CHAPTER II: DERIVATION OF ANDROGENIC AND GYNOGENIC MONOPLOID POTATO POPULATIONS

2.1 Introduction

Monoploid potato can be derived through two different mechanisms, androgenesis and gynogenesis. Although the total gametic array giving rise to a monoploid population is expected to be the same regardless of the mechanism used, differences could arise between the populations due to differential recombination rates, gametic selection influencing segregation ratios, and gametoclonal and somaclonal variation. Such differences between androgenic and gynogenic doubled haploid populations have been documented in several species of the genus *Nicotiana* and in *Hordeum vulgare* (Kumashiro and Oinuma 1985, Bjornstad et al. 1993). These factors are of great importance in potato breeding as well as in plant genetics and molecular biology in general.

In order to ascertain whether androgenic and gynogenic potato monoploids are equivalent, it is necessary to compare monoploid populations derived in similar environments from the same parental clones. Since potato is a cross-pollinated species, selected clones are highly heterozygous. Therefore, meiotic segregation results in a diverse array of monoploids in either case, so populations representing a diverse array of geneotypes must be compared to determine a general effect of the process. Knowing whether or not a mechanism of haploid derivation carries an inadvertent selection pressure or induces genomic change can influence the decision about which method to use if such selection or genomic alteration is desirable or undesirable to the objectives of the breeding program.

2.2 Androgenesis

2.2.1 Introduction

Anther culture response is affected by many factors including genotype, environment, and culture conditions. One factor that has been shown to influence androgenesis directly is the composition of the basal medium (See chapter 1). This influence could be exerted either through stimulation of embryo initiation, fulfillment of nutritional requirements during embryo development, or osmotic balance. Since a plant's response to different media is at least in part genotype dependent, a single anther culture medium may not result in optimal embryogenesis across, or even within, species. Media have been developed during this century for different tissue culture techniques in a wide variety of crop plants. Although developed for specific applications, several of these have been effective in many crops and procedures. We wished to test three commonly utilized media to determine if the salt composition affected embryogenesis of a particular *S. phureja* clone.

In routine anther culture initiation, anthers are frequently injured when they are excised from the buds. Several authors have noted that care was exercised when initiating cultures to avoid injuring the anthers (Johansson 1988, Sopory and Maheshwari 1976) and injured anthers are typically discarded. Presumably, injury will adversely affect embryo formation, possibly through the wound response or release of deleterious substances from the anther wall.

However, it is commonly thought that androgenesis is stimulated by stress (Sangwan-Norreel 1977), though stresses applied to anthers are typically environmental in nature, i.e., temperature treatments. There are few examples of intentionally injuring or exerting similar physical strains on anthers prior to culture. Sopory and Maheshwari (1972) noted that

transversely cutting anthers of *Datura innoxia* two to four times prior to culture did not eliminate embryo production. In an attempt to cryo-preserve cultured *Nicotiana tabacum* anthers, it was found that more embryos and plantlets were produced from anthers that were sliced longitudinally prior to cold storage than those stored whole. In this system, injuring anthers positively affected the later stages of embryo development and regeneration (Bajaj 1978). Centrifuging whole anthers of *Datura innoxia* 1-2 days after the first pollen mitosis, at 150g for 5 min increased the percentage of embryogenic pollen grains from 0.71% in the control to 4.24% in the centrifugation treatment (Sangwan-Norreel 1977).

Cutting anthers should result in greater microspore contact with the medium and may facilitate release of a fraction of the microspores into the medium. Therefore, it is conceivable that cutting anthers may have a neutral or stimulating influence upon embryogenesis. The effect of anther injury upon embryogenesis has not been systematically studied. If injury negatively impacts embryogenesis, utilization of injured anthers in anther culture experiments should be carefully avoided. However, if injury is inert, anther culture efficiency may be increased as less precision would be required.

During the anther culture process, the hypocotyl of primary embryos can initiate numerous secondary embryos (Leela and Rao 1982). Secondary embryos have been reported to arise from single epidermal cells on the hypocotyl of *Brassica napus* embryos (Keller and Armstrong 1977). As secondary embryos enlarge, they can detach from the primary embryo and mature independently. At harvest, secondary embryos cannot be discerned from primary embryos. This leads to the production of multiple regenerants of the same genotype in potato anther culture.

