DERIVATION, FERTILITY AND BREEDING VALUE OF DOUBLED MONOPLOIDS FROM THE DIPLOID POTATO SPECIES, SOLANUM PHUREJA

by

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Dissertation submitted to the Faculty of the

Virginia Polytechnic Institute and State University

in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

in

Horticulture

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August 20, 1990

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(ABSTRACT)

Thirty-two monoploids *(2n=x=12)* derived by anther culture of ten diploid clones of *Solanum phureja* were used to generate doubled monoploids through in vitro shoot regeneration. Doubled monoploids were compared to the anther donor and progenitor monoploids for morphological characteristics, and were evaluated for fertility in the greenhouse and progeny performance under field conditions.

Monoploids varied for frequency and earliness of shoot regeneration, number of shoots formed per explant and frequency of chromosome doubling among regenerated shoots. Regeneration was greater when stock plantlets were frequently subcultured (2- or 4-week intervals) and maintained under a 16 h photoperiod, and when explants were incubated at 20°C compared to 25°C. In addition, leaf explants regenerated at higher frequencies than stem explants.

Significant high correlations between monoploids and their doubled monoploids were observed for 14 of 17 characters in the greenhouse. Doubled monoploids were significantly greater than monoploids for 15 characters, indicating a

positive effect of increasing gene dosage from monoploid to diploid. The anther donor was not significantly greater than the mean of doubled monoploids for 10 characters; therefore, for specific characters, doubled monoploids without homozygote depression can be obtained.

Doubled monoploids varied for number of days to flower, duration of flowering, abundance of flowers, flower quality, fruit set and seed set; they had lower fruit and seed set than the anther donor. A few clones produced low levels of stainable pollen which had high $2n$ pollen frequency but did not germinate in vitro. Therefore, they were considered male-sterile for practical purposes. Used as female parents, doubled monoploids were able to transmit the $2n$ pollen trait to their progenies.

Two of four doubled monoploids exhibited superior general combining ability over the anther donor under field conditions. This demonstrates the potential of passage of a heterozygous genotype through the monoploid sieve. The advantage of the monoploid sieve may be more or less evident depending on the combining ability of the crossing partner and variable performance can be expected among doubled monoploids from an unselected anther donor. The performance of unselected doubled monoploids demonstrates the potential for their utilization in breeding and warrants further research in the area.

Dedication

To my mother Alice, my late father M'Ribu and late uncle Karema.

Acknowledgements

This work was made possible by contributions from various persons whom I wish to thank very much.

First, I wish to express my gratitude to my advisor, Dr. Richard E. Veilleux, for his commitment to professional development, his friendly approach and for that extra 'minute' that I always asked for (and got). I also thank the members of my advisory committee, Drs. Glenn R. Buss, Khidir W. Hilu, Mosbah Kushad, Bruce Wallace and Thomas A. Fretz for their suggestions at various stages of my work.

Thanks to the Government of Kenya and the USAID for financial support through the IDAT project; the Administration at Egerton University for nominating me for the Scholarship; and the staff of the International Agriculture office at the University of Illinois Urbana-Champaign for the coordination. Special thanks to Dr. John Santas for frequent consultations and the regular cheque.

Thanks to Dr. John Caldwell for letting me use his car; to Dr. Thomas Fretz the former head of Horticulture, for assistance to attend the American Society for Horticultural Science meeting at Michigan State University; to Dr. Robert Wright the current head, for the field trip to the Eastern shore. To all the faculty, staff and graduate students of the Horticulture Department, thanks for the friendly atmosphere that made me feel I was in the right place.

To Karen, Zachary and Micah Veilleux for making me part of them; to Rita Lugogo, David Mbogo and Kenneth Kyamulesire for keeping the soul near home; to Suzanne Piovano, Lucy Taylor, Carol Leda, Chi Wang, Thomas Taylor, Michael Compton, Jianping Cheng and Chong Singsit for the times shared in Saunders Hall; to Elbert Perfater and Walter Reynolds for keeping the greenhouse green; to Michele Marini for computer assistance; and to Joyce Shelton for secretarial assistance.

And finally, special appreciation to my wife Kajuju, daughter Makena, and sons Mutua, Mwenda and Mbabu for their encouraging letters, for understanding that I had to be away from them 'for a while' and for bearing the loneliness.

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Chapter 1: Literature review

Introduction

The potato, *Solanum tuberosum* L., ranks fourth among major world food crops, after wheat, rice and maize. In addition, it produces more food per unit area and time, and is nutritionally superior to most others. Although the crop is native to South America (Correll 1962; Simmonds 1976; Hawkes 1978; Hosaka & Hanneman 1988; Hanneman 1989), commercial production is predominantly in temperate climates of Europe and North America where leading commercial cultivars have been developed. In tropical regions, production has been growing rapidly and the crop now ranks high among the food crops; however, yields are generally low (International Potato Center 1984; Horton 1987). Cultivars that have been selected primarily in temperate climates have generally been found to be unsuitable for tropical conditions. Therefore, with improvement of production

practices and selection of suitable cultivars, there is potential for increasing potato production in warm tropical climates.

The potato gene pool comprises more than 200 tuber-bearing species in section Tuberarium of the genus *Solanum* (Correll 1962; Simmonds 1976; Hawkes 1978; Hanneman 1989). These species are indigenous to the Western Hemisphere in the region stretching from southwestern United States to the southern tip of Chile; the majority is found in the Andes mountain chain of South America. They belong to a ploidy series ranging from diploid to hexaploid, with a basic chromosome number of $x = 12$. Most wild species are diploid and fewer than ten are relevant to the development of the crop (Simmonds 1976; Hosaka et al. 1984; Hosaka & Hanneman 1988; Hanneman 1989; Plaisted & Hoopes 1989). About eight of the species are cultivated, often in primitive forms, in the highlands of South America. Both the wild and cultivated primitive *Solanum* species represent a diverse gene pool which can be utilised for potato breeding. Only a small fraction of the genetic diversity has been utilised in the development of existing cultivars (Howard 1970; Haynes 1972; Mendoza & Haynes 1974; Hanneman 1989; Peloquin et al. 1989; Plaisted & Hoopes 1989). Therefore, there is tremendous potential for development of potatoes suitable for diverse environments.

Potato breeding

Low genetic diversity in *S. tuberosum* (Howard 1970; Mendoza & Haynes 1974; Mendoza 1989; Plaisted & Hoopes 1989) has caused slow progress in potato breeding; therefore, introduction of genetic diversity from other species is vital. Interspecific hybridization has often been utilized in breeding programs mainly to introduce specific genes for pest resistance. Crossing barriers may exist due to incompatibility, ploidy differences, genomic dissimilarity, endosperm-embryo imbalance or meiotic irregularities in hybrids (Hermsen 1979; Landeo & Hanneman 1979; Smith & Desborough 1986; Singsit & Hanneman 1987; Ehlenfeldt & Hanneman 1988). Ploidy barriers may be overcome by use of diploids which produce unreduced gametes or by haploidization of the tetraploid. *Solanum phureja* Juz. & Buk. ($2n=2x=24$) is one of the cultivated primitive diploid species which has often been used. This species has a high potential for making $4x$-$2x$ hybrids due to its tendency to form $2n$ gametes (Mok & Peloquin 1975; McHale & Lauer 1981; Veilleux & Lauer 1981b; Veilleux et al. 1985; Ross 1986). Hybrids between *S. tuberosum* and diploid species have been shown to perform satisfactorily with regard to some factors of economic importance even without prior selection for these factors in the diploids (Mok & Peloquin 1975; De Jong et al. 1981; McHale & Lauer 1981; Veilleux & Lauer 1981a, Hermundstad & Peloquin 1985; Kidane-Mariam et al. 1985). This demonstrates a high potential for utilization of the wild and cultivated primitive species in cultivar development

programs, after selection and improvement for characters of economic importance.

Classical potato breeding is carried out at the tetraploid ($2n=4x=48$) level. Complications due to multiallelic segregation lead to unreliable predictability of progeny performance (Chase 1963; Neele & Louwes 1986). The analytic breeding scheme proposed by Chase (1963) and later modified by Mendiburu et al. (1974) can alleviate some difficulties experienced in breeding at the tetraploid level. The approach requires selection and breeding at the diploid ($2n=2x=24$) level followed by reversion to the tetraploid for cultivar development. In addition to providing the breeder with a simpler genetic system, breeding at the diploid level facilitates utilization of the large gene pool of diploid tuber-bearing species (Neele & Louwes 1986). Reversion to the tetraploid level may be achieved through direct doubling of the chromosome number, somatic polyploidization or sexual polyploidization. Direct doubling of the diploid chromosome number may not give superior tetraploids (De, Maine 1984, 1985; Neele & Louwes 1986; Uijtewaal et al. 1987b) and thus it is not recommended for cultivar breeding. Somatic polyploidization can be applied for sexually incompatible genotypes which respond to protoplast fusion techniques. Sexual polyploidization [unilateral ($4x$-$2x$) or bilateral ($2x$-$2x$)] is achieved by selection of diploids which produce $2n$ gametes (Mok & Peloquin 1975; Nijs & Peloquin 1975; Mendiburu & Peloquin 1977; McHale & Lauer 1981; Veilleux 1985).

Unreduced ($2n$) gametes have been reported in various *Solanum* species (Quinn et al. 1974; Mok & Peloquin 1975; Nijs & Peloquin 1975; McHale & Lauer 1981;

Veilleux & Lauer 1981b; Veilleux et al. 1981; Hermundstad & Peloquin 1985; Stelly & Peloquin 1986a, 1986b; Owen et al. 1988a; Watanabe & Peloquin 1989). Their role in potato evolution, genetics and breeding has received much attention (Mendiburu et al. 1974; Mok & Peloquin 1975; Nijs & Peloquin 1975; Mendiburu & Peloquin 1977; Ramanna 1979; De Jong et al. 1981; McHale & Lauer 1981; Veilleux & Lauer 1981a, 1981b; Iwanaga & Peloquin 1982; Peloquin 1982; Ehlenfeldt & Hanneman 1984; Iwanaga 1984; Kidane-Mariam et al. 1985; Veilleux 1985; Johnston et al. 1986; Ross 1986; Douches & Quiros 1987, 1988; Singsit et al. 1990). Because 2*n* gametes may carry two alleles simultaneously, they facilitate the transfer and combination in a tetraploid of four alleles from different genomes. This would give maximum heterozygosity for the resultant tetraploid. Heterosis would be realized if the 2*n* gametes are produced through first division restitution (FDR) whereas this approach may not be suitable where the unreduced gametes are produced by second division restitution (Mendiburu et al. 1974; Mok & Peloquin 1975; Mendiburu & Peloquin 1977; Ramanna 1979; McHale & Lauer 1981; Iwanaga 1984; Veilleux 1985; Johnston et al. 1986; Stelly & Peloquin 1986a, 1986b).

Application of haploids in breeding programs requires efficient procedures for producing large numbers of haploids and doubled haploids to provide a diverse pool of genotypes from which selection can be made (Griffing 1975; Hermsen & Ramanna 1981; Yonezawa et al. 1987; Gallais 1988). Haploids have been produced in several *Solanum* species through various methods, including pollination with haploid-inducing genotypes (Hermsen & Verdenius 1973; Breukelen 1981;

Wenzel et al. 1982; Uijtewaal & Hermsen 1986; Uijtewaal et al. 1987a; Caligari et al. 1988) and anther culture (Irikura 1975; Sopory et al. 1978; Weatherhead & Henshaw 1979; Wenzel et al. 1979; Wenzel & Uhrig 1981; Cappadocia et al. 1984; Uhrig 1985; Veilleux et al. 1985; Johansson 1986; Powell & Uhrig 1987; Singsit & Hanneman 1987; Sopory & Bajaj 1987; Uhrig & Salamini 1987; Cappadocia & Ahmim 1988; Owen et al. 1988a; Singsit & Veilleux 1989). Although relatively few haploids per plant have been produced with either method, these approaches seem to have potential for mass production of haploids. In barley, it has been shown that the two procedures may be equally efficient (Devaux 1987) and may produce similar plants (Devaux 1988) whereas, in tobacco, androgenetic haploids derived from inbred lines have been found to be more variable and inferior to gynogenetic ones (De Paepe et al. 1981; Kumashiro & Oinuma 1985; Deaton et al. 1986b; Wernsman et al. 1989). The cause of the androgenic variation among haploids derived from inbred lines was not known; it may be due to mutations during androgenesis (Kumashiro & Oinuma 1985; Deaton et al. 1986b). It appears that, for some species and probably some genotypes within species, the two haploidization procedures may supplement each other in a breeding program.

Both processes of haploidization (i.e., haploid-inducing pollination and anther culture) have been shown to be genetically controlled (Hermsen & Verdenius 1973; Wenzel & Uhrig 1981; Wenzel et al. 1982; Heberle-Bors 1985; Uhrig 1985; Veilleux et al. 1985; Powell & Uhrig 1987; Uhrig & Salamini 1987; Caligari et al. 1988; Singsit & Veilleux 1989). Wenzel et al. (1982) suggested that genotypic

variations in the frequency of haploid production may be related to the action of lethal genes. This means that a genotype with a high genetic load may have poor response. Uhrig & Salamini (1987) and Singsit & Veilleux (1989) have demonstrated that anther culture competence can be bred into nonresponsive genotypes within and across species. Therefore, desirable genotypes that produce adequate numbers of haploids can be developed through selection and breeding.

Utilization of monoploids

An extension of the analytic breeding scheme for potato was proposed by Wenzel et al. (1979), whereby the ploidy may be further reduced to the monoploid (monohaploid, $2n = x = 12$) level. Because monoploids carry a single set of chromosomes, allelic interactions such as dominance effects are absent. All genes are expressed and thus the phenotype is a direct reflection of the genotype in a particular environment. In addition, desirable recessive alleles that are suppressed at higher ploidy can be observed. Selection for vigor at this level, therefore, implies selection for a favorable combination of genes. Because of predominant vegetative propagation and a gametophytic self-incompatibility system, potatoes are highly heterozygous. Some recessives arising from mutations are not expressed but are maintained in the population; therefore the population is likely to carry a high genetic load. In monoploids, genotypes bearing lethal and severely deleterious alleles will not survive. Therefore, viable monoploid plants are expected to possess genomes which are free of lethal alleles; this has been

termed the 'monoploid sieve' (Wenzel et al. 1979). Wenzel et al. (1982) noted that plants derived from microspores are vigorous and may not express inbreeding depression.

