemic hypersensitivity. *Ny*-genes can be met with in some cvs., such as Pentland Crown, and in hybrids with the wild species *S. chacoense, S. demissum* and *S. microdontum*. The genes are generally of medium expressivity. Occasionally, diseased plants can be found in the field. A better localization of the virus can be accomplished when suitable minor genes are present in the genetical background. Moreover, the reaction of some *Ny*-genes depends on the virus strain, while other *Ny*-genes seem to cover the whole range of strains (Cockerham 1970, Solomon 1978, 1983, Davidson 1979/80, Brown & Fernandez-Northcote 1981).

Ry-genes, on the other hand, controlling the extreme resistance type, provide a complete protection. Extreme resistance to PVY was first detected by Stelzner in *S. stoloniferum* (1944, publ. 1950) and the inheritance found to be monomere dominant (gene *Ry*) by Ross (1958 b, c; 1961 a, b) and Cockerham (1970). The gene is strain non-specific (e. g. Fernandez-Northcote 1983). Some genotypes carrying *Ry* react with extreme resistance also to PVA, a virus related to PVY, while others do not. The genetical reason is still unknown. – According to Barker & Harrison (1984) *Ry*-resistance is expressed in protoplasts. Only 0.1 % of protoplasts from cv. Corine and Pirola became infected in contrast to up to 11 % of the susceptible cv. Kerr's Pink.

Selection can be performed among young seedlings in a tray by means of a spray gun with virus containing sap (Ross 1960, Dziewońska & Pochitonow 1971, Wiersema 1961, 1972, Brown & Fernandez-Northcote 1981). In order to avoid spreading infection by possibly latently infected seedlings to other places, a back test of the symptom-less seedlings for freedom from PVY is recommended. The segregation in most cases deviates only little from

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Selection can be performed among young seedlings in a tray by means of a spray gun with virus containing sap (Ross 1960, DZIEWOŃKSA & POCHITONOW 1971, WIERSEMA 1961, 1972, BROWN & FERNANDEZ-NORTHCOTE 1981). In order to avoid spreading infection by possibly latently infected seedlings to other places, a back test of the symptom-less seedlings for freedom from PVY is recommended. The segregation in most cases deviates only little from

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the expected values when unpreselected progenies are tested (COCKERHAM 1970, ZADINA 1977 a, PFEFFER et al. 1977, BROWN & FERNANDEZ-NORTHCOTE 1981). According to WIERSEMA (pers. comm.), deviations are often influenced by the susceptible parent (minor genes). In order to assess the presence of the gene *Ry*, a grafting test is necessary at the end of the selection work *(Fig. 20)* (Section 7.2).

The following cvs. carrying the gene *Ry* are included in the variety lists of the last years: Corine, Santé (The Netherlands), Bóbr, Brda, Bzura, Pilica, San (Poland), Magyar Roza, Szignal (Hungary), Barbara, Bison, Cordia, Esta, Fanal, Forelle, Franzi, Heidrun, Pirola, Wega (Federal Republic of Germany). The cv. Barbara additionally carries the gene *Rx* for extreme resistance to PVX. These cvs., with the possible exception of Corine, are based on proveniences of *S. stoloniferum* conferring plasmatic male sterility. They can be used as seed parents only. However, a restorer gene has been found (Ross unpubl.). The listed cvs. are third to seventh back-cross generation of the F_1 with ssp. *tuberosum*. One of the more frequently used Ry-parents is the hybrid MPI 61.303/34 which harbours genes from ssp. *andigena, S. acaule* (gene *Rx*), *S. demissum, S. spegazzinii* (gene *Fb*) and *S. stoloniferum* (gene *Ry*). Extreme resistance to PVY has been detected in cvs. of ssp. *andigena* where it is inherited by one dominant major gene as well (MUÑOZ et al. 1975, GALVEZ & BROWN 1980). Ry_{adg} in contrast to Ry_{sto} is not brought into action with PVA (Anon. 1984 b).

7.2.3 Virus X (common mosaic virus, PVX)

PVX is transmitted by wound contact and, occasionally, by soil fungi. Its infectivity is very high. Breeding is based on the types: infection resistance, localized hypersensitivity and extreme resistance. The resistance to infection in some cvs. can reach high levels. However, it must be recognized, that, in crossing, highly resistant seedlings turn out only when both parents possess the same high level of resistance. Whereas from a cross between an extremely resistant *(Rx)* and a susceptible parent, 50 % extremely resistant progeny are obtained.

The strains of PVX can be divided into four groups *(Tab. 12)*, according to their reactions with the genes for localized hypersensitivity *(Nb, Nx)* and extreme resistance *(Rx)*. Strains of group 1 are characterized by whitish-green rings on the surface of tobacco leaves followed by a light green-dark green mottle. These strains mostly occur in association with strains of group 3. Group 2 comprises strains harboured earlier by the cv. Duke of York (Erstling). Since this cv. was freed from PVX contamination, group 2 strains are met with only occasionally. Group 2 strains can change in grafting experiments (JONES 1982 a). The strains X_{CP} and X_{DP}, found by FRIBOURG (1975) in a Peruvian cv. of ssp. *andigena*, also belong to this group. These strains are not observed in North America and Europe. Group 3 comprises the strains occurring most frequently. They cause a mild mottle on tobacco leaves,