Secondary embryogenesis has been reported in several crop species including potato and appears to be a widespread phenomenon, though it may often go undetected. When 19 anther-derived regenerants of *S. phureja* × *S. chacoense* ‘CP2' were examined, only two individuals were unique, and the other 17 fell into four groups of apparently duplicate genotypes based upon RAPD marker segregation patterns (Veilleux et al. 1995). In maize, nine duplicated genotypes were observed in a population of 118 anther-derived doubled haploid lines and were also attributed to secondary embryogenesis (Murigneux et al. 1993).

Without identification and elimination of secondary regenerants, false segregation ratios and recombination rates may be obtained when developing linkage maps and in other genetic studies. In addition, unintentional duplication of genotypes could result in inefficient screening of anther-derived lines in field tests and may decrease diversity in a breeding program due to selection of multiple individuals of the same genotype.

In potato anther culture, several anthers are generally cultured in the same flask of liquid medium. Embryos often protrude through the anther wall and become detached in the medium, making it impossible to identify the anther from which an embryo regenerated. In order to select unique anther-derived genotypes from a flask, all regenerants must be screened with molecular markers (isozymes, RFLPs, or PCR-based) and those with duplicate band patterns eliminated. This process is both expensive and time-consuming and may inhibit increased usage of doubled haploids in plant breeding programs and genetic studies.

It is highly unlikely that two microspores of the same genotype would result from meiosis of a heterozygous individual. Therefore, selection of a single regenerant from each anther culture vessel would guarantee that all individuals were unique, since the same genotype will not arise in

different vessels. Although this would eliminate the need for molecular marker analysis, it would greatly decrease anther-culture efficiency. In contrast, culturing anthers individually would allow selection of one regenerant from each responsive anther while maintaining efficiency. If anthers respond similarly to culture in isolation as in the presence of other anthers, culturing anthers individually would provide a viable solution to secondary embryogenesis, particularly for moderately to highly responsive genotypes.

Our objectives in conducting anther culture were primarily to derive androgenic monoploid populations from three tuber-bearing *Solanum* clones to facilitate comparison of the two mechanisms of haploid derivation in potato. Secondarily, we wished to determine the effects of three different basal media, anther injury, and isolated culture on *S. phureja* anther cultures.

2.2.2 Materials and Methods

2.2.2.1 Basal salts experiment

Solanum phureja clone 'PP5' was grown in a greenhouse during the spring of 1997 under 16 h daylength and day and night temperatures of 24°C and 15°C, respectively. Buds were collected when anthers were 2.5-4 mm in length, which corresponds with the late uninucleate phase of microspore development (Veilleux 1990). Cold pretreatment at 4-5°C was applied for 3 days prior to culture.

The anther culture procedure was as described by Taylor and Veilleux (1992). Buds were surface sterilized in 80% EtOH for 1 min, 100% bleach with 'Tween-Twenty' detergent for 6 min, followed by two rinses in ddH_20. Anthers were excised, and 30 anthers were cultured in each 150 ml Erlenmeyer flask containing 15 ml medium. The anthers from each bud were

distributed across treatments within a replication in order to prevent confounding of bud effect with treatment effect.

The following basal salts were tested: one-half strength Linsmaier-Skoog (LS, Linsmaier and Skoog 1965), Gamborg's B_5 (GB_5, Gamborg et al. 1968), and Nitsch and Nitsch (NN, Nitsch and Nitsch 1969); components are listed in Table 2.1. The LS salts were prepared from frozen stocks, while the GB_5 and NN salts were purchased from Sigma. In addition to the inorganic and organic components listed in Table 2.1, all media contained 60 g sucrose, 2.5 g activated charcoal, 0.1 mg IAA, and 2.5 mg BA per liter as recommended by Uhrig (1985) and modified by Taylor and Veilleux (1992). The pH of all media was adjusted to 5.8 with HCl or KOH, and media were sterilized for 20 min in an autoclave at 110 kPa pressure (AMSCO Scientific).