Selected monoploid genotypes can be incorporated in the breeding program through somatic or sexual hybridization. For sexual hybridization, the chromosome number must be doubled to give doubled monoploids (homozygous diploids) which can be used as inbred lines. Somatic hybridization utilizes protoplast fusion which may be employed to combine genomes of sexually incompatible clones (Wenzel et al. 1979, 1982; Roest & Puite 1986; Uijtewaal & Hermsen 1986; Kumar & Cocking 1987). Protoplast culture is a relatively new tool in plant science which has great potential for use in genetic research. Application of protoplast techniques for potato has been attempted in a number of laboratories with varying degrees of success (de Vries & Bokelmann 1986; Debnath & Wenzel 1987; Masson et al. 1987, 1989; Rech et al. 1987; Uijtewaal et al. 1987c; Deimling et al. 1988; Fish et al. 1988; Puite et al. 1988; Masson et al. 1989; Sree Ramulu et al. 1989; Waara et al. 1989; Cheng 1990). Response to protoplast techniques is genetically determined and can be transmitted to nonresponding clones through breeding (Cheng 1990). Although protoplast techniques have been successfully applied to only a few potato genotypes, it is expected that, with development of new techniques, more genotypes can be fused and regenerated.

Utilization of doubled monoploids

It is difficult to predict the performance of the ultimate tetraploid from the phenotypes of its heterozygous diploid progenitors; therefore, selection at the diploid level may be unreliable for tetraploid cultivar development. Selection for desirable traits can be simplified by using homozygous diploid lines. However, because of frequent sterility and self-incompatibility occuring in most diploid *Solanum* species (Simmonds 1976; Hermundstad & Peloquin 1985), it is not practical to produce inbred lines by self-pollination. Doubled monoploids have therefore been proposed as an alternative source of homozygous lines (Wenzel et al. 1979; Neele & Louwes 1986; Ross 1986; Uijtewaal & Hermsen 1986). Doubled monoploids should be completely homozygous, equivalent to pure lines which have been inbred for several generations. Selection at this level would be efficient because the doubled monoploid phenotype is a direct reflection of the genotype. However, there may be phenotypic variation caused by the increased gene dosage following chromosome doubling (De, Maine 1985; Uijtewaal et al. 1987b).

Utilization of doubled monoploids in practical plant breeding requires efficient methods of doubling the chromosome number. This can be achieved by treatment with antimitotic agents such as colchicine (Weatherhead & Henshaw 1979; De, Maine & Fantes 1983; De, Maine 1985) or through adventitious shoot regeneration from callus (Jacobsen 1981; Webb et al. 1983; Karp et al. 1984; Wheeler et al. 1985; Fish & Jones 1988; Hovenkamp-Hermelink et al. 1988).

Both methods may give varying responses among genotypes and may yield small proportions of plantlets at the desired ploidy level. The potato is particularly amenable to cell and tissue culture manipulations (Espinoza et al. 1986; Karp et al. 1989; Lizarraga et al. 1989), therefore, in vitro techniques may be applied to double chromosomes, especially for clones maintained in vitro.

Favorable genotypes may be selected at the monoploid stage where allelic interactions are absent. The desired characters should remain stable through the diploidization process and be expressed in the doubled monoploid and later generations. Genetic stability of the monoploids and doubled monoploids is important; stability at this stage may be related to stability in later generations. Because genetic changes often occur during in vitro processes (Larkin & Scowcroft 1981; Austin & Cassells 1983; Wheeler et al. 1985; Evans et al. 1986; Sree Ramulu & Dijkhuis 1986; Allicchio et al. 1987; Karp et al. 1989), characters selected in the monoploid may not be expressed in the doubled monoploid. Whereas somaclonal variation would increase variability among the doubled monoploids, it would be undesirable following selection at the monoploid level. Spontaneous ploidy changes from monoploid to diploid may not hinder application of doubled monoploids but would interfere with utilization of monoploids through protoplast fusion. Changes from diploid to higher ploidy levels would be undesirable in both cases. Ploidy stability seems to be influenced by genotype and thus can be selected (Uijtewaal & Hermsen 1986; Owen et al. 1988b). Monoploid selections of *S. phureja* have been stable over several years, both in vitro and in vivo (Veilleux et al. 1985).

Fertile doubled monoploids selected for characters of economic importance may be incorporated into breeding programs through sexual hybridization. Partial or full fertility is a prerequisite to sexual hybridization. Variation for fertility has been observed in doubled haploids of various crop species including *Solanum verrucosum* (Weatherhead & Henshaw 1979), *Nicotiana sylvestris* (De Paepe et al. 1981), *Solanum tuberosum* (Breukelen 1981; Uijtewaal et al. 1987b), *Nicotiana tabacum* (Kumashiro & Oinuma 1985; Deaton et al. 1986a, 1986b), *Solanum chacoense* (Cappadocia et al. 1986), *Solanum phureja* (Owen 1987), *Theobroma cacao* (Lanaud 1987), *Hordeum vulgare* (Devaux 1988) and *Zea mays* (Wan et al. 1989). It appears that fertile doubled haploids can be selected from both heterozygous and inbred clones. It is not known how such doubled haploids or their progeny would perform.

Doubled monoploid lines can be used in recurrent selection (Griffing 1975; Gallais 1988) or to produce second cycle monoploids (Deaton et al. 1986a, 1986b; Wernsman et al. 1989). Gallais (1988) recommended line development by selection of a single doubled monoploid per plant. For tetraploids, Uhrig and Salamini (1987) proposed a cyclic breeding procedure involving anther culture and alternation of ploidy level. There is conflicting evidence on the performance of doubled haploids relative to conventionally derived homozygous lines (Fehr 1987). Some evidence suggests that doubled haploid lines may be inferior to conventional inbred lines of tobacco (Kumashiro & Oinuma 1985; Deaton et al. 1986a; Wernsman et al. 1989) and barley (Powell et al. 1986) whereas other evidence suggests that there may be no difference in performance in barley (Park

et al. 1976; Reinbergs et al. 1978) or cotton (Mahill et al. 1984). Deaton et al. (1986b) and Wernsman et al. (1989) did not observe any loss in vigor in the second-cycle doubled haploids compared to inbred lines of tobacco. Variation and loss in vigor are related to spontaneous variation present in the gametic pool of the diploid plant from which the haploids had been extracted (De Paepe et al. 1981; Kumashiro & Oinuma 1985; Deaton et al. 1986a, 1986b; Wernsman et al. 1989). If the doubling process does not induce changes, this variation can be fixed at the haploid stage. Differences between androgenetic, gynogenetic or conventionally derived homozygous lines would be related to the selection intensity of the procedure and whether there is residual heterozygosity. In vitro methods may allow genotypes to survive that would not survive under in vivo conditions. In addition, conventionally derived inbred lines have continuously been selected for fertility and seed set and they may have some residual heterozygosity.

Objectives

The objectives of this research were: 1) to determine the influence of various factors on regeneration of doubled monoploid shoots from in vitro monoploids of *Solanum phureja*; 2) to examine morphological and yield-attributing traits of monoploids and their corresponding doubled monoploids in order to assess the predictive ability of monoploids on doubled monoploid performance; 3) to study male and female fertility of doubled monoploids in order to determine their po-

tential in breeding programs; and 4) to examine the combining ability of selected doubled monoploids in crosses with heterozygous diploid clones of *S. phureja.*

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Chapter 2: Effect of genotype, explant, subculture interval and environmental conditions on regeneration of shoots from in vitro monoploids

Summary

In vitro anther-derived monoploids *(2n = x = 12)* of *Solanum phureja* were compared for shoot regeneration from leaf and stem explants under various environmental conditions. Monoploids from the same or different diploid clones varied for frequency and earliness of shoot regeneration and number of shoots formed per explant. Leaf explants regenerated at higher frequencies than stem explants. Explants from stock plantlets subcultured at a 2- or 4-week interval regenerated earlier and at a higher frequency than those from plantlets subcultured at longer intervals. Regeneration frequency and number of shoots per explant were greater when explants were incubated at 20°C compared to 25°C.

Explants from stock plantlets maintained under a 16 h as opposed to an 11 h photoperiod exhibited increased shoot regeneration; however, neither photoperiod nor the maintenance temperature of the stock plantlets influenced regeneration frequency. Genotypic differences were observed for the frequency of chromosome doubling among regenerated shoots whereas temperature treatments had no influence on chromosome doubling.

Key words: Anther-derived plants, chromosome doubling, monoploid, potato, regeneration, *Solanum phureja*

Abbreviations: BA - benzyladenine; GA_3 - gibberellic acid; IAA - indole-3-acetic acid; NAA - α-naphthaleneacetic acid; ZEA - zeatin

Introduction

Application of doubled haploids in plant breeding bypasses barriers to repeated self-pollination, enables rapid production of homozygous lines and may permit reliable selection in early generations (Hermsen and Ramanna 1981). In addition, passage of germplasm through a monoploid cycle can eliminate lethal and deleterious genes through the "monoploid sieve" (Wenzel et al. 1979).

Success of this approach in practical plant breeding depends primarily on the efficiency of producing both haploids and doubled haploids. Haploid plants have been produced through various methods in several *Solanum* species (van Breukelen 1981; Sopory and Bajaj 1987). The chromosome number can be doubled

by treatment with antimitotic agents such as colchicine (De, Maine and Fantes 1983) or through adventitious shoot regeneration from callus (Jacobsen 1981; Karp et al. 1984; Wheeler et al. 1985). Colchicine treatment is a tedious process, which often yields few plants at the desired ploidy level and gives varying responses among genotypes. In addition, periclinal chimeras may be formed (De, Maine and Fantes 1983).

For successful chromosome doubling through callus culture, the frequency of doubled cells must be sufficient to contribute to meristematic centers preceding shoot regeneration. Regeneration is influenced by various factors including the physical environment, culture medium, genotype and type of explant (Ahloowalia 1982; Webb et al. 1983; Wheeler et al. 1985; Ochatt and Caso 1986; Fish and Jones 1988; Lillo 1989). The technique fails if a genotype does not regenerate or if regeneration occurs without doubling. Somaclonal variants may also occur among the regenerants (Wheeler et al. 1985; Karp et al. 1989).

Determining the conditions that enhance shoot regeneration for various genotypes would increase the practicability of this approach. High regeneration rates would increase the chances of acquiring shoots at the desired ploidy and would also facilitate studies in somaclonal variation. The objectives of this study were: a) to determine the variability for morphogenesis among in vitro monoploids derived from a single diploid clone; and b) to investigate the effect of various factors including genotype, type of explant, subculture interval, growth regulator, photoperiod and temperature on shoot regeneration from in vitro monoploids of potato.

Materials and Methods

Plant materials

Explants were taken from monoploids *(2n=x=12)* of *Solanum phureja* Juz. & Buk. that had been derived by anther culture of diploid clones selected for photoperiod adaptation (Haynes 1972), *2n* pollen production (Veilleux and Lauer 1981) and anther culture competence (Veilleux et al. 1985). The various monoploid sources are shown in Table 1. Monoploids from clones PP5, D, F and H had been in culture for three years, those from AMB3, BC1, BC13, 2PP-2 and PP2-5 for two years, and those from A3P4-3 for seven months. Stock plantlets were maintained in vitro through periodic (approximately 4 week) shoot subculture on MS basal medium (Murashige and Skoog 1962) + 3% sucrose + 0.7% agar (Gibco Phytagar), at 25°C and 16 hour photoperiod (50 μmol m^{-2} s^{-1}). In these experiments, unless otherwise stated, the cultures were incubated under these conditions.

Variability for shoot regeneration among monoploids from one clone

Seventeen monoploids derived from a heterozygous diploid clone, PP5 (Veilleux et al. 1985), were compared for morphogenesis using a two-step procedure (Webb et al. 1983; Karp et al. 1984). Leaf disc explants (approximately 4 mm wide, 10-20 mg) were wounded by slicing lightly on the surface with a scalpel and cultured in 50 × 10 mm petri plates containing 7.5 ml of callus induction medium: MS salts and vitamins + 10 μM BA + 1 μM NAA + 3% sucrose + 100 mg/l

myo-inositol + 0.6% agar (Gibco Phytagar); pH adjusted to 5.8. After callus induction, the explants were transferred to 25 × 150 mm tubes containing 15 ml of shoot regeneration medium, similar in composition to the callus medium, except for exclusion of NAA and addition of 14.4 μM GA_3. Transfers were made after 14 days on callus medium or when callus had formed over most of the explant surface. Each monoploid was replicated four times.

The number of days from the initial culture to the beginning of shoot formation was recorded. Shoot formation was considered to have started when at least one shoot with visible leaf primordia was observed. The number of explants that formed shoots and the number of shoots per explant were determined after four weeks on the shoot induction medium.

Effect of monoploid genotype, subculture interval, type of explant and growth regulator on shoot regeneration

Four monoploids that regenerated poorly in the previous experiment (AM2, AM7, AM14 and AM16), one that regenerated well (AM26) and three additional unrelated ones (DM10, FM2 and HX2) were used. Shoots were subcultured on MS medium and maintained for four, seven or ten weeks before explants were taken. Five leaf pieces (approximately 2-4 mm diameter) and stem internodes (3-5 mm length) were cultured as in the previous experiment. Four callus induction media were used, differing by growth regulator concentration as follows: a) 10 μM BA + 1 μM NAA; b) 10 μM BA + 0.5 μM NAA; c) 10 μM BA + 1 μM IAA; and d) 10 μM zeatin + 1 μM NAA. After 14 days, the explants were

transferred to shoot regeneration media similar to the respective callus media, with the exclusion of auxin and inclusion of 14.4 μM GA_3. Each treatment combination (genotype × subculture interval × type of explant × growth regulator) was replicated twice. The number of explants exhibiting shoot regeneration and number of shoots per explant were determined after six weeks on shoot regeneration medium.

Effect of photoperiod and temperature at which source plantlets were maintained

Five sibling monoploids (AM2, AM3, AM16, AM20 and AM29), and an unrelated one (DM10) were used. Shoots were subcultured on MS medium and maintained in growth chambers at 20 or 25°C and 11 or 16 h photoperiods for two or four weeks in a factorial combination. Five leaf pieces (approximately 2-4 mm diameter) were cultured on callus induction medium and transferred after two weeks to shoot regeneration medium following the same procedure as in the first experiment. Each treatment combination (genotype × photoperiod × temperature × subculture interval) was replicated twice. After six weeks on shoot regeneration medium, the number of explants exhibiting regeneration and the number of shoots per explant were determined.

Effect of preculture and culture temperature on shoot regeneration

Ten monoploids (AM1, AM33, AMB3-3, AMB3-4, A3P4-3-1, A3P4-3-4, BC1-4, BC13-14, PP2-5-2, 2PP-2-2) derived from seven different diploid clones were used. Shoots were subcultured on MS medium and incubated in growth

chambers at 20 or 25°C and 16 h photoperiod for three weeks. Whole leaf explants were then cultured on callus induction medium as above, with NAA concentrations of 0.5 or 1 μM, at 20 or 25°C. Each treatment combination (genotype × preculture temperature × culture temperature × auxin concentration) was replicated twice. The number of explants exhibiting shoot regeneration and number of shoots per explant were determined after six weeks on shoot regeneration medium.

