

National Research
Council Canada

Conseil national
de recherches Canada

5503383

W1

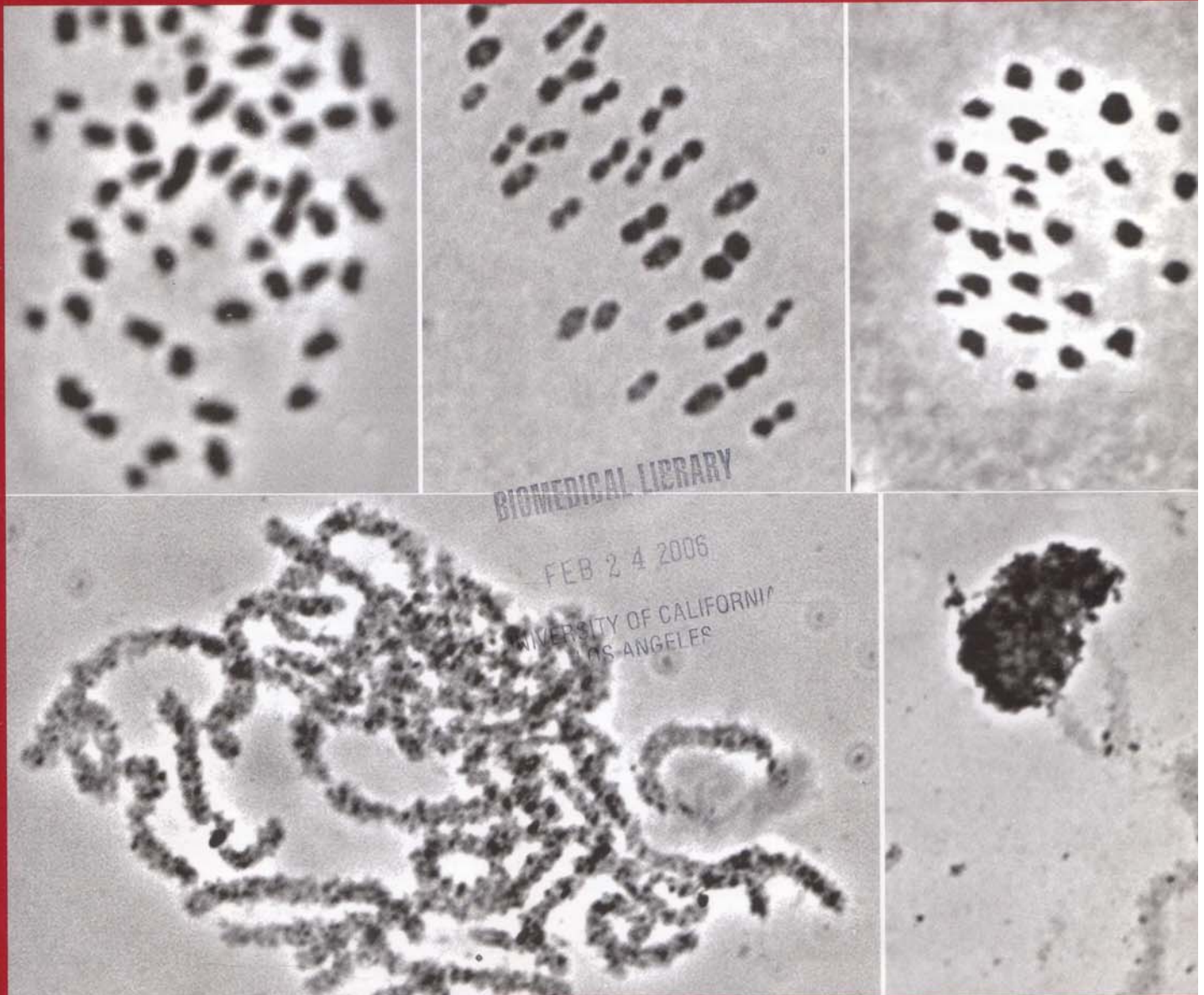
GE 382

NC-CMRC
Genome

Génome

Volume 48, Number 6, December 2005

Volume 48, numéro 6, décembre 2005



<http://genome.nrc.ca>

<http://genome.cnrc.ca>

Genome

Génome

Published by the National Research Council of Canada

Publié par le Conseil national de recherches du Canada

Editor / Directeur scientifique Peter B. Moens

Assistant to the Editor / Adjointe aux directeur scientifique Alistair Coulthard

Editorial Board / Comité de rédaction

R. Appels
J.B. Bell
F. Belzile
L. Bonen
T.E. Bureau
A. Civetta
J.H. de Jong

R. Danzmann
P. Donini
B. Golding
J.P. Gustafson
A.J. Hilliker
G. Jenkins
M. Puertas

J.S. Quinn
T. Schwarzacher
G.J. Scoles
D.J. Somers
R.S. Singh
W. Traut
S. Varmuza

NRC Research Press / Presses scientifiques du CNRC

Editor-in-chief / Directeur scientifique en chef Bruce P. Dancik

Assistant Editor-in-chief / Adjoint au directeur scientifique en chef Iain E.P. Taylor

Journals Program / Programme des revues

(Fax / Télécopieur : 613-952-7656; E-mail / Courriel : pubs@nrc-cnrc.gc.ca; URL / URL : <http://pubs.nrc-cnrc.gc.ca>)

Director / Directeur Cameron D. Macdonald

Business Manager / Gestionnaire des affaires Jason Charron

Manager, Journals / Gestionnaire, Revues Carolyn Brown

Managing Editor / Rédactrice administrative Angela Murphy (613-998-4485)

Publication Officer / Agente de publication Mary Seligly

Advertising and Reprints / Annonces publicitaires et tirés à part Roxanne Landriault (613-990-2254)

Subscriptions / Abonnements Eileen Evans-Nantais (613-990-7873)

NRC accepts no responsibility for statements or opinions expressed by a contributor. Acceptance of an advertisement, announcement, or other material does not imply endorsement by either the Journal's Editors or NRC.

© National Research Council of Canada 2005. All rights reserved.

No part of this publication may be reproduced, stored, or transmitted in any form or by any means, without the written permission of the publisher, except as stated below.

The National Research Council of Canada grants permission to individuals to make single copies of articles for personal use. Any person may reproduce short excerpts from articles in the journal for any purpose that respects the moral rights of authors, provided that the source is fully acknowledged. As a courtesy, the consent of authors of such material should be obtained directly from the author.

Authorization to reproduce items for other than personal use, as stated above, is granted by the National Research Council of Canada provided that the copyright fee of \$7.00 per copy is paid directly to CANCOPY, One Yonge Street, Suite 1900, Toronto, ON M5E 1E5, Canada.

The above permission does not extend to other kinds of copying, such as copying for general distribution, for advertising, or promotional purposes, for creating new collective works, or for resale. For such copying, arrangements must be made with the publisher, National Research Council of Canada, Ottawa, ON K1A 0R6, Canada.

Le CNRC ne se tient en aucune façon responsable des opinions exprimées par un collaborateur ou des déclarations que pourrait faire ce dernier. La publication d'annonces publicitaires, d'avis et autres n'implique pas l'endossement de leur contenu par les directeurs de la Revue ou de CNRC.

© Conseil national de recherches du Canada 2005. Tous droits réservés.