Tab. 12: Classification of PVX-strains
Einteilung der PVX-Stämme (COCKERHAM 1955, modified)

gene *Gen*	cultivar *Sorte*	groups of strains – *Stammgruppen* 1	2	3	4	strain HB *Stamm HB*
nxnb	Arran Banner	+	+	+	+	+
Nxnb	Epicure	−[1]	+	−[1]	+	+
nxNb	Arran Victory	−[1]	−[1]	+	+	+
NxNb	Craigs Defiance	−[1]	−[1]	−[1]	+	+
Rx	Saco	−[2]	−[2]	−[2]	−[2]	+
Rx_{adg}	Astarte					
Rx_{acl}	Saphir					

+ susceptible – *anfällig;* [1] hypersensitive – *überempfindlich;* [2] type extreme resistance – *Typus extreme Resistenz*

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while in potato genotypes carrying the gene *Nx* black local lesions appear on the leaves after sap infection. When infected by way of grafting, *Nx* carriers react with top necrosis (acronecrosis). Such cvs. remain free of PVX in the field with very rare exceptions. Group 4 strains fail to activate the *N*-genes. They can infect cvs. with *Nx* and *Nb* but not those with *Rx*. They are observed in field plants in some cases. They are weak in competition and usually do not spread in North America and Europe.

A further strain, PVX_{HB}, found in 7 % of Bolivian clones of ssp. *andigena,* invades readily the genotypes *Nb, Nx, Rx* and Rx_{acl} (MOREIRA et al. 1980, JONES 1982 a). BROWN et al. (1984 a) found a dominant gene for extreme resistance against this strain in *S.* × *sucrense*. The distribution of PVX_{HB} outside of Bolivia is unknown. At the moment the assumption is justified that breeding with the genes *Nx, Rx,* Rx_{adg} or Rx_{acl} conveys sufficient protection against all important strains in the fields of North America and Europe (compare ADAMS et al. 1984).

Selection for hypersensitivity and extreme resistance can be performed by the spray gun method applicable to seedlings and field plants as well as to isolated leaves. Necrotic local lesions showing up after infection with PVX-1 or PVX-3 are indicative of the presence of the gene *Nx,* while a zero reaction demonstrates *Rx*. The spray infection in the field is less sensitive to low temperatures than that with PVY. For the final statement of resistance, graft tests are necessary.

The genes *Nb, Nx, Rx,* Rx_{acl} and Rx_{adg} are introduced into many cvs. *Nx* has long belonged to the genetical outfit of European and North American cvs. and is often linked with the genes *Na* and *Nc* (HOWARD & FULLER 1965). The most wide spread German cv. with localized hypersensitivity to PVX is the cv. Clivia. Kind and origin of the responsible gene are unknown.

The gene *Rx* (MILLS 1965, COCKERHAM 1970) originates from the Chilean cv. Villaroela of ssp. *tuberosum* and found entrance into US-cvs. Atlantic, Carlton, Jemsec, Reliance, Saco, Shoshoni, Tawa via the USA-breeding line 41956.

The gene Rx_{adg} was detected by WIERSEMA (1961) in a progeny from ssp. *andigena* CPC 1673. This line is also resistant to the pathotypes Ro-1 and Ro-4 of *Globodera rostochiensis.* Because of these two important traits, CPC 1673 is used in breeding often (see a. o. HOWARD et al. 1978; *Tab. 15*).

The gene Rx_{acl} was found in *S. acaule* (Ross 1954, COCKERHAM 1970, DELHEY 1974). It was introduced into the German cvs. Aguti, Assia, Barbara, Moni, Natalie, Roeslau, Saphir and the Argentine cv. Serrana Inta via the back-cross hybrid MPI 44.1016/10. Hybrids of the constitution *(S. acaule* × *S. stoloniferum)* × ssp. *tuberosum* with both of the genes *Ry* and Rx_{acl} are also used as parents in cv. breeding (Ross 1954, DAVIDSON 1979/80).

Another gene for extreme resistance (or perhaps localized hypersensitivity) of unknown origin is carried by the nematode resistant *S. vernei* back-cross hybrid 62-33-3 and introduced in the Dutch cvs. Atrela, Darwina, Pansta, Produzent, Promesse, Proton and Santé (*Tab. 15,* Section 7.3).

7.2.4 Virus M (leafrolling mosaic virus, PVM)

PVM is characterized by a leafrolling that is extended to the younger leaves and accompanied by a mosaic. The leaves are flexible and not stiff, in contrast to PLRV. PVM has its main distribution in East and Southeast Europe, but since 1972 it is migrating westwards (WEIDEMANN 1978). PVM is transmitted by aphids in the non-persistent mode and more readily than PVY (KOSTIV 1981). Several strains can be distinguished by their symptoms in cvs. and indicator plants such as *Lycopersicum chilense, Gomphrena globosa, S. stoloniferum* EBS 2630 etc. (KOWALSKA 1978).

Potato cvs. differ in degrees of infection resistance (SCHOLZ 1965, OERTEL et al. 1978, see also STEGEMANN & SCHNICK 1985). Selection for infection resistance is performed among field plants with the spray gun method (Ross 1960). Back-tests are made on indicator plants or by serological methods.

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