Effect of genotype and temperature on chromosome doubling

Shoots regenerated from explants given the control treatment (Karp et al. 1984) described in the first experiment were selected for ploidy study from the second and third experiments above and from a concurrent experiment (not reported here). Several shoots from each genotype (5-30 shoots depending on the number available for each genotype) were randomly selected and cultured on MS medium for six weeks. Single in vitro leaflets were examined under a fluorescence microscope at 200× to determine the number of chloroplasts for 10 pairs of guard cells (Singsit & Veilleux, in review). The mean number of chloroplasts per pair of guard cells was used to estimate ploidy: <12, 12-17 and >17, for monoploid, diploid and polyploid, respectively.

From each of the four temperature combinations in the fourth experiment, ten regenerated shoots were randomly sampled from two clones (A3P4-3-1 and 2PP-2-2) and cultured on MS medium for ploidy determination.

Data analysis

Data on days before shoot formation, regeneration frequency and number of shoots per explant were analysed by SAS General Linear Models procedure (SAS Institute 1985).

Results and Discussion

Variability among monoploids derived from one diploid clone

Shoot regeneration was generally high among the sibling monoploids with 10 of 17 forming shoots on all four explants and a mean of 24 shoots per explant (Table 2). Variation among monoploids was apparent; AM16 did not regenerate whereas AM29 yielded a mean of 45 shoots per explant. The number of days before shoot formation and the number of regenerated shoots per explant varied significantly among the monoploids and were negatively correlated ($r = -0.62$, $P < 0.001$). Thus, cultures that formed shoots early also tended to have more shoots. Some monoploids tended to form shoots over the entire surface of the explant, whereas others formed shoots in one or a few clumps on the callus. Similarly, some monoploids continued to regenerate shoots over an extended period whereas others formed shoots only during a brief interval.

Fish & Jones (1988) also observed variation for shoot regeneration among dihaploids derived from the same tetraploid parents. These variations indicate genetic variability for shoot formation within a single clone. The variation among sibling monoploids implies that shoot formation is a quantitative trait. Although

AM16 did not regenerate in this experiment, it did so in other experiments (Table 3). Therefore the capacity to regenerate was not observed to segregate among the sibling monoploids.

Variability among monoploids from different diploid clones

Genotypic differences were observed for regeneration frequency and number of shoots per explant (Table 3). All explants from AM20 and AM29 again regenerated with many shoots as observed in the first experiment. Most explants from AM3 regenerated but with few shoots whereas few explants from AM7 responded but with many shoots. Regeneration frequency and number of shoots per explant were erratic for most of the other genotypes. AM16 was no longer recalcitrant whereas DM10 was completely recalcitrant.

A range of responsiveness was again observed in an expanded range of untested genotypes in the fourth experiment (Table 3). Significant differences were observed in regeneration frequency and shoot number among the genotypes. Although shoot number per explant was highly variable, all the genotypes in this set regenerated at high frequencies.

Effect of subculture interval of source plantlets

Explants taken after 4 weeks since the last subculture of source plantlets formed shoots earlier and had higher regeneration frequencies than those taken after 10 weeks (Table 4). Explants taken after 7 weeks were intermediate and did not differ significantly from those taken after 4 or 10 weeks. The number of

shoots per explant was not significantly influenced by the subculture interval. There was no significant difference between the 2 and 4 week subculture intervals in the third experiment. Therefore, subculture interval may not be critical under routine monthly subculturing but may have a negative influence if cultures are neglected for longer than 4 weeks. There was significant interaction between genotype and subculture interval; some genotypes regenerated more from recently subcultured plantlets whereas others regenerated equally for the three subculture intervals.

Effect of type of explant

Leaf explants exhibited higher regeneration frequency than stems (Table 5). There were no significant differences in the number of days before shoot formation or the number of shoots formed on the two types of explants. Stem explants derived from recently subcultured plantlets tended to have higher regeneration frequency.

Shoot regeneration from leaf, stem, pith, rachis and tuber explants has previously been reported in several potato cultivars (Wheeler et al. 1985; Ochatt and Caso 1986). Because of morphological differences, the explants do not represent equivalent tissues; therefore direct comparison of explants may not be appropriate. It is important however, that various tissues can be used as explants for shoot formation. In vitro monoploids vary in the relative number and size of stems and leaves, therefore the more abundant tissue can be used.

Effect of type and concentration of growth regulator in culture media

The auxin:cytokinin combinations in the culture media had no significant effect on days before shoot formation, regeneration frequency or shoot number (Table 6). Poor survival of explants was observed on the medium containing IAA, confirming Ahloowalia's (1982) report. Webb et al. (1983) and Wheeler et al. (1985) reported variation among cultivars for morphogenetic response to changes in amounts and types of growth regulators in the media. High auxin concentration in the callus stage could inhibit subsequent shoot formation; therefore a reduction in the concentration of auxin during the callus stage may be expected to increase regeneration. However, reduction of NAA concentration by half in the present study did not significantly increase regeneration. Zeatin did not enhance shoot formation compared to BA. This was similar to the results of Wheeler et al. (1985).

Effect of photoperiod and temperature of the source plantlets

Explants from plantlets maintained under a 16 h photoperiod regenerated significantly more shoots than those from plantlets maintained under an 11 h photoperiod whereas photoperiod did not significantly influence regeneration frequency (Table 7). Preculture temperature did not significantly influence regeneration frequency or shoot number. Masson et al. (1987) reported that plantlets of interspecific hybrids between *S. tuberosum* dihaploids and *S. phureja, S. vernei* and *S. stenotomum* were more amenable to protoplast division and subsequent shoot regeneration when maintained under a 13 h photoperiod and

20°C than under a 16 h photoperiod or 24°C. In the present study, a combination of 11 h photoperiod and 25°C inhibited regeneration. However there was no significant interaction between photoperiod and preculture temperature.

Effect of culture incubation temperature

Explants incubated at 20°C had higher regeneration frequency and more shoots per explant than those at 25°C (Table 8). All explants incubated at 20°C regenerated whereas those at 25°C had a mean regeneration frequency of 69%. Shoots were formed earlier on explants incubated at 20°C; after six weeks on shoot regeneration medium, these shoots had grown to a mean length of 17 mm compared to 6 mm for those at 25°C. Lillo (1989) also observed higher shoot regeneration from protoplast calli of *S. tuberosum* incubated at 20°C compared to 25°C. An incubation temperature of 20°C was therefore more suitable than 25°C for shoot regeneration.

Effect of genotype and temperature on ploidy of regenerated shoots

The ploidy of regenerated shoots was dependent upon the monoploid genotype (Table 9). For some genotypes (AM7, AM16), no shoots of higher ploidy were recovered whereas for others (AM26, AM27), all the sampled shoots were diploid or polyploid. The ability to regenerate shoots with higher ploidy may be determined by the level of mixoploidy in the explant tissue and karyological changes during the callus process (Jacobsen 1981; Karp et al. 1984; Wheeler et al. 1985; Sree Ramulu and Dijkhuis 1986; Owen et al. 1988; Pijnacker et al. 1989).

Genotypic differences for frequencies of endopolyploid cells within specific monoploid clones therefore affect the relative ease with which they can be doubled (Sree Ramulu and Dijkhuis 1986; Owen et al. 1988; Karp et al. 1989; Pijnacker et al. 1989). This approach to doubling the chromosome number may not be suitable for some genotypes.

There was no significant difference for ploidy of regenerated shoots among the four temperature regimes. Therefore, any of these treatments may be applied to regenerate shoots with higher ploidy. However, because of earlier and greater shoot regeneration, incubation of explants at 20°C is more suitable for obtaining doubled monoploids through this procedure.

Conclusion

Both related and unrelated monoploids varied for earliness of shoot formation, shoot regeneration frequency, number of shoots formed and pattern and duration of shoot formation. There was no direct relationship between the regeneration frequency and the number of shoots per explant. The observed variations imply that shoot formation is a quantitative process in which several genes may be involved. These variations can be utilized in selecting competent monoploid clones.

Shoot formation was influenced by the subculture interval of the source plantlets. Explants from recently subcultured plantlets regenerated earlier and at a higher frequency; therefore regular frequent (1 month) subculture of stock plantlets is essential, especially for stem explants. Leaf explants regenerated at

higher frequencies than stem explants; however both types of explants may be used effectively.

Explants from stock plantlets maintained under a 16 h as opposed to an 11 h photoperiod before culture exhibited increased shoot regeneration; however neither photoperiod nor the preculture temperature of stock plantlets influenced regeneration frequency.

Both regeneration frequency and the number of shoots per explant increased when explants were incubated at 20°C compared to 25°C. Temperature treatments did not influence chromosome doubling. Therefore, incubation at 20°C is more suitable for obtaining doubled monoploids because of greater shoot regeneration.

Genotypic differences were observed for shoot regeneration and the frequency of chromosome doubling among regenerated shoots. Some monoploids did not regenerate whereas others did not double despite a high regeneration frequency. Although doubled monoploids could be obtained for most of the genotypes examined, alternative methods are required for recalcitrant clones.

Acknowledgements

This research was supported in part by the Virginia Agricultural Experiment Station Project No. 6128030 and the USAID/Kenya Government IDAT project (Egerton University and University of Illinois).

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Table 1. Description of plant materials - all monoploids were derived from diploid clones by anther culture

Monoploid	Diploid source	Pedigree of diploid clone
AM1, AM2, AM3, AM4, AM6, AM7, AM9, AM14, AM16 AM18, AM20, AM21, AM22, AM24, AM26, AM27, AM28, AM29, AM33, AM37, AM39	PP5	*S. phureja* PI 225682 × PI 225669
DM10	D	MN 2999.79-53 × MN 2999.79-137[a]
FM2	F	MN 2999.79-207 × MN 2999.79-155[a]
HX2	H	MN 2999.79-207 × MN 2999.79-155[a]
AMB3-1, AMB3-4	AMB3[b]	AM3-8[c] × B14 *S. berthaultii* PI 265858
A3P4-3-1, A3P4-3-4	A3P4-3	AM3-8 × NBP4[d]
BC1-4	BC1[b]	(PP5 × 1.22) × 1.22[e]
BC13-14	BC13[b]	(PP5 × 1.22) × 1.22[e]
PP2-5-2	PP2-5[b]	PP5 × P20 (*S. phureja* PI 320349)
2PP-2-2	2PP-2[b]	P20 (PI 320349) × PP5

[a]S. *phureja* composite received from F. I. Lauer, University of Minnesota, St. Paul
[b]Selected by C. Singsit
[c]AM3-8 is a doubled monoploid of AM3
[d]NBP4 is an adapted clone of *S. phureja* received as clone 79-36 from H. DeJong, Agriculture Canada, Fredericton, New Brunswick
[e]Haploid inducing pollinator of *S. phureja* received from the Inter-Regional Potato Introduction Station, Sturgeon Bay, Wisconsin

Table 2. Shoot regeneration after four weeks for seventeen monoploid clones derived from one diploid clone

Monoploid clone	Number of explants	Days before shoot formation	Regeneration frequency		Shoot number per explant
			number	%	
AM29	4	25	4	100	45
AM21	4	25	4	100	39
AM9	4	25	4	100	35
AM20	4	29	4	100	32
AM18	4	31	4	100	32
AM27	4	31	4	100	29
AM4	4	31	4	100	23
AM22	4	29	4	100	18
AM3	4	26	1	25	17
AM28	4	31	4	100	15
AM26	4	33	4	100	13
AM24	4	26	3	75	12
AM7	4	36	3	75	8
AM6	4	36	3	75	7
AM2	3	29	1	33	6
AM14	4	42	1	25	3
AM16	4	-	0	0	0
LSD 5%	-	6	-	-	18

Table 3. Shoot regeneration after six weeks for monoploid clones derived from various diploid clones

Monoploid clone	Number of explants	Days before shoot formation[a]	Regeneration frequency		Shoot number per explant
			number	%	
Experiment 2					
AM7	48	23	2	4.2	33
AM16	47	33	5	10.6	14
AM26	47	43	11	23.4	5
HX2	46	48	10	21.7	4
AM2	48	31	6	12.5	4
FM2	24	42	2	8.3	3
AM14	47	48	2	4.3	2
DM10	48	-	0	0.0	0
LSD 5%	-	14	-	12.6	9
Experiment 3					
AM29	14	-	14	100.0	40
AM20	12	-	12	100.0	36
AM3	16	-	15	93.8	8
AM16	16	-	4	25.0	2
AM2	16	-	4	25.0	2
DM10	16	-	0	0.0	0
LSD 5%	-	-	-	20.9	12
Experiment 4					
A3P4-3-1	13	-	12	92.3	31
2PP-2-2	16	-	16	100.0	27
AMB3-3	14	-	9	64.3	20
PP2-5-2	16	-	14	87.5	19
AMB3-4	16	-	15	93.8	18
AM33	16	-	10	62.5	17
AM1	16	-	16	100.0	13
A3P4-3-4	16	-	15	93.8	9
BC1-4	16	-	10	62.5	4
BC13-14	16	-	14	87.5	4
LSD 5%	-	-	-	21.2	7

[a]For Experiments 3 and 4, no data were taken on days before shoot formation

Table 4. Shoot regeneration after six weeks for explants that were taken at various intervals since the last subculture of source plantlets

Weeks after subculture	Number of explants	Days before shoot formation	Regeneration frequency		Shoot number per explant
			number	%	
Experiment 2[a]					
4	112	38	20	17.9	5
7	119	40	13	10.9	9
10	124	48	5	4.0	8
LSD 5%	-	9	-	7.5	NS
Experiment 3[a]					
2	47	-	29	61.7	23
4	43	-	20	46.5	23
LSD 5%	-	-	-	NS	NS

[a]Same data as presented for Experiments 2 and 3 in Table 3, summarized over 8 and 6 genotypes, respectively

Table 5. Shoot regeneration after six weeks for leaf and stem explants

Type of explant[a]	Number of explants	Days before shoot formation	Regeneration frequency		Shoot number per explant
			number	%	
Leaf	180	41	26	14.4	6
Stem	175	38	12	6.9	9
LSD 5%	-	NS	-	6.1	NS

[a]Same data as presented for Experiment 2 in Table 3, summarized over 8 genotypes

Table 6. Shoot regeneration after six weeks for various types and concentrations of growth regulators in the media