Il est interdit de reproduire, de sauvegarder ou de transmettre quelque partie de la présente publication dans quelque format ou de quelque façon sans le consentement écrit de l'éditeur, à l'exception des conditions suivantes.

Le Conseil national de recherches du Canada accorde aux particuliers le droit de faire des copies individuelles pour leur usage personnel. Toute personne peut reproduire de courts extraits des articles de la Revue pour autant que les droits moraux de l'auteur soient respectés et que la mention de provenance soit clairement indiquée. Il est de convenance de demander le consentement de l'auteur avant de faire mention de son travail.

On obtiendra l'autorisation de reproduire des articles à d'autres fins que l'usage personnel (tel qu'on l'a décrit ci-dessus) auprès du Conseil national de recherches du Canada pour autant que des redevances de droits d'auteurs de 7,00 \$/copie soient versées au CANCOPY, 1, rue Yonge, bureau 1900, Toronto, ON M5E 1E5, Canada.

La permission ci-dessus ne s'applique pas à la reproduction à grande échelle, telle que la reproduction aux fins de distribution générale, publicitaire ou promotionnelle, de compilation de nouveaux ouvrages collectifs ou de revente. Dans de tels cas, il faudra ratifier une entente particulière avec l'éditeur, le Conseil national de recherches du Canada, Ottawa, ON K1A 0R6, Canada.

The Genetics Society of Canada has chosen Genome as its principal medium for the publication of scientific papers.

La Société de génétique du Canada a choisi Génome comme son principal organe de publication des articles scientifiques.



National Research
Council Canada

Ottawa, Canada
K1A 0R6

Conseil national
de recherches Canada

Ottawa, Canada
K1A 0R6

Postage paid at Ottawa
Publications mail
Registration No. 40062591

Port payé à Ottawa
Poste-publication
Enregistrement n° 40062591

USPS periodical postage paid at Champlain, NY 12919, USA

Genome

Volume 48, Number 6, December 2005

Génome

Volume 48, numéro 6, décembre 2005

	v–vi	Introduction / Introduction
	MINIREVIEW	MINISYNTHESE
	J. Xu 951–958	The inheritance of organelle genes and genomes: patterns and mechanisms
	ARTICLES	ARTICLES
I.G. Adonina, E.A. Salina, E.G. Pestsova, and M.S. Röder	959–970	Transferability of wheat microsatellites to diploid <i>Aegilops</i> species and determination of chromosomal localizations of microsatellites in the S genome
S.M. Pensel, J.C. Vilardi, and M.I. Remis	971–976	Mitochondrial DNA variation in the grasshopper <i>Sinipta dalmani</i> : application of long-PCR to the development of a homologous probe
C. Phumichai, M. Mori, A. Kobayashi, O. Kamijima, and K. Hosaka	977–984	Toward the development of highly homozygous diploid potato lines using the self-compatibility controlling <i>Sli</i> gene
S.P. Kumpatla and S. Mukhopadhyay	985–998	Mining and survey of simple sequence repeats in expressed sequence tags of dicotyledonous species
J.M. González, L.M. Muñiz, and N. Jouve	999–1009	Mapping of QTLs for androgenetic response based on a molecular genetic map of $\times$ <i>Triticosecale</i> Wittmack
P. Oliver, J. Balanyà, M.M. Ramon, A. Picornell, L. Serra, A. Moya, and J.A. Castro	1010–1018	Population dynamics of the 2 major mitochondrial DNA haplotypes in experimental populations of <i>Drosophila subobscura</i>
N. Gouin, S.J. Westenberger, S.M. Mahaney, P. Lindley, J.L. VandeBerg, and P.B. Samollow	1019–1027	Development, inheritance, and linkage-group assignment of 60 novel microsatellite markers for the gray, short-tailed opossum <i>Monodelphis domestica</i>
P. Ling and X.M. Chen	1028–1036	Construction of a hexaploid wheat (<i>Triticum aestivum</i> L.) bacterial artificial chromosome library for cloning genes for stripe rust resistance
R.G. Danzmann, M. Cairney, W.S. Davidson, M.M. Ferguson, K. Gharbi, R. Guyomard, L.-E. Holm, E. Leder, N. Okamoto, A. Ozaki, C.E. Rexroad, III, T. Sakamoto, J.B. Taggart, and R.A. Woram	1037–1051	A comparative analysis of the rainbow trout genome with 2 other species of fish (Arctic charr and Atlantic salmon) within the tetraploid derivative Salmonidae family (subfamily: Salmoninae)

Continued on inside back cover / Suite au verso

Front cover: Chromosome preparations of testes of codling moth larvae stained with either orcein or AgNO₃. For further details, please see Fuková et al., this issue.

Page couverture : Préparations chromosomiques des testicules de larves du carpocapse colorées soit avec l'orceïne ou l'AgNO₃. Pour plus de détails, voir Fukova et al. dans ce numéro.

Canada

Printed in Canada by / Imprimé au Canada par
Dolco Printing Inc.
Page production by / Mise en page par
NRC Research Press / Presses scientifiques du CNRC



0831-2796(200512)48:6:1-Z

Aardevo Exhibit 1005
Page 0003

J.C. Kuhl, M.J. Havey, W.J. Martin, F. Cheung, Q. Yuan, L. Landherr, Y. Hu, J. Leebens-Mack, C.D. Town, and K.C. Sink	1052–1060	Comparative genomic analyses in <i>Asparagus</i>
E.V. Ananiev, M.A. Chamberlin, J. Klaiber, and S. Svitashv	1061–1069	Microsatellite megatracts in the maize (<i>Zea mays</i> L.) genome
A. Schneider, G. Linc, I. Molnár, and M. Molnár-Láng	1070–1082	Molecular cytogenetic characterization of <i>Aegilops biuncialis</i> and its use for the identification of 5 derived wheat – <i>Aegilops biuncialis</i> disomic addition lines
I. Fuková, P. Nguyen, and F. Marec	1083–1092	Codling moth cytogenetics: karyotype, chromosomal location of rDNA, and molecular differentiation of sex chromosomes
E.C. Howell, S.J. Armstrong, G.C. Barker, G.H. Jones, G.J. King, C.D. Ryder, and M.J. Kearsey	1093–1103	Physical organization of the major duplication on <i>Brassica oleracea</i> chromosome O6 revealed through fluorescence in situ hybridization with <i>Arabidopsis</i> and <i>Brassica</i> BAC probes
B.S. Lee, M.Y. Kim, R.R.-C. Wang, and B.L. Waldron	1104–1115	Relationships among 3 <i>Kochia</i> species based on PCR-generated molecular sequences and molecular cytogenetics
	NOTES	NOTES
I. Cross and L. Rebordinos	1116–1119	5S rDNA and U2 snRNA are linked in the genome of <i>Crassostrea angulata</i> and <i>Crassostrea gigas</i> oysters: does the (CT) _n -(GA) _n microsatellite stabilize this novel linkage of large tandem arrays?
D. Lamoureux, D.G. Peterson, W. Li, J.P. Fellers, and B.S. Gill	1120–1126	The efficacy of Cot-based gene enrichment in wheat (<i>Triticum aestivum</i> L.)