Growth regulators (μM)	Number of explants	Days before shoot formation	Regeneration frequency		Shoot number per explant
			number	%	
Experiment 2[a]					
10 BA:0.5 NAA	87	40	14	16.1	10
10 BA:1 IAA	89	39	7	7.9	7
10 BA:1 NAA	89	41	7	7.9	5
10 ZEA:1 NAA	90	41	10	11.1	4
LSD 5%	-	NS	-	NS	NS
Experiment 4[a]					
10 BA:0.5 NAA	77	-	62	80.5	16
10 BA:1 NAA	78	-	69	88.5	16
LSD 5%	-	-	-	NS	NS

[a]Same data as presented for Experiments 2 and 4 in Table 3, summarized over 8 and 10 genotypes, respectively

Table 7. Shoot regeneration after six weeks for explants from plantlets grown under different photoperiods

Photoperiod[a] (hr)	Number of explants	Regeneration frequency		Number of shoots per explant
		number	%	
16	47	27	57.4	27
11	43	22	51.2	18
LSD 5%	-	-	NS	6

[a]Same data as presented for Experiment 3 in Table 3, summarized over 6 genotypes

Table 8. Shoot regeneration after six weeks for explants incubated at two temperature levels

Culture temperature[a] (°C)	Number of explants	Regeneration frequency		Number of shoots per explant
		number	%	
20	78	78	100.0	22
25	77	53	68.8	8
LSD 5%	-	-	9.4	3

[a]Same data as presented for Experiment 4 in Table 3, summarized over 10 genotypes

Table 9. Frequencies of estimated ploidy for regenerated shoots of various genotypes based on chloroplast counts in guard cells

Genotype[a]	Number of shoots examined	Frequency of estimated ploidy		
		Monoploid	Diploid	Polyploid
AM1	10	0.40	0.30	0.30
AM2	7	0.43	0.14	0.43
AM3	5	0.40	0.60	0.00
AM7	30	1.00	0.00	0.00
AM16	13	1.00	0.00	0.00
AM26	5	0.00	0.80	0.20
AM27	9	0.00	0.78	0.22
AM28	7	0.43	0.43	0.14
AM37	14	0.36	0.36	0.29
AM39	8	0.75	0.25	0.00
A3P4-3-1	38	0.08	0.55	0.37
2PP-2-2	39	0.23	0.59	0.18

[a]For AM1 through AM39 shoots were randomly sampled from three experiments; for A3P4-3-1 and 2PP-2-2 shoots were sampled from the four temperature treatments in Experiment four

Chapter 3: Phenotypic variation and correlations between monoploids, doubled monoploids and the anther donor of Solanum phureja

Summary

Ten anther-derived monoploids, twelve doubled monoploids and the heterozygous anther donor clone of *Solanum phureja* were compared for six vegetative, eight reproductive and three yield characters in the greenhouse. Significant high correlations were observed between the monoploids and their doubled monoploids for 14 of 17 characters. Therefore, doubled monoploids presumably express the same alleles as monoploids and the phenotypic expression of a monoploid can be used to predict accurately the phenotype of the derived doubled monoploid. Doubled monoploids flowered earlier and were significantly greater than the monoploids for 15 of 17 characters, indicating a positive effect

of increasing gene dosage from monoploid to diploid. Morphological traits can be used to distinguish plants of the two ploidy levels. Significant genotypic differences were observed for all parameters within each ploidy level. The anther donor was not significantly different from the mean of doubled monoploids for 10 of 17 characters. For each of 14 characters, some doubled monoploids exceeded the anther donor. Therefore, doubled monoploids which do not express homozygote depression can be obtained.

Key words: Doubled monoploid, homozygote depression, monoploid, potato, *Solanum phureja*

Introduction

The analytic breeding scheme proposed for potato (Chase 1963) can be enhanced by selection at the monoploid level (monohaploid, $2n = x = 12$) where allelic interactions are absent. Genotypes bearing lethal or severely deleterious alleles are expected to be eliminated through the monoploid sieve (Wenzel et al. 1979). This approach provides a means of producing homozygous lines for self-incompatible species and a rapid alternative to self-pollination for several generations for producing inbred lines of compatible species. Monoploids which express desirable characters can be incorporated into a breeding scheme through protoplast fusion or sexual hybridization after chromosome doubling. Chromosome doubling may be achieved by treatment with colchicine or by adventitious

shoot regeneration from callus (Karp et al. 1984). The latter procedure appears to be practical for potato (M'Ribu & Veilleux 1990).

Desirable characters selected at the monoploid level should be expressed in the derived doubled monoploids; therefore, prediction of the doubled monoploid phenotype from the monoploid phenotype would facilitate selection. This is particularly important for reproductive and yield factors in order to eliminate sterile or low-yielding genotypes early. Stability of traits during diploidization is essential in order that desirable monoploid characters are preserved. Because genetic changes may occur in vitro (Allichio et al. 1987; Karp et al. 1989), doubled monoploid phenotypes may be unpredictable. In addition, the increased gene dosage may influence the phenotype of the presumably homozygous diploid. The objectives of this study were: to determine the correlations for vegetative, reproductive and yield factors between monoploids and their derived doubled monoploids; to determine the phenotypic influence of diploidization; and to compare morphological features of the monoploids and doubled monoploids with the heterozygous diploid anther donor.

Materials and methods

Monoploids of *Solanum phureja* Juz. & Buk. were previously obtained by anther culture of a heterozygous clone selected for 2*n* pollen production (Veilleux & Lauer 1981) and anther culture competence (Veilleux et al. 1985). Doubled monoploids were obtained by adventitious shoot regeneration from monoploids

in a two-step in vitro procedure (Karp et al. 1984; M'Ribu and Veilleux 1990). In vitro plantlets of twelve monoploids, their corresponding doubled monoploids and the anther donor were transferred to the greenhouse. Two of the monoploid clones could not be successfully established in vivo. Several shoot tip cuttings (approximately 5 cm) from recently established in vivo plants were rooted on peat moss under mist. Six cuttings per clone were transferred individually to 4-liter pots three weeks after planting. After establishment in pots, three vigorous plants per clone were selected and placed on benches in a completely random design.

The following vegetative parameters were determined thirteen weeks after potting: plant height from soil level to the highest shoot apex; length of internode as the ratio of height to number of internodes; stem diameter at an internode position approximately half-way up the length of the main stem; leaf length from the leaf axil to the tip of the terminal leaflet on three fully expanded leaves; leaflet length and width along the midrib and across the widest section, respectively, of the terminal leaflet on three fully expanded leaves. The number of days to flowering was determined from transplanting to pots until anthesis of the first flower. The following reproductive parameters were determined during the flowering period for each plant (3-5 samples per plant depending on the flowering potential of each clone): number of flower buds on a representative inflorescence before anthesis; length and diameter of flower buds just before anthesis; number of open flowers on a mature inflorescence; corolla diameter as the maximum distance across a flower one day after anthesis; anther and style lengths at anthesis. Tu-

bers were harvested 22 weeks after the plants were potted. All tubers approximately 1 cm or larger were counted and weighed.

Analysis of variance was performed by the SAS GLM procedure (SAS Institute 1985). Correlations among the various parameters within and between the ploidy levels were determined by the SAS Correlation procedure, employing clonal means.

Results and discussion

Significant high correlations between the monoploids and their corresponding doubled monoploids were observed for all but three parameters (Table 10). Monoploids and their doubled monoploids also corresponded very closely for morphological characters such as branching and general growth patterns, leaf patterns and texture, foliage pigmentation, flowering patterns, flower morphology and pigmentation, stolon development and tuber shape (Figures 1 & 2). Thus, doubled monoploids and their monoploid progenitors could be easily recognized by observation of the flowering pattern, flower morphology and pigmentation. The similarity between monoploids and their corresponding doubled monoploids suggests that the diploidization process had not induced observable mutations; therefore, alleles expressed in the monoploid were generally expressed in the doubled monoploid as well. The phenotype of the monoploid could be used to predict that of the derived doubled monoploid. Therefore, selection of vigorous high-yielding monoploids can be expected to provide vigorous high-yielding dou-

bled monoploids. Monoploids which express poor phenotypes can be eliminated before diploidization.

The doubled monoploids flowered earlier and were significantly greater than the monoploids for all parameters except number of flower buds and number of tubers (Table 11). These results confirmed the report of Uijtewaal et al. (1987) who observed an increase in vigor after doubling monohaploids of *S. tuberosum*. Pehu et al. (1987) also reported various morphological differences between monoploid and diploid anther-derived genotypes. Therefore, a positive effect of increasing gene dosage from monoploid to diploid can be expected. However, a further increase to the tetraploid level may not have a similar effect (De, Maine 1985; Uijtewaal et al. 1987). The observed morphological differences between monoploids and diploids can be utilized to distinguish in vivo monoploids from diploids following anther culture or chromosome doubling. Such screening would reduce the number of plants that need to be examined cytologically.

Significant phenotypic differences were observed among clones for all parameters at both ploidy levels. Uijtewaal et al. (1987) also observed variation among monohaploids derived from a single dihaploid. The monoploid clones in the present study have been shown to vary for regeneration response in tissue culture (M'Ribu & Veilleux 1990). Variability among clones derived from a single heterozygous diploid may be a function of heterozygosity in the anther donor. However, variation has been observed even among haploids derived from highly inbred lines of tobacco (Kumashiro & Oinuma 1985; Deaton et al. 1986a,

1986b). These authors suggested that such variation was partly due to spontaneous mutation during androgenesis. For heterozygous plants, the contribution of such mutations to gametoclonal variation would be difficult to distinguish from that due to meiotic recombination.

The anther donor flowered earlier and was greater than the mean of the doubled monoploids for six characters; it was not significantly different for the other ten characters (Table 11). In general, the doubled monoploid means were distributed around the means of the anther donor clone. Relatively high performance may be expected for the doubled monoploids because they represent a selected gametic sample of the anther donor; however, their performance may be affected by homozygote depression. Homozygote depression is a function of the genetic makeup of the diploid source and the character under observation. Thus doubled monoploids may exhibit homozygote depression for some traits and not others (Weatherhead and Henshaw 1979; Mahill et al. 1984).

In addition to reflecting the gametic pool of the heterozygous diploid donor, the relative performance of doubled monoploids is determined by dominance or additive gene effects on the specific character. For characters determined by additive gene effects, a response equivalent to or greater than the heterozygous diploid can be expected; thus, higher tuber yield can be expected for some doubled monoploids. Except for three reproductive parameters (number of flowers, anther length and style length), there were some doubled monoploids that were superior to the anther donor for each trait (Table 11). This can be interpreted to mean that, for most of the characters examined, additive gene effects may be

more important than dominance effects. Caligari et al. (1987) also reported that some doubled haploid lines of barley surpassed their progenitor F_1 hybrids for all the characters examined. The superiority of some doubled monoploids over their heterozygous source implies that favorable meiotic recombinations had occured during microsporogenesis followed by selection through survival at the monoploid level. Superior performance of homozygous lines also supports the idea that heterosis may result from dispersion of favorable dominant alleles rather than overdominance (Caligari et al. 1987; Gallais 1988).

Reduction in vigor, yield or fertility has been observed in doubled haploids of *Solanum verrucosum* (Weatherhead and Henshaw 1979), *Gossypium hirsutum* (Mahill et al. 1984), *Solanum chacoense* (Cappadocia et al. 1986), *Nicotiana tabacum* (Deaton et al. 1986a; Wernsman et al. 1989), *Theobroma cacao* (Lanaud 1987) and *Solanum tuberosum* (Uijtewaal et al. 1987). No homozygote depression was observed in *Hordeum vulgare* (Reinbergs et al. 1978; Caligari et al. 1987), *X Triticosecale* (Charmet & Branlard 1985), *Triticum aestivum* (Winzeler et al. 1987) or second cycle doubled haploids of *Nicotiana tabacum* (Deaton et al. 1986b; Wernsman et al. 1989). Greater depression may be expected in cross-pollinated heterozygous plants which have accumulated high proportions of sublethal alleles. Because these sublethals may not be eliminated by the monoploid sieve, some of the derived doubled monoploids are likely to be inferior to the heterozygous diploid source.

In order that doubled monoploids be useful in potato breeding, clones which do not flower or have malformed, sterile flowers need to be eliminated. Some

flower malformations may not necessarily be associated with sterility whereas others, such as fused or shrivelled anthers and styles, effectively prevent pollination and/or fruit set. Unless such genotypes have other special characters that warrant their utilization in somatic hybridization schemes, they may be discarded. Knowledge of the number of days before anthesis may be important for programming of operations. Flowering and yield characters in the doubled monoploids may be predicted from the corresponding characters in the monoploids (Table 12). Most of the other monoploid characters did not correlate well with flowering or yield characters in the doubled monoploid. The number of days to anthesis and the number of flowers per inflorescence of the monoploid were found to predict both parameters in the doubled monoploid. In addition, because the patterns, morphology and pigmentation of the monoploid flowers resembled those of the corresponding doubled monoploids, observation of flowering in the monoploids would facilitate elimination of genotypes that are likely to be sterile as diploids. Similarly, the number of tubers, total tuber yield and the average tuber weight in the monoploids may be used to predict the same parameters in the doubled monoploids. Therefore, clones with potentially high breeding value can be selected at the monoploid level.

Acknowledgements

This research was supported in part by the Virginia Agricultural Experiment Station Project No. 6128030 and the USAID/Kenya Government IDAT project (Egerton University and University of Illinois).

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Table 10. Pearson correlation coefficients for vegetative, reproductive and yield parameters between ten anther-derived monoploid clones and their corresponding doubled monoploids

Parameter	Correlation coefficient (r)	Probability (r = 0)
Vegetative		
Height (cm)	0.96	< 0.01
Internode length (cm)	0.95	< 0.01
Stem diameter (mm)	0.91	< 0.01
Leaflet width (cm)	0.91	< 0.01
Leaf length (cm)	0.90	< 0.01
Leaflet length (cm)	0.88	< 0.01
Reproductive		
Flower buds/inflorescence	0.94	< 0.01
Flowers per inflorescence	0.88	< 0.01
Flower bud width (mm)	0.85	< 0.01
Corolla diameter (mm)	0.85	< 0.01
Days to anthesis	0.84	< 0.01
Flower bud length (mm)	0.56	0.09
Anther length (mm)	0.29	0.45
Style length (mm)	0.16	0.69
Yield		
Number of tubers	0.88	< 0.01
Total tuber weight (g)	0.75	0.02
Average tuber weight (g)	0.69	0.04

Table 11. Means and ranges for vegetative, reproductive and yield parameters of ten monoploids, twelve doubled monoploids and the heterozygous anther donor clone

Parameter	Monoploid		Doubled monoploid		Anther donor
	Mean[a]	Range[b]	Mean[a]	Range[b]	Mean
Vegetative					
Height (cm)	68.0	33.7-104.5	79.1	52.7-123.1	103.4 *
Internode length (cm)	1.8	1.1-3.3	2.5	1.5-4.3	3.6 *
Stem diameter (mm)	5.6	4.3-7.1	6.8	4.8-8.8	6.6
Leaf length (cm)	14.9	12.2-17.9	17.9	12.2-22.2	18.2
Leaflet length (cm)	4.4	3.3-5.3	5.4	4.1-6.9	5.4
Leaflet width (cm)	2.1	1.5-2.8	3.0	2.1-4.7	3.0
Reproductive					
Days to anthesis	89.2	65.7-98.7	77.8	47.3-99.0	50.0 *
Flw. buds/inflorescence	8.8	6.3-13.7	8.5	6.0-13.0	10.3
Flower bud length (mm)	8.1	6.9-10.0	9.3	7.4-10.4	9.3
Flower bud width (mm)	5.1	3.8-7.4	6.1	5.0-8.0	6.3
Flowers/inflorescence	3.8	2.0-6.3	5.1	3.0-7.3	9.0 *
Corolla diameter (mm)	22.3	17.3-29.0	26.9	18.7-33.7	26.3
Anther length (mm)	3.6	2.9-4.0	5.0	4.2-5.8	6.4 *
Style length (mm)	5.2	3.6-6.2	6.3	4.6-9.0	9.5 *
Yield					
Number of tubers	14.3	9.0-19.3	11.4	6.5-21.3	11.0
Total tuber weight (g)	117.2	66.0-172.7	204.7	100.5-300.3	254.0 *
Ave. tuber weight (g)	9.2	4.9-16.5	21.3	7.4-43.9	24.7

[a]Significant differences ($P \leq 0.05$) between means of monoploids and doubled monoploids for all parameters except number of flower buds and number of tubers
[b]Range for means of individual monoploid or doubled monoploid clones
*Significant difference ($P \leq 0.05$) between anther donor and mean of doubled monoploids

Table 12. Correlation of monoploid parameters with flowering and yield parameters in the corresponding doubled monoploids

Monoploid parameter	Correlation with doubled monoploid parameter			
	Flowering		Yield	
	Days to anthesis	Number of flowers	Tuber weight	Tuber number
	(r)	(r)	(r)	(r)
Vegetative				
Leaflet length (cm)	-0.70 *	0.41	-0.01	-0.35
Leaflet width (cm)	-0.63	0.67 *	-0.31	0.10
Stem diameter (mm)	-0.52	0.37	-0.05	-0.36
Internode length (cm)	0.49	-0.27	0.31	0.46
Height (cm)	0.37	-0.18	0.29	0.59
Leaf length (cm)	0.13	-0.46	0.38	-0.59
Reproductive				
Days to anthesis	0.84 **	-0.67 *	0.03	0.10
Corolla diameter (mm)	-0.84 **	0.56	-0.03	0.33
Flowers/inflorescence	-0.80 *	0.88 **	-0.22	0.30
Flower bud length (mm)	-0.60	0.47	0.09	0.39
Flower bud width (mm)	-0.50	0.06	0.14	-0.06
Style length (mm)	0.23	-0.73 *	0.46	-0.19
Flw. buds/inflorescence	-0.22	0.28	-0.01	0.11
Anther length (mm)	0.06	-0.44	0.01	-0.46
Yield				
Number of tubers	0.51	0.51	-0.02	0.88 **
Total tuber weight (g)	-0.36	-0.75 *	0.75 *	-0.14
Average tuber weight (g)	0.60	-0.87 **	0.53	-0.56

*, **Significant correlation coefficient at $P \leq 0.05$ and $P \leq 0.01$, respectively

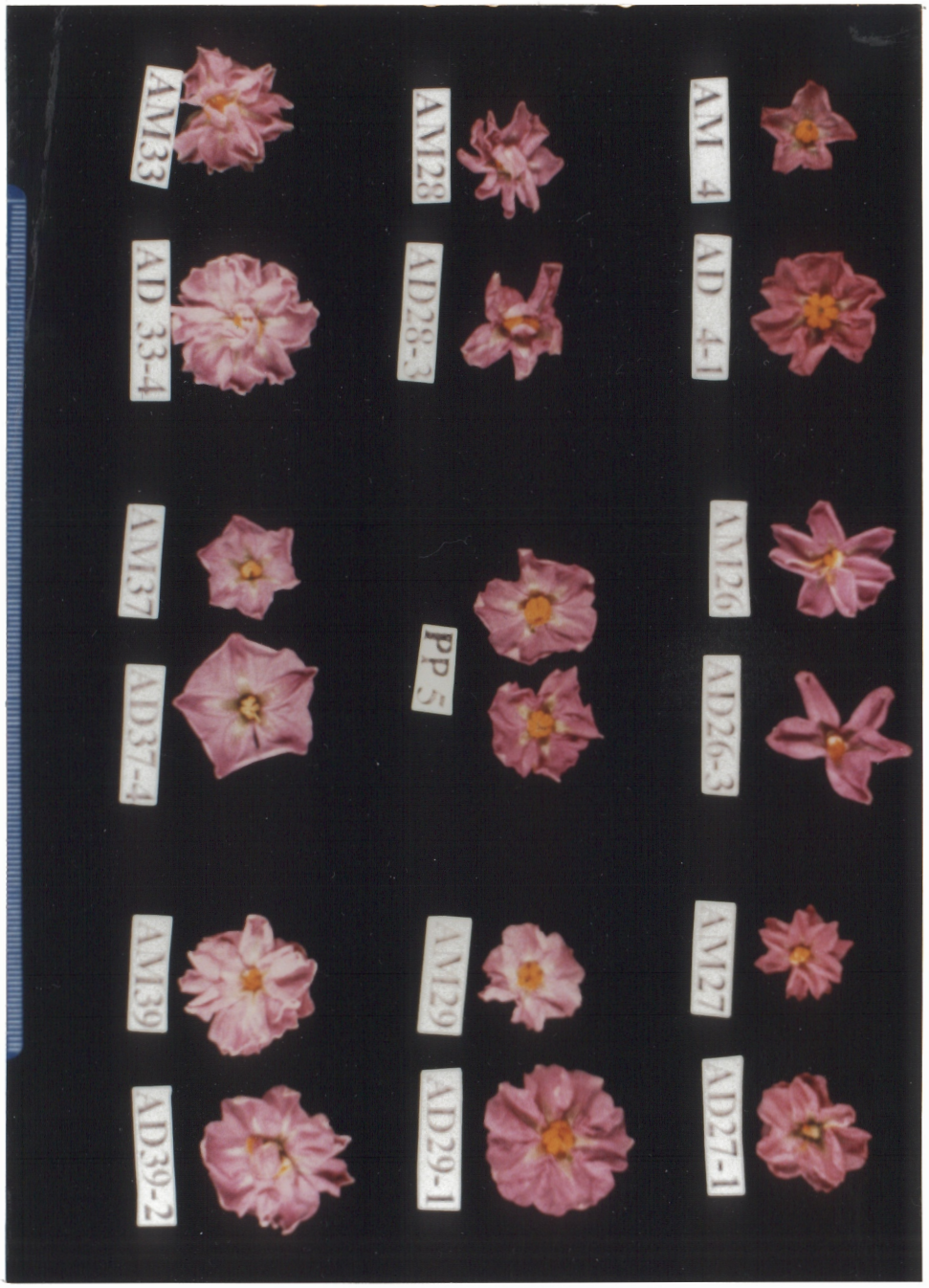

Figure 1. Flowers of eight monoploids, their corresponding doubled monoploids and the anther donor (PP5): the monoploid flower is on the left in each pair

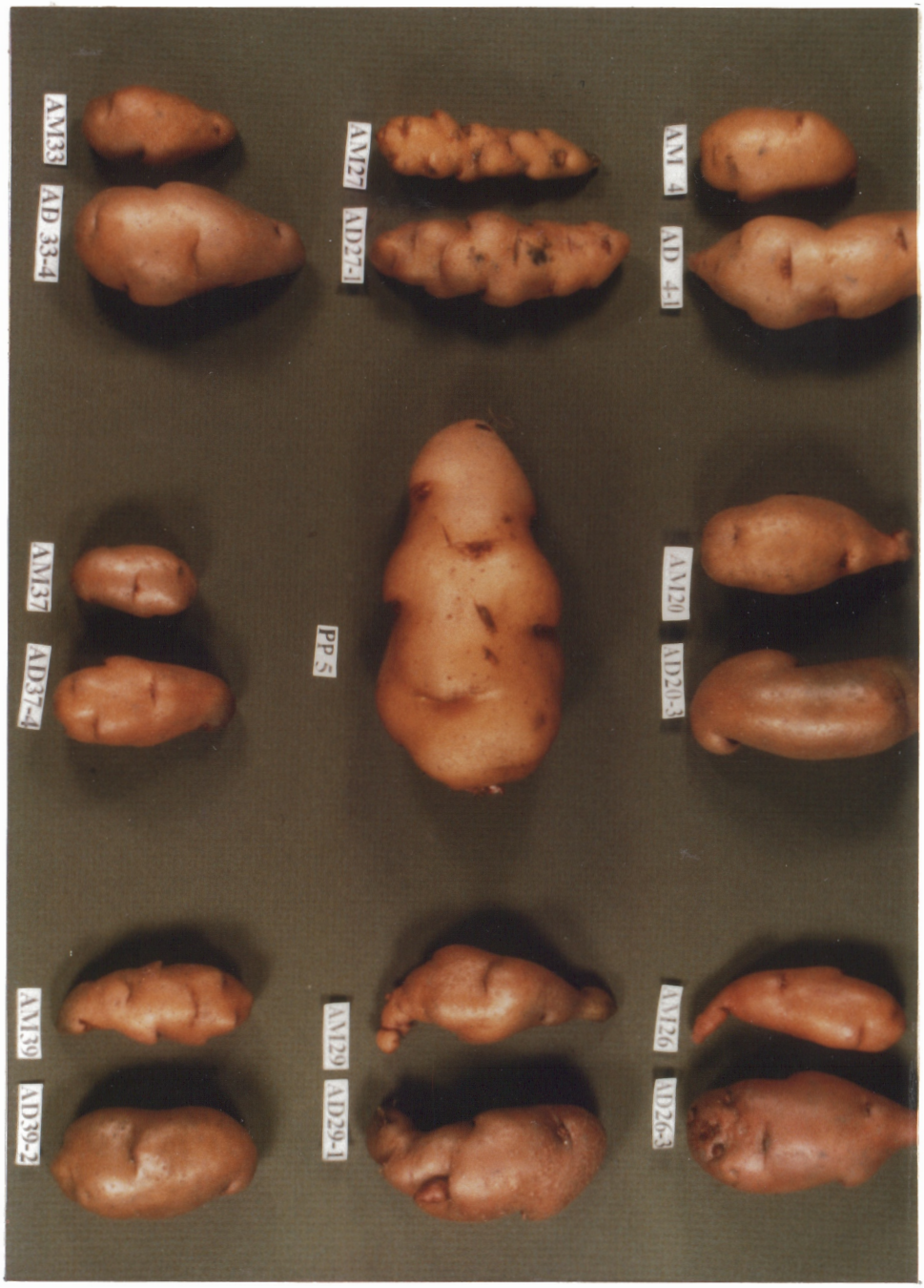

Figure 2. Tubers of eight monoploids, their corresponding doubled monoploids and the anther donor (PP5): the monoploid tuber is on the left in each pair

Chapter 4: Fertility of doubled monoploids of *Solanum phureja*

Summary

Thirteen anther-derived doubled monoploids and their heterozygous diploid anther donor were evaluated in the greenhouse for flowering, fruit set, seed set and pollen fertility. Doubled monoploids varied for number of days to flower, duration of flowering, abundance of flowers and general flower quality. Fruit set on the doubled monoploids varied from 0 to 73% with a mean of 29% compared to the anther donor with 58%. Seed set varied from 9 to 214 seeds per fruit and was significantly lower than the anther donor (380 seeds/fruit). Five doubled monoploids that shed low quantities of pollen exhibited pollen stainability of 6-60% and 2*n* pollen frequency of 20-55%. However, the pollen did not germinate in vitro. It was concluded that doubled monoploids had lower female fer-

tility compared to the anther donor but that they produced sufficient seed to facilitate utilization in a breeding program as female parents. However, they were considered to be male-sterile for practical purposes.

Key words: Doubled monoploid, fertility, homozygote depression, monoploid, potato, *Solanum phureja*

Introduction

Potato germplasm is generally highly heterozygous because of predominant cross-pollination, self-incompatibility and frequent interspecific hybridization. Heterozygosity is also retained by asexual propagation. Specific populations may therefore carry a high genetic load. Breeding populations can be improved by development of homozygous lines which reduce the frequency of lethal and deleterious alleles. Because of frequent sterility and self-incompatibility in *Solanum* species (Hermundstad and Peloquin 1985; Cappadocia et al. 1986; Birhman and Kaul 1989), it is not practical to produce inbred lines of potato by self-pollination. Doubling of haploids derived through parthenogenesis or anther culture appears to be viable for development of inbred lines in potato.

Doubled haploids enable rapid production of homozygous lines and permit early selection (Hermsen & Ramanna 1981; Yonezawa et al. 1987; Gallais 1988a). It is a more efficient means to inbred lines than traditional methods when the total number of plants is restricted (Griffing 1975; Yonezawa et al.

1987). The method bypasses barriers to repeated self-pollination and therefore enables production of homozygous lines even in self-incompatible plants. In addition, passing the genome through the monoploid phase can eliminate deleterious alleles (Wenzel et al. 1979). Doubled monoploids have been successfully produced in various potato species; in a few cases, their hybridization potential has been tested (Breukelen 1981; Cappadocia et al. 1986; Uijtewaal et al. 1987). Desirable clones can be developed through recurrent selection and incorporated into programs for line and hybrid breeding (Griffing 1975; Yonezawa et al. 1987; Gallais 1988a, 1988b).

Fertility of doubled monoploids is prerequisite to their utilization in sexual hybridization. Variation in fertility has been observed in doubled monoploids of potato ranging from full fertility to complete sterility (Weatherhead and Henshaw 1979; Breukelen 1981; Cappadocia et al. 1986; Uijtewaal et al. 1987). It appears that although homozygotization may increase the frequency of sterility, fertile clones can be selected. The objective of this study was to assess the fertility of doubled monoploids derived from a clone of *Solanum phureja* in order to determine their potential for breeding of potato.