Toward the development of highly homozygous diploid potato lines using the self-compatibility controlling *Sli* gene

Chalernpol Phumichai, Motoyuki Mori, Akira Kobayashi, Osamu Kamijima, and Kazuyoshi Hosaka

Abstract: Cultivated diploid potatoes ($2n = 2x = 24$) are self-incompatible, but can be altered to become self-compatible using the *Sli* gene. Previously, a diploid clone 97H32-6 was selfed up to S_3 using the *Sli* gene. To explore the usefulness of the *Sli* gene for the production of highly homozygous diploid potatoes, 2 S_4 families from the above 97H32-6 derived S_3 lines (inbred series A) and 3 S_5 families by continuous selfings from a different F_1 ($= S_0$) plant (inbred series B) were developed. The level of heterozygosity and the location of heterozygous loci on the genetic map were investigated using RFLP and AFLP markers. The average heterozygosity levels of the originally heterozygous loci decreased from 100% in S_0 to 10.7% in S_4 and 8.6% in S_5 (inbred series A and B, respectively). The average rate of reduction in heterozygosity per generation (38.4% and 38.5% for inbred series A and B, respectively) was lower than the theoretically expected rate (50%). However, none of the loci or chromosome sections was exclusively heterozygous in the advanced self-progeny. Thus, highly homozygous and seed-propagated diploid potatoes could be obtained by repeated selfing using the *Sli* gene.

Key words: Selfing, heterozygosity, diploid potato, *Sli* gene, *Solanum*.

Résumé : Les pommes de terre diploïdes cultivées ($2n = 2x = 24$) sont autoincompatibles mais cela peut être modifié grâce au gène d'autocompatibilité *Sli*. Antérieurement, 1 clone diploïde, 97H32-6, a été autofécondé jusqu'en S_3 à l'aide du gène *Sli*. Afin d'explorer l'utilité de ce gène pour la production de pommes de terre diploïdes fortement homozygotes, 2 familles S_4 dérivées des lignées S_3 mentionnées plus haut (série A de lignées autofécondées) et 3 familles S_5 obtenues par autofécondation continue d'une plante F_1 ($= S_0$) différente (série B) ont été produites. Le niveau d'hétérozygotie et la position des locus hétérozygotes sur la carte génétique ont été déterminés à l'aide de marqueurs RFLP et AFLP. Les niveaux moyens d'hétérozygotie pour les locus initialement hétérozygotes sont passés de 100 % en S_0 à 10,7 % en S_4 et 8,6 % en S_5 chez les séries A et B, respectivement. Les taux moyens de réduction de l'hétérozygotie à chaque génération (38,4 % et 38,5 % respectivement) étaient plus faibles que l'attente théorique (50 %). Cependant, aucun des locus ou segments chromosomiques n'était exclusivement hétérozygote chez les lignées autofécondées. Ainsi, des pommes de terre largement homozygotes et reproduites via les graines ont été obtenues par autofécondation répétée à l'aide du gène *Sli*.

Mots clés : autofécondation, hétérozygotie, pomme de terre diploïde, gène *Sli*, *Solanum*.

[Traduit par la Rédaction]

Introduction

Potato (*Solanum tuberosum* L., $2n = 4x = 48$) is an auto- or segmental allo-tetraploid showing tetrasomic inheritance, which results in complicated genetic segregation (Howard 1970; Ross 1986; Matsubayashi 1991). Since potatoes are propagated vegetatively, highly heterotic genotypes can be maintained, which facilitates the use of heterosis, an essential factor in potato breeding, particularly for tuber yield (Ross 1986; Peloquin et al. 1989). Alternatively, open-pollinated true potato seed (TPS) can be used as a low-cost planting material for potato production (Shonnard and Peloquin 1991). However, the introduction of TPS in developed countries is difficult because of the lack of homogeneity in tuber shape and size (Ross 1986). Highly homozygous inbreds, once amply produced, would be highly useful for the development of TPS varieties with genetic uniformity, as well as for the investigation of heterosis (Birhman and Hosaka 2000).

Received 19 November 2004. Accepted 22 June 2005.
Published on the NRC Research Press Web site at
<http://genome.nrc.ca> on 29 November 2005.

Corresponding Editor: J.H. de Jong.

C. Phumichai. Graduate School of Science and Technology, Kobe University, Rokkodai, Nada, Kobe 657-8501, Japan.

M. Mori and A. Kobayashi. Potato Breeding Laboratory, National Agricultural Research Center for Hokkaido Region, NARO, Shinsei, Memuro, Hokkaido 082-0071, Japan.

O. Kamijima. Faculty of Agriculture, Kobe University, Rokkodai, Nada, Kobe 657-8501, Japan.

K. Hosaka.¹ Food Resources Education and Research Center, Kobe University, 1348 Uzurano, Kasai, Hyogo 675-2103, Japan.

¹Corresponding author (e-mail: hosaka@kobe-u.ac.jp).

To obtain homozygous lines, tetraploid potatoes can be selfed, but the selfing causes marked tuber yield reduction and loss of fertility, since these traits are controlled largely by heterotic genetic effects (Krantz 1924, 1946; Mendiburu and Peloquin 1977; Golmirzaie et al. 1998a; 1998b). The use of closely related diploid species and dihaploid potatoes is hampered by gametophytic self-incompatibility controlled by multiallelic *S* genes (Pushkarnath 1942; Pandey 1962; Cipar et al. 1964a). Self-compatible variants have often been described in essentially self-incompatible diploid species (Pushkarnath 1942; Cipar et al. 1964b; De Jong and Rowe 1971; Olsder and Hermesen 1976; De Jong 1977; Cappadocia et al. 1986; Birhman 1986). However, as far as we could determine, these variants were not successively selfed over generations.

Monoploidization by parthenogenetic reduction using a *Solanum phureja* haploid inducer or anther culture has been extensively conducted (Irikura 1975; van Breukelen et al. 1975; Foroughi-Wehr et al. 1977; Uijtewaal et al. 1987a). Spontaneously doubled or colchicine-doubled monohaploids (complete homozygous diploids) were often isolated from monohaploids of dihaploid *S. tuberosum* (van Breukelen et al. 1977; Uijtewaal et al. 1987b), *S. phureja* Juz. et Buk. (Veilleux et al. 1985), or *Solanum chacoense* Bitter (Cappadocia et al. 1986). However, most of these homozygous diploids were either male or female sterile (van Breukelen et al. 1977; Uijtewaal et al. 1987b; Singh et al. 1988; Paz and Veilleux 1999), except for a few exceptional self-fertile plants (Wenzel et al. 1979; Cappadocia et al. 1986).

A scheme for hybrid breeding using homozygous diploids and subsequent somatic hybridization has been proposed, for which the availability of highly homozygous diploid (or pure) lines is a key for success (Meyer et al. 1992). In considering the use of pure lines for the development of genetically uniform TPS varieties, self-fertile, seed-propagated lines are highly desirable, and simple techniques to develop such lines are necessary.