Materials and methods

Plant materials

Doubled monoploids were obtained through in vitro shoot regeneration (Karp et al. 1984) from monoploids that were derived by anther culture of a heterozy-

gous clone of *Solanum phureja* (Veilleux et al. 1985). Thirteen doubled monoploids and the anther donor clone were established in vivo from in vitro plantlets. Several shoot tip cuttings (approximately 5 cm) taken 4-8 weeks after establishment of the plantlets in vivo were rooted on peat moss under mist in the greenhouse. Three weeks after planting, six to eight cuttings of each clone were transferred individually into 4-liter pots placed on sand beds. After establishment in pots, four vigorous plants per clone were selected. Four plantings were made: Nov 1988-May 1989, May-Aug 1989, Oct 1989-May 1990 and Jan-June 1990. For each planting, plants were arranged in a completely random design. The plants were fertilized once a week with liquid compound fertilizer (20:10:20) applied in the irrigation water. The flowering potential, female fertility and male fertility were evaluated as described below.

Flowering potential

Flowering data were obtained for the second, third and fourth plantings. The number of days to flowering was determined from transplanting to pots until anthesis of the first flower. The following counts were made during the first flowering phase: number of inflorescences per plant approximately two weeks after the beginning of anthesis; number of flower buds on three inflorescences; and number of open flowers on three mature inflorescences. Length of the flowering period was estimated as short (<3 weeks), moderate (3-6 weeks) or long (>6 weeks). The general appearance of flower parts was examined during each season.

Female fertility

A preliminary crossing study was carried out in the first planting season. Eight doubled monoploids were used as pistillate parents in crosses with 10 hybrid clones of *S. phureja* × *S. chacoense* (PC) and 5 clones of *S. phureja* selected for heat tolerance and 2*n* pollen production. A variable number of pollinations was made (5-133 per doubled monoploid) between March 18 and May 5, 1989. Fruits were harvested approximately eight weeks after pollination and stored for three weeks before seeds were extracted.

Four clones of *S. phureja* (PB1-3, PB8-15, PB10-5 and PB13-15) were selected as pollinators in the subsequent study. Eleven doubled monoploids and the anther donor were planted in Oct 1989. The daylength was extended to approximately 16 hours by artificial lighting from 5 to 11 PM with four mercury vapor lamps (80-165 μmol m^{-2} s^{-1}) until mid Feb 1990. Depending on the flowering potential of each doubled monoploid, 30-247 pollinations per clone (1-69 per specific cross) were made between Dec 25, 1989 and April 28, 1990. Fruits and seeds were handled as above.

Male fertility

Anthers from several flowers were examined for size, shape, presence of pollen pores, presence of pollen and ability to shed pollen at anthesis. Pollen samples were collected on each of three dates from the anther donor (control) and five clones that were found to shed some pollen in Spring 1990. Samples were stained with acetocarmine, cotton blue and fluorescein diacetate. Between 500 and 1000

pollen grains were examined under the microscope (100×) to determine the percentage of stainable pollen. The frequency of $2n$ pollen among the stainable grains was recorded.

Pollen samples of the anther donor and five doubled monoploids were collected at anthesis from several anthers for in vitro germination. Because of low pollen shed, pollen was extracted by lightly tapping the anthers. Pollen germination media was prepared according to Janssen and Hermsen (1976): 20 g sucrose + 5 mg H_3BO_3 + 5 mg $Ca(OH)_2$ in 100 ml H_2O, pH adjusted to 7.0. Each sample was placed on a drop of media on a microscope slide and incubated at 25°C in a covered petri plate humidified with a moist paper towel. After 24, 48 and 72 hours, samples were stained with acetocarmine or cotton blue for microscopic examination. Pollen grains were considered to have germinated if the pollen tube had grown to approximately one fifth the diameter of the grain.

Results and discussion

Flowering potential

Doubled monoploids varied for number of days to flower, duration of flowering, number of inflorescences, number of flower buds and number of open flowers per inflorescence. They could be grouped into three categories according to the overall abundance of flowers (Table 13). AD2-4 and AD3-4 flowered early and produced abundant flowers approximately equal to the anther donor whereas AD20-3 and AD28-3 flowered late and for only a short period thus producing

only a few flowers. All the clones had two flowering phases; AD2-4 and AD21-1 had a less pronounced phasic pattern whereas AD1-3 and AD37-4 had distinct flowering phases.

Doubled monoploids varied for the number of flower buds reaching maturity (Table 13). AD1-3, AD2-4 and AD27-1 retained a high percentage of flower buds whereas most buds aborted on AD28-3, AD20-3 and AD26-3. The clones also varied for the pattern of bud maturity and longevity of flowers after anthesis. In some clones (AD2-4, AD3-4, AD37-4), most flower buds in a single inflorescence opened within two or three days whereas in others (AD29-1, AD33-4 and AD39-2), the flowers opened singly over a 7-10 day period. Some clones retained flowers for several days after anthesis whereas flowers on other clones were short-lived. Because it is convenient to pollinate all flowers in an inflorescence at one time, clones with extended periods of bud maturity pose difficulties in hybridization. On the other hand, clones with short-lived flowers need critical timing for pollination. Clones with long-lasting flowers could be successfully pollinated a few days after anthesis.

The morphology of flowers varied from normal (AD1-3) to predominantly deformed (AD33-4, Fig 3). Some clones had normal pistils whereas others had thin, crooked, short or fused styles often without a stigma. Similarly, anther morphology varied from normal with some pollen shed to short, shrivelled or fused anthers; in some cases, anthers were absent. AD33-4 and AD39-2 had large whorled corollas which were often fused to the anthers and styles. In these clones, the stigma was often absent or generally inaccessible for pollination.

Based on the abundance and quality of flowers, the doubled monoploids were grouped into four categories that reflected their potential for hybridization (Table 14). Clones in the first category with morphologically normal flowers may be expected to be fertile whereas all the others have little or no potential for male fertility. The second, third and fourth categories have high, moderate and low potential for seed set on cross pollination.

Fruit and seed set

In the preliminary experiment, pollinations resulting in fruit set varied among the doubled monoploids from 29 to 68% or 0 to 69% in crosses with the PC hybrid and *S. phureja* pollinators, respectively (Table 15). The number of pollinations resulting in fruit set appeared to be higher for the PC than the *S. phureja* pollinators (48% vs. 28%). Mean seed set varied from 31 to 80 and 31 to 149 seeds per fruit for the PC and *S. phureja* pollinators, respectively. Seed set was generally lower for the PC pollinators. Dissimilarity between the *S. phureja* genome of the doubled monoploids and the genomic contribution of *S. chacoense* to the PC hybrids may be responsible for the lower seed set.

In the second crossing experiment, some clones (AD2-4, AD3-4, AD21-1, AD39-2) flowered for long periods whereas others (AD28-3, AD37-4) flowered only briefly; therefore only a few pollinations could be made on the latter. Pollinations resulting in fruit set varied among the doubled monoploids from 0 to 73% (Table 16). The overall mean for the doubled monoploids was 29% compared to 58% for the anther donor. Therefore, doubled monoploids were less

fertile than the anther donor. In general, clones categorized as having high hybridization potential from the flowering data bore more fruit mainly because more flowers were available for pollination.

Mean seed set varied among the doubled monoploids from 9 to 214 seeds per fruit (Table 17). All the doubled monoploids set fewer seeds than the anther donor (average of 103 and 380 seeds per fruit, respectively). This implies that there was homozygote depression for seed set in the doubled monoploids. Variable seed set has been previously reported in doubled monoploids of potato and other open-pollinated species (Weatherhead and Henshaw 1979; Breukelen 1981; Cappadocia et al. 1986; Lanaud 1987; Uijtewaal et al. 1987). High or low seed set can be expected in doubled haploids from a heterozygous cross-pollinated plant. Although the clones represent a sample of gametes selected by survival at the monoploid level, some may possess sublethal alleles that were not eliminated by the monoploid sieve. In inbred plants where the genetic load is low, doubled monoploids without homozygote depression can be obtained. Doubled haploids of barley (Park et al. 1976; Reinbergs et al. 1978; Caligari et al. 1987; Devaux 1988) and wheat (Buyser et al. 1985; Winzeler et al. 1987) have been shown to produce seed yield equivalent to or greater than standard cultivars or inbred lines produced through conventional methods.

Sterility is a frequent feature in heterozygous potato and can be expected to increase with homozygosity. Homozygote depression and variation in flowering, fruit set and seed set were observed among inbred lines derived from an unselected heterozygous diploid. Although fertility was lower in the doubled mono-

ploids than the anther donor, most of the clones set sufficient fruit and seed for breeding purposes. Low seed set occurring early in a selection program should not be a barrier to further breeding; similar responses were encountered during development of inbred lines of maize. With population improvement and selection of fertile breeding lines, fertility of doubled monoploids can be improved. Some doubled monoploids have been shown to supercede the anther donor in general combining ability for vigor and tuber yield under field conditions (M'Ribu and Veilleux 1990). The progeny, obtained by outcrossing these doubled monoploids with heterozygous clones, flowered profusely and set fruit in the field. It is possible, therefore, that a second cycle of doubled monoploids derived from these progeny would be more fertile.

Pollen fertility

Six of the thirteen doubled monoploids (AD1-3, AD2-4, AD3-4, AD4-1, AD21-1 and AD29-1) had normal-appearing anthers and shed pollen lightly. AD37-4 had a few anthers that occasionally contained pollen but were indehiscent. All other clones had malformed anthers or none at all; these were considered completely male sterile. It appeared that male fertility was more drastically affected by homozygotization. Similar results have been reported for other species (Weatherhead and Henshaw 1979; Cappadocia et al. 1986; Lanaud 1987).

Using three staining methods, we observed high levels of stainable pollen for the anther donor but considerably reduced levels for doubled monoploids that shed pollen (Table 18). The percentage of $2n$ pollen within the stainable class

varied from 20 to 55%, with the doubled monoploids generally having higher frequencies than the anther donor. However, all pollen samples from the doubled monoploids failed to germinate in vitro even after 72 hours whereas in vitro pollen tube growth was readily apparent for the anther donor. Because in vitro germination is better correlated to seed set than stainability (Janssen and Hermsen 1976), the doubled monoploids may be considered to produce sterile pollen. However, germination in vitro can fail even with functional pollen (Heslop-Harrison et al. 1984). In addition, only 22% of pollen of the anther donor germinated in vitro although the clone is known to give high seed set. Therefore, it cannot be concluded categorically that the doubled monoploids were completely male-sterile. Because of insufficient pollen shed, these clones could not be used for in vivo pollinations; therefore, they may be considered male-sterile for practical purposes.

Conclusion

Flowering pattern, abundance of flowers and flower quality varied among thirteen doubled monoploids. The clones expressed various levels of fertility and exhibited poorer flowering, lower fruit set and lower seed set than the anther donor. One clone set no fruit. A few clones produced stainable pollen which did not germinate in vitro. Some of the doubled monoploids were sufficiently fertile to be incorporated into a breeding program as female parents; however, there appears to be low potential for utilization of any of them as male parents. The

observed low fertility may be expected due to homozygote depression in highly heterozygous species. Population improvement probably can facilitate production of suitable breeding lines for extraction of more fertile doubled monoploids. The occurrence of female fertility among first cycle doubled monoploids provides the opportunity for selection of more fertile doubled monoploids in future cycles.

The doubled monoploids of *S. phureja* were more fertile compared to the previously reported doubled monohaploids of *S. tuberosum* for which only a few clones flowered and produced relatively few seeds (Breukelen 1981; Uijtewaal et al. 1987). Because *S. tuberosum* has been selected for vegetative propagation at the tetraploid level, reduced fertility may be expected among its homozygous lines compared to those of sexually propagated diploid species. Inclusion of genomes from diploid species such as *S. phureja* could enhance fertility in the resultant hybrids and increase the potential for breeding TPS and clonal cultivars. Development of adapted genotypes of *S. phureja* as a component of tetraploid breeding lines through analytic breeding schemes is warranted.

Acknowledgements

We thank Drs. F. I. Lauer and F. Bani-Aameur for providing the *S. phureja* × *S. chacoense* pollinator clones. This research was supported in part by the Virginia Agricultural Experiment Station Project No. 6128030, the USAID/Kenya Government IDAT project (Egerton University and University of Illinois), and BARD project No. US-1196-86.

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Table 13. Flowering potential for thirteen doubled monoploids and the anther donor (PP5) grown in the greenhouse in three seasons

Doubled monoploid or anther donor	Days to anthesis	Duration of flowering	Number of inflore-scences	Buds per inflore-scence	Flowers per inflore-scence[a]	Abundance of flowers
PP5	57	long	10	11	10 = 89%	high
AD2-4	58	long	13	8	7 = 85%	high
AD3-4	67	long	8	8	4 = 49%	high
AD39-2	62	long	4	11	8 = 76%	high
AD4-1	83	long	7	7	4 = 62%	high
AD27-1	72	long	3	11	9 = 83%	moderate
AD33-4	73	long	4	9	6 = 67%	moderate
AD21-1	73	long	5	5	3 = 65%	moderate
AD29-1	77	moderate	4	7	4 = 59%	moderate
AD1-3	73	moderate	3	7	6 = 86%	moderate
AD26-3	66	moderate	4	13	6 = 45%	low
AD37-4	74	short	5	12	6 = 52%	low
AD20-3	82	short	2	11	4 = 37%	low
AD28-3	89	short	3	10	3 = 31%	low
LSD 5%	19		3	3	3	

[a]Percentage value is the ratio between the number of open flowers and the number of flower buds per inflorescence

Table 14. Grouping of thirteen doubled monoploid clones for overall hybridization potential as determined by their flowering characteristics

Group flowering characteristics	Hybridization potential	Clones classified in group
Moderate to many flowers Normal pistil and stamens Low pollen shed Normal corolla	High	AD1-3, AD2-4, AD3-4, AD4-1, AD21-1, AD29-1
Few flowers Normal pistil Anthers shrivelled or absent Slightly deformed corolla	Moderate	AD20-3, AD28-3
Few flowers Thin, crooked or normal styles Shrivelled or normal anthers without pollen pores Normal or whorled corolla	Moderate/Low	AD27-1, AD37-4
Few to many flowers Short crooked styles Stigma often absent Anthers few or lacking Anthers shrivelled and fused to pistil or petals Whorled or serrate corolla	Low	AD26-3, AD33-4, AD39-2

Table 15. Pollination, fruit set and seed set for eight doubled monoploids pollinated with ten Phureja × Chacoense (PC) hybrid and five Phureja clones