Recently, we found a dominant allele (the *S* locus inhibitor gene *Sli*) from a self-compatible variant in a wild diploid species, *S. chacoense*, which can alter self-incompatible plants to self-compatible ones (Hosaka and Hanneman 1998a). The *Sli* gene was mapped at the most distal end of chromosome 12 (Hosaka and Hanneman 1998b), and its possible tight linkage with a recessive lethality gene was proposed (Hosaka and Hanneman 1998a). Birhman and Hosaka (2000) introduced the *Sli* gene by crossing various cultivated diploid potatoes and successfully developed *S*₂ progeny. The *S*₂ inbreds were later accidentally pollinated with bulked pollen grains from other *S*₂ plants, producing *F*₁ hybrids from which a new series of inbred progeny was developed (Phumichai et al. 2004). Restriction fragment length polymorphism (RFLP) analysis was carried out to monitor the inbreeding process of this new inbred series from *F*₁ (= *S*₀) to *S*₃ progeny, the results of which suggested a possible association between fertility and heterozygosity, resulting in a much higher observed percentage of heterozygosity in *S*₃ progeny than theoretically expected (Phumichai et al. 2004).

In this study, 2 *S*₄ families from the above *S*₃ progeny and 3 *S*₅ families of a new inbred series were developed, and their inbreeding processes were monitored using RFLP and

AFLP markers. The specific objectives were as follows: (i) to understand the process of inbreeding in terms of inbreeding depression as measured by fertility; (ii) to measure the degree of heterozygosity reduction and identify heterozygous loci that resist universal homozygosity; and (iii) to discuss the usefulness of *Sli* gene in developing of highly homozygous lines of diploid potato.

Materials and methods

Plant material

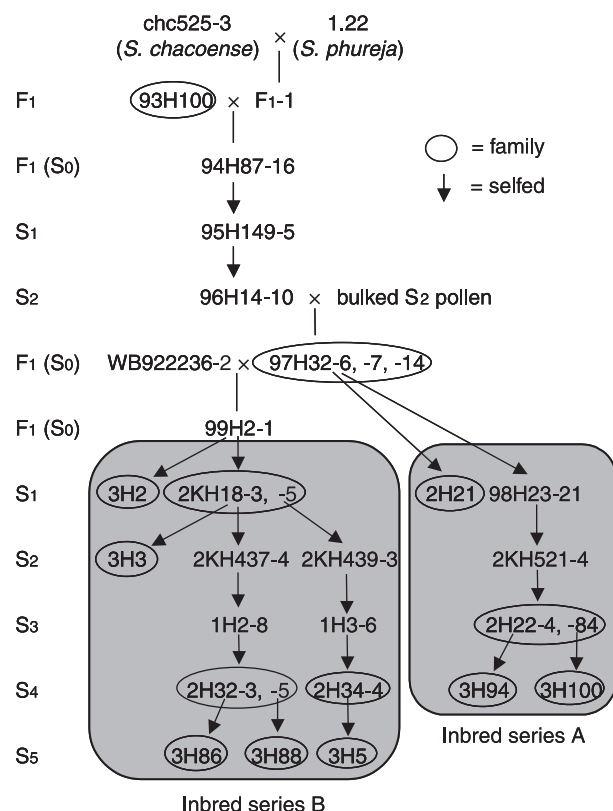
The first series of inbred lines (inbred series A) was obtained from a diploid clone 97H32-6 (composed of 75% cultivated and 25% wild germplasm with 100% cultivated cytoplasm), which was self-compatible due to the function of the *Sli* gene originally derived from a self-compatible variant of *S. chacoense* (Hosaka and Hanneman 1998a). Two randomly chosen *S*₄ families, 3H94 (15 plants) and 3H100 (15 plants), were analyzed for heterozygosity by DNA analysis and compared with that of their *S*₁ (15 plants from the 2H21 family) and *S*₃ (15 plants from the 2H22 family) ancestors (Fig. 1). The *S*₂ family was not available for DNA analysis.

The second series of inbred lines (inbred series B) were started from 2 advanced diploid clones, WB922236-2 and WB922236-3 (full sibs of W822227-1 × W882204-8 that possess at least 37.5% of the chromosomal and 100% of the cytoplasmic information from *S. phureja* PI 225683) by crossing with the pollen of *Sli* gene donors *F*₁-1, 97H32-6, 97H32-7, or 97H32-14 (the last 3 clones were full sibs descended from *F*₁-1; see Fig. 1). Three randomly chosen *S*₅ families, 3H86 (15 plants), 3H88 (15 plants), and 3H5 (12 plants), were used for DNA analysis; their pedigrees are shown in Fig. 1. Their parental populations, *S*₁ (12 plants from family 3H2), *S*₂ (14 plants from 3H3), and *S*₄ (7 plants from 2H32 and 16 plants from 2H34) were used for comparison. The *S*₃ family was not available for DNA analysis.

Plants were grown in a green house in the fall and winter or in an insect-proof screen house in the spring. To ensure optimal growing conditions for flowering, light was supplemented to maintain 16 h of daylight from October to March, while from April to July, plants were grown in natural daylight. To advance selfed generations quickly, only seedlings, except for the initial population of inbred series B, were used without harvesting tubers. Self-pollinations were made on as many flowers as possible until the plants set berries during the season.

RFLP analysis

Total DNA isolation and RFLP detection procedures were all as described in Hosaka and Hanneman (1998b). Samples of total DNA from *S*₁ plants (from 3H2 and 2H21 families) were digested with restriction endonucleases *Dra*I, *Eco*RI, *Eco*RV, or *Hind*III and surveyed to obtain heterozygous loci in *S*₀ plants. Only heterozygous loci detected with specific probe and enzyme combinations were used to analyze the subsequent inbred progeny. RFLP probes used were either single-copy tomato probes obtained from Dr. S.D. Tanksley, Cornell University (Tanksley et al. 1992) or potato probes (Hosaka and Spooner 1992).

Fig. 1. Two inbred series, A and B, developed from 97H32-6.**AFLP analysis**

The same sets of DNA samples used for RFLP analysis were also used for AFLP analysis using procedures that are essentially the same as the protocols used in the AFLP[®] Analysis System (Invitrogen, Carlsbad, Calif.), described previously (Phumichai et al. 2004).

Data analysis

The locus-based observed percentage of heterozygosity (LOPH) was given by the percentage of the heterozygous RFLP loci for each individual plant, and the mean of the family was calculated. For the parental clone, the LOPH was estimated from the percentage of segregating loci in its selfed progeny using RFLP or AFLP markers. Section-based observed percentage of heterozygosity (SOPH) was defined as the percentage of the sections (each of 12 chromosomes was trisected into sections *a*, *b*, and *c*, based on the map length) containing 1 or more heterozygous RFLP loci, and calculated in the same way as the LOPH was for parents and their progeny. For RFLP loci, segregation ratios of homozygotes and heterozygotes were compared with the expected segregation ratios of 1:1 and 1:2:1, respectively, using chi-square tests.

Results**Production of selfed progeny**

The initial selfings up to 96H14-10 were reported by Birhman and Hosaka (2000). Reproductive traits and heterozygosity levels in subsequent selfing progeny from

97H32-6 (S_0) to the 2H22 family (S_3) in inbred series A were investigated by Phumichai et al. (2004). For this study, 424 S_4 plants of 7 families derived from the 2H22 family by selfing were raised, of which only 30 plants flowered and produced enough pollen grains for self-pollination during the season. As many flowers as possible were self-pollinated (a total of 132 flowers), but only 8 plants produced seeds (Table 1).

Production of inbred series B was started with 5040 F_1 plants from 7 families: WB922236-2 $\times$ F_1 -1 (294 plants), WB922236-2 $\times$ 97H32-6 (808 plants), WB922236-2 $\times$ 97H32-7 (510 plants), WB922236-2 $\times$ 97H32-14 (687 plants), WB922236-3 $\times$ F_1 -1 (1050 plants), WB922236-3 $\times$ 97H32-7 (1087 plants), and WB922236-3 $\times$ 97H32-6 (604 plants). Only a fraction of these flowered, among which 68 (1.3%) formed tubers. Since a drastic decrease in progeny number as a result of poor fertility and growth had been experienced, we saved an additional 141 plants that did not flower but formed good tubers. These 209 plants grown from tubers became an initial population for selfing in inbred series B. The result of self-pollinations for each generation is summarized in Table 1. As the selfed generation proceeded, many families failed to produce selfed progeny, resulting in an arrival at S_5 only from 99H2-1, 1 of the 14 F_1 hybrids from WB922236-2 $\times$ 97H32-6. Forty-three S_5 plants successfully produced selfed seeds.

Fertility and self-compatibility

In the selfed progeny of inbred series A, the percentage of available plants for self-pollination drastically decreased from 62.6% in S_1 to 7.1% in S_4 (Table 1). Many S_4 plants did not form buds, and even if buds were formed, they dropped at the very young developmental stage. The percentage of selfers (= plants that set seeds by self-pollination) among pollinated plants decreased steadily from 82.5% in S_1 to 26.7% in S_4 .

Such steady decreases in either the percentage of self-pollinated plants or the percentage of selfers were not found in inbred series B. Both percentages fluctuated greatly among generations, with the average decrease being 32.5% for pollinated plants and 43.0% for selfers from S_0 to S_5 . Many S_5 plants flowered, but most of them showed very poor or no pollen shedding.

Heterozygosity in inbred series A

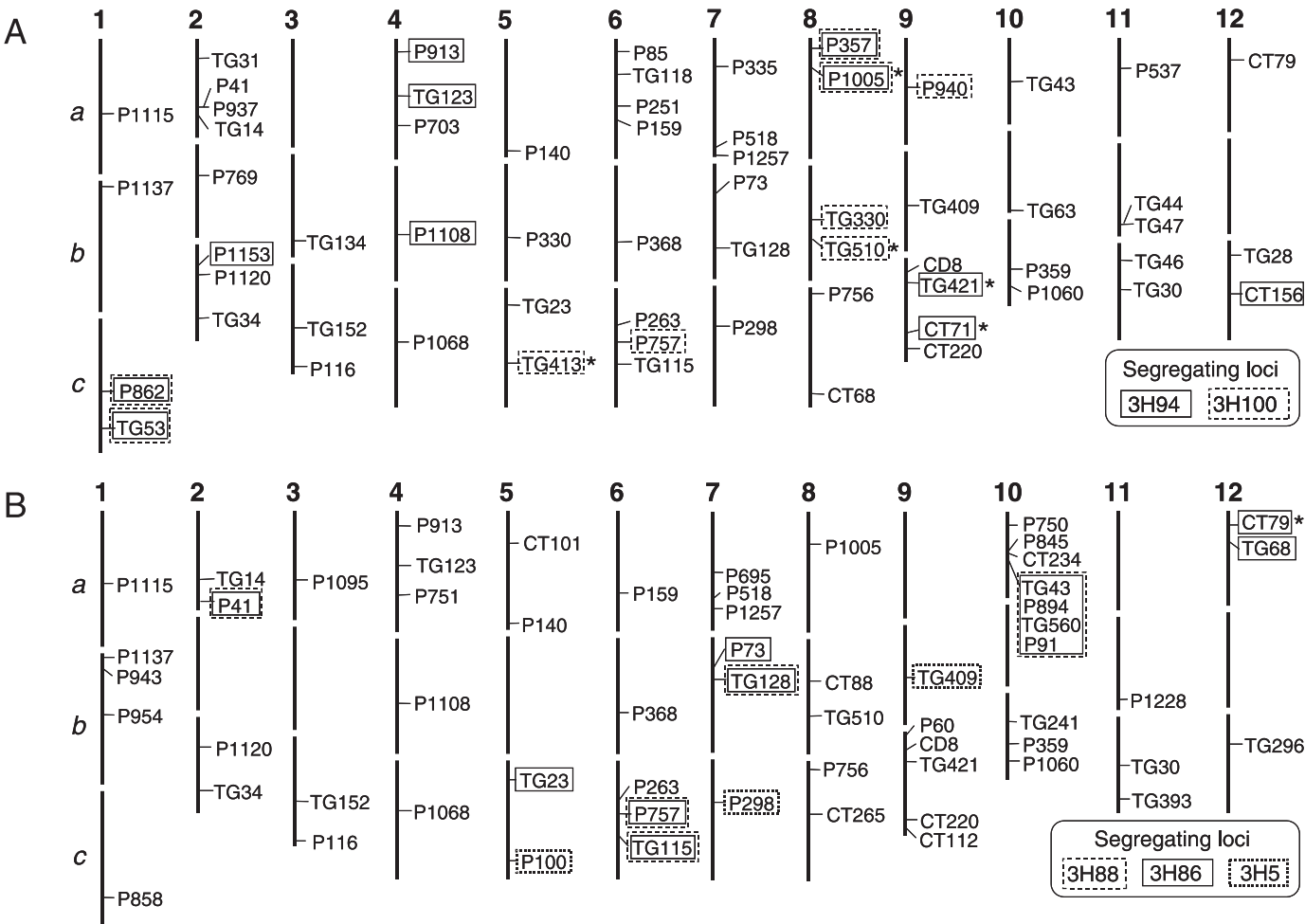
Out of 123 single-copy RFLP probes surveyed, 62 on 34 chromosome sections (Fig. 2A) segregated in the S_1 population (2H21), which were used to monitor the subsequent inbreeding process. Since some loci were localized together within a short map distance or cosegregated, SOPH was thereafter more weighted than LOPH.

The S_2 clone 2KH521-4 showed a slightly lower SOPH (52.9%), or an even higher LOPH (50.0%) than those of the parental S_1 population (the mean SOPH of 57.4%, or LOPH of 49.6%), indicating no reduction in heterozygosity from S_1 to S_2 . Thus, 2KH521-4 and its parental clone 98H23-21 were probably unintentional selections with higher heterozygosity in S_2 and S_1 populations, respectively (Phumichai et al. 2004). In contrast, 2H22-4 and 2H22-84, showing SOPH values of 20.6% and 17.7%, respectively, were likely unintentional selections with lower heterozygosity in the S_3 fam-

Table 1. Total number and percentage of plants that were self-pollinated and those that successfully produced seeds by self-pollination (= selfers). See text for details.