Doubled monoploid	PC hybrid pollinators			Phureja pollinators		
	Fruits per pollination	Seeds per fruit		Fruits per pollination	Seeds per fruit	
		Mean	Range[a]		Mean	Range[a]
AD2-4	7/12 = 58%	62	54-78	9/13 = 69%	149	128-157
AD3-8	91/133 = 68%	71	35-123	17/52 = 33%	81	63-94
AD27-1	28/49 = 57%	46	33-61	5/14 = 36%	31	21-43
AD20-1	13/43 = 30%	31	7-58	4/17 = 24%	42	29-55
AD29-1	21/72 = 29%	80	39-115	17/76 = 22%	117	96-141
AD6-2	42/112 = 38%	54	36-108	0/16 = 0%	-	-
AD39-2	2/5 = 40%	50	39-61	0/0 = NC[b]	-	-
AD26-1	6/17 = 35%	58	30-74	0/0 = NC[b]	-	-

[a]Range for means of specific crosses
[b]NC - no crossing was made

Table 16. Number of pollinations and fruit set for eleven doubled monoploids and the anther donor (PP5) in crosses with four S. phureja pollinators (PB1-3, PB8-15, PB10-5, PB13-15)

Doubled monoploid or anther donor	Number of fruits per pollination				
	PB1-3	PB8-15	PB10-5	PB13-15	Total
PP5	18/32 = 56%	18/31 = 58%	16/27 = 59%	16/27 = 59%	68/117 = 58%
AD28-3	4/5 = 80%	2/3 = 67%	2/4 = 50%	3/3 = 100%	11/15 = 73%
AD3-4	9/14 = 64%	2/5 = 40%	21/34 = 62%	16/37 = 43%	48/90 = 53%
AD21-1	5/17 = 29%	12/20 = 60%	12/29 = 41%	10/26 = 39%	39/92 = 42%
AD1-3	1/4 = 25%	1/1 = 100%	5/10 = 50%	5/15 = 33%	12/30 = 40%
AD29-1	4/16 = 25%	6/19 = 32%	8/18 = 44%	7/14 = 50%	25/67 = 37%
AD2-4	24/61 = 39%	15/58 = 26%	15/69 = 22%	24/59 = 41%	78/247 = 32%
AD4-1	7/39 = 18%	4/34 = 12%	17/46 = 37%	18/37 = 49%	46/156 = 30%
AD37-4	0/5 = 0%	0/5 = 0%	1/2 = 50%	2/2 = 100%	3/14 = 21%
AD39-2	3/46 = 7%	5/37 = 14%	1/38 = 3%	7/32 = 22%	16/153 = 11%
AD26-3	0/8 = 0%	0/3 = 0%	1/17 = 6%	0/23 = 0%	1/51 = 2%
AD33-4	0/8 = 0%	0/6 = 0%	0/10 = 0%	0/9 = 0%	0/33 = 0%
Total	75/255 = 29%	65/222 = 29%	99/304 = 33%	108/284 = 38%	

Table 17. Seed set for ten doubled monoploids and the anther donor (PP5) in crosses with four S. phureja clones (PB1-3, PB8-15, PB10-5, PB13-15)

Doubled monoploid or anther donor	Number of seeds per fruit				
	PB1-3	PB8-15	PB10-5	PB13-15	Mean
PP5	341	398	381	398	380
AD3-4	208	248	201	199	214
AD37-4	*	*	142	162	152
AD2-4	129	142	114	169	139
AD28-3	90	137	159	152	135
AD29-1	33	142	170	162	127
AD1-3	30	69	176	127	101
AD21-1	54	82	72	53	62
AD39-2	89	71	28	34	56
AD4-1	31	29	40	52	38
AD26-3	*	*	9	*	9
Mean	112	146	136	151	

*No fruit

Table 18. Percent of stainable and 2n pollen for five doubled monoploids and the anther donor (PP5) stained with acetocarmine, cotton blue and fluorescein diacetate

Doubled monoploid or anther donor	% stainable pollen			% 2*n* pollen
	Acetocarmine	Cotton blue	Fluorescein diacetate	
PP5	87	91	86	22
AD1-3	60	20	11	42
AD4-1	22	14	13	45
AD3-4	13	18	10	55
AD2-4	18	10	6	36
AD29-1	15	9	7	20

Figure 3. Flowers of the anther donor (PP5) and eleven doubled monoploids

Chapter 5: Field evaluation of the breeding value of doubled monoploids of Solanum phureja

Summary

Five anther-derived doubled monoploids and the heterozygous anther donor of adapted *S. phureja* were crossed with four heterozygous clones. The doubled monoploids exhibited various forms of male sterility and had less flowering, fruit set and seed set than the anther donor. Tubers produced by F_1 seedlings from crosses of four doubled monoploids and the anther donor with two pollinators were planted in the field in three randomized complete blocks. The plants were evaluated for frequency of *2n* pollen, vigor, tuber yield and tuber morphology. Two doubled monoploids exhibited superior general combining ability over the anther donor. This demonstrates the potential of passage of a genotype through the monoploid sieve. Although the monoploid sieve may rid the genotype of le-

thal alleles, it may not remove nonlethal additive recessives responsible for low vigor and yield. Therefore doubled monoploids with high or low performance can be obtained from an unselected anther donor. Progeny of one pollinator exhibited higher vigor and yield than that of the other. Therefore, the advantage of the monoploid sieve may be more or less evident depending on the combining ability of the crossing partner. Clones within each family could be selected for high frequency of *2n* pollen. Therefore, although the doubled monoploids were apparently pollen sterile, they could transmit the *2n* pollen trait which had been derived from the anther donor.

Key words: Doubled monoploid, *2n* pollen, haploid, potato, *Solanum phureja*

Introduction

The analytic breeding scheme proposed for potato by Chase (1963) can alleviate the difficulties experienced in classical breeding at the tetraploid level ($2n = 4x = 48$). In addition to providing the breeder with a simpler genetic system, breeding potato at the diploid level ($2n = 2x = 24$) facilitates utilization of the large gene pool of diploid tuber-bearing species (Neele and Louwes 1986). Reversion to the tetraploid level may be achieved through unilateral (*4x-2x*) or bilateral (*2x-2x*) sexual polyploidization by selection of diploids which produce *2n* gametes (Mendiburu and Peloquin 1977; McHale and Lauer 1981; Veilleux 1985).

An extension of the analytic breeding scheme for potato was proposed by Wenzel et al. (1979), whereby the ploidy may be further reduced to the monoploid (monohaploid, $2n=x=12$) level. Because monoploids carry only a single set of chromosomes, allelic interactions are absent; therefore the phenotype is a direct reflection of the genotype. Selection for vigor at this level implies selection for a favorable combination of genes. In addition, genotypes bearing lethal or severely deleterious alleles will not be viable as monoploids; therefore, viable monoploid plants are expected to possess genomes which are free of lethal alleles. This elimination of deleterious alleles simply by survival of monoploid genotypes has been termed the "monoploid sieve" (Wenzel et al. 1979).

Selected monoploid genotypes may be incorporated into a breeding program through sexual or somatic hybridization. The latter utilizes protoplast fusion techniques which may be employed to combine genomes of sexually incompatible clones. For sexual hybridization, monoploids may be doubled by treatment with colchicine or by adventitious shoot regeneration from callus (Karp et al. 1984). Doubled monoploids should be completely homozygous, equivalent to inbred lines which are otherwise difficult to obtain in potato due to self-incompatibilities and sterilities. They provide an alternative method for production of inbred lines (Uijtewaal and Hermsen 1986).

Variations for fertility have been observed in doubled haploids of various species including *Nicotiana sylvestris* (De Paepe et al. 1981), *Nicotiana tabacum* (Kumashiro and Oinuma 1985; Deaton et al. 1986), *Theobroma cacao* (Lanaud 1987), *Hordeum vulgare* (Devaux 1988) and *Zea mays* (Wan et al. 1989). In

potato, low levels of fertility have been reported in doubled monoploids of *S. verrucosum* (Weatherhead and Henshaw 1979), *S. tuberosum* (van Breukelen 1981; Uijtewaal et al. 1987), *S. chacoense* (Cappadocia et al. 1986) and *S. phureja* (Owen 1987). It appears that male fertility was more drastically reduced by the increased homozygosity; in some cases complete male sterility was observed whereas female fertility varied. Progeny tests of these doubled monoploids have not been reported.

Doubled monoploids derived from a clone of *S. phureja* have shown variable vigor and reproductive abnormalities including poor flowering, flower bud abortion and flower deformations (Owen 1987). Because these plants produced variable numbers of flowers with normal pistils, it is possible that they can be used as female parents. The objectives of this study were: 1) to assess female fertility of androgenetic doubled monoploids derived from a heterozygous clone of *S. phureja*, 2) to compare the progenies of the doubled monoploids under field conditions with those of the diplandrous parent clone which had been crossed to the same pollinators, and 3) to observe occurrence of *2n* pollen in the progenies of the doubled monoploids.

Materials and methods

Monoploid genotypes of *Solanum phureja* Juz. & Buk. were previously obtained by anther culture of a heterozygous diploid clone (PP5) selected for *2n* pollen production (Veilleux and Lauer 1981) and response to anther culture

(Veilleux et al. 1985). Doubled monoploids were obtained by doubling the chromosome number in a two-step shoot regeneration procedure (Karp et al. 1984). Three plants each of 5 doubled monoploids (AD3-8, AD6-2, AD9-4, AD20-1 and AD29-1) and the anther donor clone were grown in 4-liter pots on soil benches in the greenhouse (Dec 1987 - May 1988). The number of days from planting until anthesis was recorded. The plants were pollinated with fresh pollen from 4 different clones of *S. phureja* (HX9 selected at VPI&SU; 75-21, 79-15 and 79-36 selected for field adaptation and generously provided by Dr. H. De Jong, Agriculture Canada, Fredericton, New Brunswick). The number of pollinations per doubled monoploid varied with its flowering potential. Fruits were harvested 8 - 10 weeks after pollination. The number of fruits and the number of seeds per fruit were recorded.

Ten families with sufficient seed set (AD3-8, AD6-2, AD9-4, AD20-1 and PP5 crosses with 75-21 and 79-36) were selected for the breeding study. Forty seeds per family were soaked for 24 h in a solution of gibberellic acid (1500 ppm), rinsed and sown in seed trays in the greenhouse. Four weeks after germination, the seedlings were transplanted into half-liter pots and placed on soil benches in the greenhouse where they remained until maturity (Sept 1988 - Feb 1989). Tubers were harvested 20 weeks after sowing and stored at 5°C for 7 weeks.

Clones with at least 3 tubers were planted in a field trial at the VPI&SU Horticultural Research Farm in three randomized complete blocks. Single-row family plots (one tuber per clone per plot) were used. The plants were spaced at 100 cm between rows and 75 cm within rows. Plot size varied from 6 to 20 plants

depending on the number of available clones per family. The field plot, which was on a heavy clay loam soil, was improved by incorporation of 250 m^3 of sand per ha before planting. Regular crop management operations were carried out; irrigation was only applied once due to the abundant rainfall in the 1989 growing season. The plot was harvested 24 weeks after planting.

Pollen samples were collected from all clones that flowered sufficiently, 11 weeks after planting. One composite sample from the three replications was obtained for each clone. Samples were prepared for flow cytometry analysis by suspending approximately 0.01 g of sample in 0.5 - 1 ml of buffer solution [1.68 g morpholinopropanesulfonic acid (MOPS) + 3.53 g sodium citrate + 3.66 g magnesium chloride + 1 ml Triton X-100 per liter]. Approximately 0.3 - 0.5 ml of the suspended sample was then filtered through a 60 μm nylon mesh and analysed in a laser flow cytometer (Epics V, Model 752, Coulter Electronics, Hialeah, FL) following the same procedure as Owen et al. (1988). Between 5 and 10 thousand pollen grains per sample were sorted by size into two populations corresponding to *1n* and *2n* pollen. The *2n* pollen frequency in samples from three clones was determined by examination of 1000 pollen grains under a microscope for use as standards.

Plant vigor was assessed 13 weeks after planting on a scale of 1 = low to 4 = high. The weight of tubers from individual plants was recorded at harvest. Tuber morphology was assessed on a scale of 1 = poor to 4 = good. Family plot means were used in analysis of variance for plant vigor, tuber yield and tuber

morphology by the SAS GLM procedure (SAS Institute 1985). Clonal means over the three replications were used to calculate the variation within families.

Results and discussion

Flowering, fruit set and seed set

Compared to the parent clone, flowering on the doubled monoploids was generally delayed and fewer flowers were formed. Flower buds were observed 60 - 70 days after planting for all the doubled monoploids but the number of days before anthesis ranged from 88 to 123 (Table 19). The plants exhibited male sterility ranging from absence of anthers to indehiscent anthers containing stainable pollen. Whereas AD3-8 and AD6-2 produced many normal flowers, AD29-1 and AD9-4 produced only a few. Flowers on AD9-4 exhibited a reduction in the number of anthers and most were shrivelled or fused to the style. A concomitant increase in corolla parts was observed. Most buds on AD20-1 aborted and the few that matured were characterized by shrivelled anthers. However, all the doubled monoploids produced some flowers with sufficiently normal pistils to enable pollination.

Fruit set was generally low for the doubled monoploids (Table 19). Those with normal flower morphology (AD3-8, AD6-2, AD29-1) were not necessarily more fertile than those with deformed flowers (AD9-4, AD20-1). It appeared that fruit set on the doubled monoploids was sensitive to environmental conditions in the greenhouse. Some fruits aborted in the early stages; more fruits formed when

cool overcast weather occured after pollination. All the doubled monoploids set some fruit but some specific crosses failed, probably because of incompatibility reactions. It is apparent that fruit set can be improved by appropriate choice of pollinators and pollination under favorable environmental conditions.

The number of seeds per fruit was generally low for the doubled monoploids compared to the anther donor. Variation for seed set among the doubled monoploids was apparent. In contrast with the low seed set reported by Uijtewaal et al. (1987) for doubled monoploids of *S. tuberosum*, the plants produced 36 - 72 seeds per fruit. Cappadocia et al. (1986) and van Breukelen (1981) also reported variable seed set on several doubled monoploids of *S. chacoense* and *S. tuberosum*, respectively. It appears that fruit and seed set vary among genotypes derived from a single clone, different clones within a species or different species. In this study, although the doubled monoploids were apparently male sterile, they exhibited sufficient female fertility for utilization in a breeding program. It is unlikely however that they could be utilized directly as inbred lines for hybrid seed production on a large scale due to the poor flowering and reduced fertility.

Progeny performance under field conditions

Significant general combining ability for plant vigor and tuber yield was observed among the doubled monoploids and anther donor. Progenies of AD20-1 and AD3-8 were more vigorous than progeny of the anther donor whereas progeny of AD9-4 was less vigorous (Table 20). Similarly, progenies of AD20-1 and

AD3-8 had higher yield than progeny of the anther donor whereas those of the other two doubled monoploids were not significantly different (Table 21).