Generation	No. of plants grown	Self-pollinating plants	Flowers	Selfers
Inbred series A				
F ₁ (S ₀)	1	1 (100%)	10	1 (100%)
S ₁	91	57 (62.6%)	401	47 (82.5%)
S ₂	11	3 (27.3%)	15	2 (66.7%)
S ₃	81	37 (45.7%)	307	20 (54.1%)
S ₄	424	30 (7.1%)	132	8 (26.7%)
Inbred series B				
F ₁ (S ₀)	209	90 (43.1%)	578	60 (66.7%)
S ₁	831	186 (22.4%)	712	67 (36.0%)
S ₂	315	58 (18.4%)	231	16 (27.6%)
S ₃	118	38 (32.2%)	282	15 (39.5%)
S ₄	106	55 (51.9%)	727	28 (50.9%)
S ₅	427	115 (26.9%)	536	43 (37.4%)

Fig. 2. Segregating RFLP loci in the most advanced selfed progenies in inbred series A and B. Asterisks indicate loci showing significant segregation distortion at the 5% level. Each chromosome linkage map was trisected into *a*, *b*, and *c*, and the chromosome section based observed percentage of heterozygosity was obtained.



ily (the mean SOPH = 30.8%) (Table 2). For the S₄ families 3H94 and 3H100, the mean LOPH values obtained were 9.6% and 8.2%, respectively, and the mean SOPH values obtained were 12.0% and 9.4%, respectively.

Eleven and 9 loci segregated in the 3H94 and 3H100 families, respectively, of which 4 loci on 2 sections (1*c* and 8*a*) were common (Fig. 2A). Four loci (TG413, TG510, TG421, and CT71) showed significant segregation distortion at the

5% level in either 1 of the 2 S_4 families, while the P1005 locus was distorted in both families but differently in each family.

AFLP banding patterns were obtained using 22 AFLP primer combinations, generating a total of 304 bands segregating (present versus absent) in the S_1 family. Assuming that each band was controlled by 1 locus, AFLP loci indicated a steady decrease in LOPH as the selfed generation proceeded (Table 2). The AFLP estimates for LOPH were often much higher than those for RFLP. The real reason is unknown, but might be that heterozygous loci were sometimes doubly counted as a result of the silver-staining method that stained both strands of a denatured DNA fragment.

Heterozygosity in inbred series B

Fifty-nine of 121 RFLP loci surveyed segregated in the S_1 family (3H2), which covered 29 chromosome sections. In addition, 326 segregating AFLP bands were used to monitor the inbreeding process of inbred series B. RFLP phenotypes provided no evidence for either contamination by accidental out-crossing or new mutations, because bands present in the selfed progeny were all present in the initial S_0 plant 99H2-1.

The SOPH decreased steadily from the original 100% in 99H2-1 (S_0) to the family means of 62.4% in 3H2 (S_1), 37.9% in 3H3 (S_2), 9.9% in 2H32 or 10.4% in 2H34 (both S_4), and 6.0–12.2% in each of 3H86, 3H88, and 3H5 (all S_5) (Table 2). The S_1 parent (SOPH = 65.5%) was more heterozygous than the population mean (SOPH = 62.4%). One of S_5 families, 3H86, showed a higher SOPH (12.2%) than the mean SOPH of its parental 2H32 population (9.9%). This was because 2H32-3, a true parental clone of the 3H86 family not used for the DNA analysis, was estimated to be SOPH = 20.7%, which was distinctly higher than the SOPH estimates from any 2H32 plants used for DNA analysis.

Genetic segregation occurred in 12 loci in 3H86, 8 loci in 3H88 and 3 loci in 3H5 families, none of which were common in the 3 S_5 families (Fig. 2B). Only locus CT79 in the 3H86 family showed distorted segregation favoring one of homozygote genotypes. The LOPH estimated by AFLP bands were somewhat similar to those by RFLP, indicating the gradual decrease of heterozygosity through selfing generations (Table 2).

Comparison between inbred series A and B

For inbred series A, all available SOPH (RFLP) and LOPH (AFLP) values for each generation (Table 2) were pooled and averaged. These averages were as follows: 100% in S_0 , 57.4% in S_1 , 57.6% in S_2 , 31.3% in S_3 , and 10.7% in S_4 , with an average of 38.4% reduction per generation. For inbred series B, the averages were 100% in S_0 , 69.0% in S_1 , 37.9% in S_2 , 24.9% in S_3 , 15.6% in S_4 , and 8.6% in S_5 , with an average of 38.5% reduction per generation. Thus, both inbred series experienced decreases in their levels of heterozygosity, with approximately the same rate by selfing, although the reduction rates fluctuated greatly among generations in series A. There was neither a heterozygous chromosome section nor a distorted locus common to all 5 of the advanced self-progeny (Fig. 2).

Table 2. Locus-based observed percentage of heterozygosity (LOPH) or chromosome section-based observed percentage of heterozygosity (SOPH) estimated using RFLP and AFLP markers. For parental clones, LOPH and SOPH were estimated by the proportion of segregating loci in their progeny, while those for the progeny were represented by the mean with the range in parentheses.

Parent			Progeny								
Generation	Clone	Expected	LOPH (RFLP)	SOPH (RFLP)	LOPH (AFLP)	Generation	Family	No.	Expected	LOPH (RFLP)	SOPH (RFLP)
Inbred series A											
S ₀	97H32-6	100	100	100	100	S ₁	2H21	15	50	49.6 (32.3-62.9)	57.4 (41.2-70.6)
S ₂	2KH521-4	25	50.0	52.9	62.2	S ₃	2H22	15	12.5	26.1 (19.4-41.9)	30.8 (20.6-44.1)
S ₃	2H22-4	12.5	17.7	20.6	45.1	S ₄	3H94	15	6.25	9.6 (3.2-14.5)	12.0 (2.9-17.6)
S ₃	2H22-84	12.5	14.5	17.7	42.4	S ₄	3H100	15	6.25	8.2 (0-14.5)	9.4 (0-17.6)
Inbred series B											
S ₀	99H2-1	100	100	100	100	S ₁	3H2	12	50	56.0 (39.0-76.3)	62.4 (48.3-75.9)
S ₁	2KH18-3	50	59.3	65.5	79.1	S ₂	3H3	14	25	29.1 (15.5-39.0)	37.9 (20.7-44.8)
S ₃	1H2-8	12.5	20.3	20.7	19.9	S ₄	2H32	7	6.25	7.5 (1.7-13.6)	9.9 (3.4-13.8)
S ₃	1H3-6	12.5	11.9	20.7	38.3	S ₄	2H34	16	6.25	5.6 (0-10.3)	10.4 (0-17.9)
S ₄	2H32-3	6.25	20.3	20.7	19.3	S ₅	3H86	15	3.125	10.7 (1.7-18.6)	12.2 (3.4-17.2)
S ₄	2H32-5	6.25	13.6	13.8	19.0	S ₅	3H88	15	3.125	6.1 (0-11.9)	7.6 (0-10.3)
S ₄	2H34-4	6.25	5.1	10.3	21.2	S ₅	3H5	12	3.125	3.0 (0-5.1)	6.0 (0-10.3)

Discussion

There have been various studies on the inbreeding of diploid cultivated potatoes using self-compatible variants (De Jong and Rowe 1971; Olsder and Hermesen 1976; De Jong 1977; Hermesen 1978; Birhman and Hosaka 2000; Phumichai et al. 2004). However, to our knowledge, inbreeding has never reached beyond S_4 , and this is the first report that self-fertile S_5 diploid inbreds with more than 90% homozygosity were produced. This demonstrated that highly homozygous and seed-propagated diploid potatoes could be obtained using the *Sli* gene. Because of the simple procedure (crossing with a *Sli* gene donor, then continued selfings), the *Sli* gene method may be a better alternative in obtaining such inbreds. In this study, we paid little attention to tuber yield because most self-pollinations were made on seedlings. Nevertheless, some S_4 plants in inbred series A produced considerable tuber yields (data not available). Thus, these advanced inbreds may be valuable genetic stocks for studying the mechanism of heterosis and for producing hybrid variety at the diploid level.

The frequencies of plants that were self-pollinated and actually selfed decreased constantly in inbred series A. Since the parental clone 97H32-6 was considered to be a *Sli* gene homozygote (Phumichai et al. 2004), the decrease of the selfer frequency seems solely due to the reduction in general fertility, as demonstrated by increasing frequencies of plants that did not flower or produce pollen. In comparison, inbred series B maintained relatively constant frequencies of flowering plants and selfers throughout selfing generations, which might be explained partly by a strong selection pressure for fertility in the initial stage (4.1% were selected before selfings started). However, the fact that only 1 of 7 cross combinations reached S_5 generation indicates that serious loss of fertility is typical in most families. Thus, inbreeding depression, typically expressed as reduced pollen viability and tuber yield (Krantz 1924, 1946; Mendiburu and Peloquin 1977; Ross 1986; Golmirzaie et al. 1998a, 1998b), will certainly limit continued selfing of the present diploid inbreds.

Continuous selfing may eliminate undesirable recessive alleles as they become homozygous and may in turn lead to the recovery of fertility with advancing generations (Busbice 1968; Pfeiffer and Bingham 1983; Ray and Bingham 1992). This expectation is not supported by the present study, because there was no indication of fertility recovery even at the S_5 generation. Fertility-related traits, such as flowering or nonflowering, pollen shedding, pollen fertility, self-compatibility, and seed fertility appear to be under polygenic control except for self-compatibility, which is controlled by the *Sli* gene. A large population would be necessary to simultaneously select desirable gene combinations and eliminate undesirable genes. Alternatively, a slower approach to homozygosity using sib mating or composite sibbing of inbreds may permit more effective selection before complete fixation occurs (Kinman 1952).

The observed average percentage of reduction of heterozygosity per generation (38.4% or 38.5%) was lower than the theoretically expected value of 50%. The slower than expected decline in heterozygosity has been reported by Brouwer

and Osborn (1997) with inbreeding diploid alfalfa and by Scotti et al. (2000) with inbreeding tetraploid alfalfa. Consequential selection for vigor and fertility during selfing has been suggested as the cause (Brouwer and Osborn 1997). We previously suggested that reproductive traits, such as flowering versus nonflowering and the degree of pollen production, were strongly correlated with heterozygosity, and selection for fertility favored the more heterozygous plants (Phumichai et al. 2004). Pollen performance might also be involved because the level of heterozygosity in the sporophyte affects the phenotypic expression of the gametophyte or pollen viability (Ortiz and Peloquin 1994; Stephenson et al. 2001). In this study, however, the levels of heterozygosity of the parental clones (selfers) were not always higher than the population means. Thus, other factors, such as seed germination ability and vegetative vigor, could have been involved in unintentional selection.

In conclusion, heterozygosity might be correlated with both fertility and viability, which agrees with numerous inbreeding studies (Krantz 1946; Wilsie 1958; Jugenheimer 1976; Ray and Bingham 1992). However, there was no apparent indication for the presence of an exclusively heterozygous locus or chromosome section, which is possibly related to plant survivability. Thus, further continuous selfings, if possible, would bring about near pure lines: 99% homozygosity at S_{10} , based on a theoretical calculation from the observed 38.4%–38.5% reduction rate in heterozygosity, or at S_7 , based on the theoretical 50% reduction rate.

Acknowledgements

We thank K. Kawano for useful comments and improvement of the manuscript, and our technical staff, M. Miyake, S. Kakehi, H. Hashizume, M. Fujimatsu, and S. Kitanishi for plant maintenance. This research was supported in part by a Grant-in-Aid for Scientific Research (No. 13556001) from the Japan Society for the Promotion of Science, and by the Ministry of Agriculture, Forestry and Fisheries (project research: general research on land use and agricultural establishment in the 21st century for the improvement in the rate of food self-sufficiency.)

References

- Birhman, R.K. 1986. Pollination mechanisms in *Solanum chacoense*. *Acta Bot. Indica*, **16**: 89–91.
- Birhman, R.K., and Hosaka, K. 2000. Production of inbred progenies of diploid potatoes using an *S*-locus inhibitor (*Sli*) gene, and their characterization. *Genome*, **43**: 495–502.
- Brouwer, D.J., and Osborn, T.C. 1997. Molecular marker analysis of the approach to homozygosity by selfing in diploid alfalfa. *Crop Sci.* **37**: 1326–1330.
- Busbice, T.H. 1968. Effects of inbreeding on fertility in *Medicago sativa* L. *Crop Sci.* **8**: 231–234.
- Cappadocia, M., Cheng, D.S.K., and Ludlum-Simonette, R. 1986. Self-compatibility in doubled haploids and their F_1 hybrids, regenerated via anther culture in self-incompatible *Solanum chacoense* Bitt. *Theor. Appl. Genet.* **72**: 66–69.
- Cipar, M.S., Peloquin, S.J., and Hougas, R.W. 1964a. Inheritance of incompatibility in hybrids between *Solanum tuberosum* haploids and diploid species. *Euphytica*, **13**: 163–172.