Progenies of the anther donor were inferior in vigor and yield to average performance of the progenies of all the doubled monoploids. This demonstrates the efficacy of the "monoploid sieve". However, the lack of superiority for the progeny of AD6-2 and the inferiority of the progeny of AD9-4 demonstrates that passage through the monoploid phase may not necessarily increase progeny vigor or yield. These characters are polygenic; therefore, progeny performance is determined by quantitative additive effects of alleles from the doubled monoploid and the pollinator. Whereas the monoploid sieve rids the genome of lethal alleles, it may not remove nonlethal recessives responsible for low vigor or yield. Through random segregation, it may be expected that some of the androgenetic microspores that regenerated into monoploids could have carried a high proportion of these nonlethal but otherwise undesirable alleles. Therefore, a wide range of genotypes can be expected among monoploids from a single heterozygous clone. Without selection for vigor or yield, the doubled monoploid sample may include some poor combiners which may result in low yield for some doubled monoploid progenies.

The heterozygous pollinator used to testcross the doubled monoploids also had a significant impact on estimation of combining ability. Families of 75-21 were more vigorous and yielded more than those of 79-36 (Tables 20 & 21). No significant differences for vigor or yield were observed among the doubled monoploids when 75-21 was the pollinator. On the other hand, families of 79-36 were

more variable and allowed detection of significant differences among the doubled monoploids. These observations suggest that clone 75-21 may have a higher proportion of dominant alleles for vigor and yield which masked differences among the doubled monoploids. Clone 79-36 may have a high proportion of recessive alleles such that its progeny reflected more strongly the differences among the doubled monoploids. Although significant differences for yield were not detected among the families of 79-21 when analyzed separately, it can be observed that the trend of higher yield for AD20-1 and AD3-8 families was consistent with the progenies of 79-36.

The coefficients of variation for both vigor and yield were greater for families of 79-36 than 75-21, especially for the families derived from the anther donor, PP5 (Tables 20 & 21). Because the doubled monoploids were presumably completely homozygous, the observed variation within their progeny families reflects the heterozygosity in the pollinator clone. These observations imply that 79-36 is relatively more heterozygous than 75-21. The generally high coefficients of variation for the PP5 families were expected because of its known heterozygosity (Veilleux et al. 1985).

No significant differences were observed among progeny of the doubled monoploids for tuber morphology (Table 22). However, families of 79-36 had more acceptable tubers than families of 75-21. Variation for tuber morphology within families did not follow any particular trend. Some clones with acceptable tubers were observed in each family. It is apparent, therefore, that high-yielding clones with acceptable tuber shape can be selected.

Occurrence of 2n pollen in the progeny

Among the 109 progeny examined by flow cytometry, the frequency of *2n* pollen ranged from less than 1% to approximately 69%. Several clones in each family exhibited high frequencies (Table 23). The anther donor, PP5, has been observed to have a variable frequency of *2n* pollen due to co-orientation of second division spindles (first division restitution or FDR mechanism) during microsporogenesis (Owen et al. 1988). The pollinators, 75-21 and 79-36, have only exhibited $<3\%$ and $<1\%$ *2n* pollen, respectively. This suggests that the capacity to produce a high frequency of *2n* pollen was inherited from the doubled monoploids which had been derived from a clone selected for the trait. The lack of segregation among the doubled monoploids for ability to convey the trait means that the anther donor clone was homozygous for the gene(s) controlling *2n* pollen production.

The distribution of clones among the *2n* pollen frequency classes differed between the families of the two pollinators. More than 35% of clones from doubled monoploids crossed with 75-21 were in the upper frequency class ($>10\%$), which may be considered sufficiently high for breeding purposes; no clones from these families were in the lowest frequency class. Families of 79-36 on the other hand had approximately 20% of clones in the highest frequency class and nearly 30% in the lowest frequency class. These differences should be related to the difference between the pollinator clones themselves because of the homozygosity of the doubled monoploids. Therefore, doubled monoploids carrying the recessive *2n*

pollen gene(s) can be used to assess the potential for expression of the trait in the progeny of a heterozygous clone.

Even though the doubled monoploids were pollen sterile, they were able to transmit the *2n* pollen trait to their progenies. This has practical implications for utilization of sexual polyploidization to return to the tetraploid level following selection at the monoploid/doubled monoploid levels. Gametes (*2n*) produced through the FDR mechanism or its equivalent preserve maximum heterozygosity present in the parent and facilitate its transfer into a tetraploid through *4x-2x* crosses (Mendiburu and Peloquin 1977; Veilleux 1985). If male-fertile doubled monoploids become available from genotypes producing *2n* gametes, they may be expected to produce *2n* pollen. With development of diplogynous (*2n* egg-producing) doubled monoploids, construction of tetra-allelic tetraploids using a double cross involving four genetically divergent doubled monoploids can be envisioned.

Conclusion

The concept of the monoploid sieve has been proposed for cross-pollinated species which carry a genetic load of lethal and sub-lethal genes. Because of difficulties in obtaining monoploids, doubled monoploids have only recently become available. Therefore, little work has been published on the breeding value of doubled monoploids from cross-pollinated plants.

Doubled monoploids were observed to express low fertility. Compared to inbred lines developed through continued self-pollination, doubled monoploids have been selected only for survival, not for fertility. Reduced fertility is an expected result of inbreeding depression but is not a barrier to continued breeding. In addition, fertility is not necessarily correlated with breeding value. Therefore, fertile doubled monoploids with high breeding value can be obtained. Because diploid species have greater fertility than dihaploids obtained from tetraploids, inclusion of selected diploid germplasm in a breeding program may improve the fertility of doubled monoploids.

Progenies of some doubled monoploids exhibited high plant vigor and high yield. For a polygenic trait, the advantage of the monoploid sieve may be observed if the monoploids were derived from a genotype selected for the relevant trait. The observed variation among doubled monoploids derived from a single clone infers that monoploids/doubled monoploids should be selected. The performance of unselected doubled monoploids as female parents in the present study shows promising potential and warrants further efforts in selection of suitable genotypes for monoploid extraction.

Acknowledgements

We thank Dr. H. De Jong for providing the pollinator clones, Haywood Pyle for his assistance with the Flow Cytometer analysis and Jon Wooge, Suzanne Piovano, Michael Compton, Jianping Cheng, Thomas Taylor, Iteu Hidayat,

Cristie Waxman and Chi Wang for their assistance with the fieldwork. This research was supported in part by the Virginia Agricultural Experiment Station Project No. 6128030, the USAID/Kenya Government IDAT project (Egerton University and University of Illinois), and BARD project No. US-1196-86.

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Table 19. Days before anthesis, number of pollinations, fruit set and seed set for five anther-derived doubled monoploids and the anther donor (PP5) under greenhouse conditions

Doubled monoploid or anther donor	Days before anthesis	Fruits per pollination[a]	Number of seeds per fruit[a]
AD3-8	88	4/74 = 5.4 %	37
AD6-2	88	4/64 = 6.3 %	37
AD9-4	119	13/46 = 28.3 %	72
AD20-1	123	7/34 = 20.6 %	36
AD29-1	101	11/39 = 28.2 %	48
PP5	81	41/53 = 77.4 %	103

[a]Combined data for four different pollinators

Table 20. Plant vigor rating (1 = low, 4 = high) and its variation among progeny families of four doubled monoploids and the anther donor (PP5) crossed with two pollinators (75-21 and 79-36)

Doubled monoploid or anther donor	Plant vigor (scale 1-4)			C V (%)		
	All progeny	75-21 progeny	79-36 progeny	All progeny	75-21 progeny	79-36 progeny
AD20-1	3.24 a[y]	3.12 a[y]	3.37 a[y]	25.0	22.7	29.5
AD3-8	3.17 a	3.50 a	2.84 b	24.5	9.5	33.4
AD6-2	2.91 b	3.19 a	2.63 b	32.6	24.3	48.3
PP5	2.79 b	3.30 a	2.27 c	38.0	17.1	60.9
AD9-4	2.45 c	3.29 a	1.62 d	40.1	24.5	51.9
Mean	2.91	3.28[z]	2.54[z]	28.6	19.5	45.3

[y]Mean separation within columns by Student-Newman-Keuls ($P \leq 0.05$)
[z]Significant difference ($P \leq 0.05$) between 75-21 and 79-36 families

Table 21. Tuber yield (kg/plant) and its variation among progeny families of four doubled monoploids and the anther donor (PP5) crossed with two pollinators (75-21 and 79-36)

Doubled monoploid or anther donor	Tuber yield (kg/plant)			C V (%)		
	All progeny	75-21 progeny	79-36 progeny	All progeny	75-21 progeny	79-36 progeny
AD20-1	1.54 a[y]	1.60 a[y]	1.48 a[y]	39.9	44.2	33.0
AD3-8	1.47 a	1.60 a	1.34 a	43.0	19.2	62.2
AD6-2	1.13 b	1.39 a	0.86 b	49.9	43.2	56.4
PP5	1.07 b	1.38 a	0.76 b	63.6	44.0	94.7
AD9-4	0.81 b	1.25 a	0.36 b	65.3	46.2	57.7
Mean	1.20	1.45[z]	0.96[z]	43.3	38.9	74.1

[y]Mean separation within columns by Student-Newman-Keuls ($P \leq 0.05$)
[z]Significant difference ($P \leq 0.05$) between 75-21 and 79-36 families

Table 22. Tuber morphology rating (1 = poor, 4 = good) and its variation among progeny families of four doubled monoploids and the anther donor (PP5) crossed with two pollinators (75-21 and 79-36)

Doubled monoploid or anther donor	Tuber morphology (scale 1-4)			C V (%)		
	All progeny	75-21 progeny	79-36 progeny	All progeny	75-21 progeny	79-36 progeny
AD20-1	2.40	2.51	2.29	23.9	21.4	28.3
PP5	2.35	1.99	2.71	36.0	35.3	29.3
AD9-4	2.29	2.03	2.56	24.5	23.2	18.0
AD3-8	1.97	1.58	2.36	37.3	25.1	33.2
AD6-2	1.95	1.71	2.19	35.6	31.6	38.3
Mean	2.19	1.96[y]	2.42[y]	29.7	31.2	30.0

[y]Significant difference ($P \leq 0.05$) between 75-21 and 79-36 families

Table 23. Number of clones in each of four 2n pollen classes among progeny families of four doubled monoploids and the anther donor (PP5) crossed with two pollinators (75-21 and 79-36)

Family	2*n* pollen frequency classes							
	0-1%		2-5%		6-10%		>10%	
	Number and frequency of clones in each class							
	n	%	n	%	n	%	n	%
AD3-8 × 75-21	0	0	5	28	5	28	8	44
AD6-2 × 75-21	0	0	4	36	2	18	5	45
AD9-4 × 75-21	0	0	7	35	5	25	8	40
AD20-1 × 75-21	0	0	5	56	3	33	1	11
PP5 × 75-21	3	20	5	33	5	33	2	13
AD3-8 × 79-36	6	38	4	25	3	19	3	19
AD6-2 × 79-36	1	20	3	60	0	0	1	20
AD9-4 × 79-36	0	0	1	100	0	0	0	0
AD20-1 × 79-36	0	0	3	50	2	33	1	17
PP5 × 79-36	4	50	1	13	2	25	1	13

Conclusion

Potato production can be increased by improvement of production practices, improvement of crop protection methods and development of new cultivars. Only a small proportion of the potato germplasm has been used in development of existing cultivars; therefore, there is tremendous potential for improvement of the crop. Utilization of the wide diversity of diploid tuber-bearing *Solanum* species can enhance potato breeding. The presence of polyploid ($2n$) gametes among potato species facilitates interploidy hybridization. In addition, alternative methods such as the use of botanical seeds (TPS) are being investigated. With development of TPS methods, hybrid potato production can be envisioned. This could lead to an increase in world food production similar to the green revolution for grain crops.

Potato populations are highly heterozygous and may carry high genetic load. The wild and primitive cultivated diploid species require selection for characters of economic importance before they can be utilized in breeding schemes for cul-

tivar development. Favorable combinations of genes derived from different genomes can be incorporated through analytic breeding schemes. To accomplish the latter, recent biotechnological techniques such as anther culture and protoplast fusion may be employed.

Vigorous homozygous genotypes represent favorable combinations of genes. However, because of self-incompatibility and frequent sterility, it is not practical to produce inbred lines of potato. Doubled monoploids have been proposed as an alternative and rapid method to produce homozygous lines of potato. The present study focused on the methods for derivation of doubled monoploids and assessment of their breeding potential.

Availability of monoploids and doubled monoploids is prerequisite to efficient selection through this procedure. By anther culture or parthenogenetic haploid induction, monoploids can be obtained from diploid clones. Although these methods have not been perfected for many species, they appear to have potential for generation of sufficient numbers of monoploids. Monoploid genotypes derived from the same or different diploid clones varied for shoot regeneration and frequency of chromosome doubling among the regenerated shoots. This variation can be used to select responsive genotypes. The two-step in vitro procedure was found suitable for generating doubled monoploids from in vitro monoploids but alternative methods are required for recalcitrant genotypes.

A comparison of monoploids with their corresponding doubled monoploids showed high correlations between the two. Therefore, the monoploid phenotype can be used to select genotypes that are likely to be vigorous and fertile as dou-

bled monoploids. The correlation between performance of the monoploid or doubled monoploid and their progenies was not investigated in this study but would be of interest.

Doubled monoploids were found to perform relatively well for vegetative growth, therefore the monoploid sieve appears to have selected favorable combinations of genes. Although fertility was generally reduced, doubled monoploids set sufficient seed to enable utilization as female parents. However, there appears to be limited potential for use as male parents. Through selection of fertile genotypes, it is possible that male-fertile clones can be derived in future cycles.

Progeny of a few unselected doubled monoploids exhibited high performance under field conditions. It was demonstrated that selection through the monoploid sieve was beneficial. In addition, the progeny produced 2*n* pollen, a trait inherited from the anther donor through the doubled monoploids. This would facilitate incorporation of diploid germplasm into tetraploids.

The high performance of unselected doubled monoploids from one clone demonstrates the potential for improving germplasm by passage through the monoploid sieve. This warrants further research to develop adapted homozygous clones of *S. phureja* and other potato species that can be used in breeding potato by analytic breeding schemes. It may be possible to realize the achievements obtained by the development of hybrid maize much more rapidly for potato by application of the techniques described in this research.

Curriculum vita

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Experience

1982-present: Lecturer in Horticulture, Department of Horticulture, Egerton University, Njoro, Kenya

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Associations

International Society for Horticultural Science
American Society for Horticultural Science
African Potato Association
Crop Science Society of Kenya

Honor Societies

Gamma Sigma Delta - Honor Society of Agriculture

Phi Sigma - Honor Society of Biology