- Cipar, M.S., Peloquin, S.J., and Hougas, R.W. 1964b. Variability in the expression of self-incompatibility in tuber-bearing diploid *Solanum* species. *Am. Potato J.* **41**: 155–162.
- De Jong, H. 1977. Self-compatibility in inbred cultivated diploid potatoes. *Incompat. Newsl.* **8**: 16–17.
- De Jong, H., and Rowe, P.R. 1971. Inbreeding in cultivated diploid potatoes. *Potato Res.* **14**: 74–83.
- Foroughi-Wehr, B., Wilson, H.M., Mix, G., and Gaul, H. 1977. Monohaploid plants from anthers of a dihaploid genotype of *Solanum tuberosum* L. *Euphytica*, **26**: 361–367.
- Golmirzaie, A.M., Ortiz, R., Atlin, G.N., and Iwanaga, M. 1998a. Inbreeding and true seed in tetraploid potato. I. Selfing and open pollination in Andean landraces (*Solanum tuberosum* Gp. *Andigena*). *Theor. Appl. Genet.* **97**: 1125–1128.
- Golmirzaie, A.M., Bretschneider, K., and Ortiz, R. 1998b. Inbreeding and true seed in tetraploid potato. II. Selfing and sib-mating in heterogeneous hybrid populations of *Solanum tuberosum*. *Theor. Appl. Genet.* **97**: 1129–1132.
- Hermesen, J.G.Th. 1978. Genetics of self-compatibility in dihaploids of *Solanum tuberosum* L. 2. Detection and identification of all possible incompatibility and compatibility genotypes in six F₁s from interdiaploid crosses. *Euphytica*, **27**: 1–11.
- Hosaka, K., and Hanneman, R.E., Jr. 1998a. Genetics of self-compatibility in a self-incompatible wild diploid potato species *Solanum chacoense*. I. Detection of an *S* locus inhibitor (*Sli*) gene. *Euphytica*, **99**: 191–197.
- Hosaka, K., and Hanneman, R.E., Jr. 1998b. Genetics of self-compatibility in a self-incompatible wild diploid potato species *Solanum chacoense*. 2. Localization of an *S* locus inhibitor (*Sli*) gene on the potato genome using DNA markers. *Euphytica*, **103**: 265–271.
- Hosaka, K., and Spooner, D.M. 1992. RFLP analysis of the wild potato species *Solanum acaule* Bitter (*Solanum* sect. *Petota*). *Theor. Appl. Genet.* **84**: 851–858.
- Howard, H.W. 1970. Genetics of the potato. Logos Press Ltd., London.
- Irikura, Y. 1975. Induction of haploid plants by anther culture in tuber-bearing species and interspecific hybrids of *Solanum*. *Potato Res.* **18**: 133–140.
- Jugenheimer, R.W. 1976. Corn: improvement, seed production, and uses. Wiley-Interscience Publications, New York.
- Kinman, M.L. 1952. Composite-sibbing versus selfing in development of corn inbred lines. *Agron. J.* **44**: 209–214.
- Krantz, F.A. 1924. Potato breeding methods. *Tech. Bull. Minn. Agric. Exp. Sta.* 25.
- Krantz, F.A. 1946. Potato breeding methods. III. A suggested procedure for potato breeding. *Tech. Bull. Minn. Agric. Exp. Sta.* 173.
- Matsubayashi, M. 1991. Phylogenetic relationships in the potato and its related species. In *Chromosome engineering in plants: genetics, breeding, evolution*. Part B. Edited by T. Tsuchiya and P.K. Gupta. Elsevier, Amsterdam. pp 93–118.
- Mendiburu, A.O., and Peloquin, S.J. 1977. Bilateral sexual polyploidization in potatoes. *Euphytica*, **26**: 573–583.
- Meyer, R., Salamini, F., and Uhrig, H. 1992. Biotechnology and plant breeding: relevance of cell genetics in potato improvement. *Proc. R. Soc. Edinb. Sect. B.* **99B**: 11–21.
- Olster, J., and Hermesen, J.G.Th. 1976. Genetics of self-compatibility in dihaploids of *Solanum tuberosum* L. 1. Breeding behaviour of two self-compatible dihaploids. *Euphytica*, **25**: 597–607.
- Ortiz, R., and Peloquin, S.J. 1994. Effect of sporophytic heterozygosity on the male gametophyte of the tetraploid potato (*Solanum tuberosum* L.). *Ann. Bot. (London)*, **73**: 61–64.
- Pandey, K.K. 1962. Interspecific incompatibility in *Solanum* species. *Am. J. Bot.* **49**: 874–882.
- Paz, M.M., and Veilleux, R.E. 1999. Influence of culture medium and in vitro conditions on shoot regeneration in *Solanum phureja* monoploids and fertility of regenerated doubled monoploids. *Plant Breed.* **118**: 53–57.
- Peloquin, S.J., Yerk, G.L., Werner, J.E., and Darmo, E. 1989. Potato breeding with haploids and $2n$ gametes. *Genome*, **31**: 1000–1004.
- Pfeiffer, T.W., and Bingham, E.T. 1983. Improvement of fertility and herbage yield by selection within two-allele populations of tetraploid alfalfa. *Crop Sci.* **23**: 633–636.
- Phumichai, C., Kamijima, O., and Hosaka, K. 2004. Inbreeding process of an outbreeding diploid potato monitored using DNA markers. *Mem. Grad. School Sci. Technol. Kobe University*, **22**: 115–121.
- Pushkarnath, 1942. Studies on sterility in potatoes. 1. The genetics of self- and cross-incompatibilities. *Indian J. Genet. Plant Breed.* **2**: 11–36.
- Ray, I.M., and Bingham, E.T. 1992. Inbreeding cultivated alfalfa at the diploid level by selfing and sib-mating. *Crop Sci.* **32**: 336–339.
- Ross, H. 1986. Potato breeding—problems and perspectives. Verlag Paul Parey, Berlin, Germany.
- Scotti, C., Pupilli, F., Salvi, S., and Arcioni, S. 2000. Variation in vigour and in RFLP-estimated heterozygosity by selfing tetraploid alfalfa: new perspectives for the use of selfing in alfalfa breeding. *Theor. Appl. Genet.* **101**: 120–125.
- Shonnard, G.C., and Peloquin, S.J. 1991. Performance of true potato seed families. 1. Effect of level of inbreeding. *Potato Res.* **34**: 397–407.
- Singh, A.K., Uhrig, H., and Salamini, F. 1988. Implications of chromosome pairing in a monohaploid and its colchidiploid of *Solanum tuberosum* ($x = 12$). *Genome*, **30**: 347–351.
- Stephenson, A.G., Hayes, C.N., Jannsson, M.H., and Winsor, J.A. 2001. The performance of microgametophytes is affected by inbreeding depression and hybrid vigor in the sporophytic generation. *Sex. Plant Reprod.* **14**: 77–83.
- Tanksley, S.D., Ganai, M.W., Prince, J.P., de Vicente, M.C., Bonierbale, M.W., Broun, P., Fulton, T.M., Giovannoni, J.J., Grandillo, S., Martin, G.B., Messeguer, R., Miller, J.C., Miller, L., Paterson, A.H., Pineda, O., Roder, M.S., Wing, R.A., Wu, W., and Young, N.D. 1992. High density molecular linkage maps of the tomato and potato genomes. *Genetics*, **132**: 1141–1160.
- Uijtewaal, B.A., Huigen, D.J., and Hermesen, J.G.Th. 1987a. Production of potato monohaploids ($2n = x = 12$) through prickles pollination. *Theor. Appl. Genet.* **73**: 751–758.
- Uijtewaal, B.A., Jacobsen, E., and Hermesen, J.G.Th. 1987b. Morphology and vigour of monohaploid potato clones, their corresponding homozygous diploids and tetraploids and their heterozygous diploid parent. *Euphytica*, **36**: 745–753.
- van Breukelen, E.W.M., Ramanna, M.S., and Hermesen, J.G.Th. 1975. Monohaploids ($n = x = 12$) from autotetraploid *Solanum tuberosum* ($2n = 4x = 48$) through two successive cycles of female parthenogenesis. *Euphytica*, **24**: 567–574.
- van Breukelen, E.W.M., Ramanna, M.S., and Hermesen, J.G.Th. 1977. Parthenogenetic monohaploids ($2n = x = 12$) from *Solanum tuberosum* L., and *S. verrucosum* Schlecht., and the production of homozygous potato diploids. *Euphytica*, **26**: 263–271.
- Veilleux, R.E., Booze-Daniels, J., and Pehu, E. 1985. Anther cul-

- ture of a $2n$ pollen producing clone of *Solanum phureja* Juz. & Buk. Can. J. Genet. Cytol. **27**: 559–564.
- Wenzel, G., Schieder, O., Przewozny, T., Sopory, S.K., and Melchers, G. 1979. Comparison of single cell culture derived *Solanum tuberosum* L. plants and a model for their application in breeding programs. Theor. Appl. Genet. **55**: 49–55.
- Wilsie, C.P. 1958. Effect of inbreeding on fertility and vigor of alfalfa. Agron. J. **50**: 182–185.