Cultures were incubated for 5-6 wk in the dark at room temperature on a gyrotory shaker (NBS Model G-10) at 130 rpm. Embryos were harvested by pouring the liquid media with anthers and embryos through a sieve, rinsing with sterile ddH2O, inverting the sieve with embryos onto a 1 × 10 cm petri plate, and counting embryos under 10× magnification. All embryos were plated on Uhrig's modification of V-1 embryo medium (Gamborg's B_5 medium with minimal organics, 50 mg $CaHPO_4$, 14.96 mg $CaCl_2 . 2H_2O$, 0.250 g NH_4NO_3, 10 g sucrose, 6 g agarose, 0.5 mg GA_3 (filter sterilized) per liter, pH=5.6) and grown in an incubation chamber (Percival) at 20-21°C with fluorescent lighting and a 16 h photoperiod. Anthers were transferred three times at 3-4 wk intervals or until regenerated. Plantlets were subcultured on MS basal medium.

The ploidy of regenerants was determined by flow cytometry (Coulter Flow Cytometer Epics-XL) of propidium iodide stained nuclei isolated from *in vitro* plants (Owen et al. 1988).

Approximately 0.1-0.5 g *in vitro* plant material was chopped with a razor blade on ice in 1.5 ml buffer (882 mg sodium citrate, 419 mg MOPS, 915 mg $MgCl_2$, 0.1 ml Triton X-100 and distilled H_2O to 250 ml). Data were analyzed using the general linear models procedure of SAS version 6.12.

2.2.2.2 Isolated culture and anther injury experiment

During the fall of 1996, *S. phureja* `BARD1-3' was grown in the greenhouse and buds were collected and pre-treated as described above. Excised anthers were evenly distributed across the five treatments, so that each anther from a single bud was placed in a different treatment.

Anther culture was carried out as above with the following modifications. To determine the effects of isolated anther culture, four anthers from each bud were cultured individually in Cell Wells 24-well microwell plates (Corning), and the remaining anther was cultured as a control (with five others from buds for which the anthers were similarly distributed) in a 150 ml Erlenmeyer flask. Microwells were 16 mm diam and contained 2 ml medium. Medium was autoclaved with activated charcoal (AC) and filter-sterilized to remove the AC. Erlenmeyer flasks contained 12 ml medium with AC.

In order to determine the effect of injury, we placed anthers in microwells and subjected them to one of the following isolated culture treatments: uninjured control, approximately 25% of the apical end excised, 25% of the basal end excised, or sliced longitudinally. The fifth anther from each bud, that was cultured with five others in a flask, was also uninjured. On four different dates, three replications were conducted. Each replication consisted of 30 anthers, and a total of 72 anthers was cultured per treatment.

All flask and CellWell cultures were incubated for 6 wks and flasks were harvested as described above. CellWell plates were harvested with the aid of a dissecting microscope by picking out embryos directly from the wells without sieving or rinsing. Embryo plating and regeneration were as described above. Data were analyzed by ANOVA with SAS version 6.12 procedure GLM (SAS Institute 1985)

2.2.2.3 Genetic analysis of BARD1-3 regenerants

The ploidy of regenerants was determined by flow cytometry (Coulter Flow Cytometer epics-XL) of propidium iodide stained cells (Owen et al. 1988). DNA from the parental clone and the monoploids was extracted utilizing a modification of the procedure outlined by Doyle and Doyle (1987). Briefly, *in vitro* or greenhouse-grown plant material was ground to a fine powder in liquid nitrogen with a mortar and pestle. Approximately 2-3 ml extraction buffer (0.1 M Tris HCl (pH = 8.0), 1.4 M NaCl, 0.02 M EDTA (pH = 8.0), 2% hexadecyltrimethylammonium bromide, and 1% 2-mercaptoethanol) was added, and approximately 0.75 ml grindate was poured into a 1.5 ml microfuge tube. After 1.5-2 h incubation at 60°C, 300 µl chloroform/isoamyl alcohol (24:1) was added and microfuge tubes were inverted 6-8 times. Grindate was centrifuged at 11,500 rpm for 15 min. Supernatant was transferred into a microfuge tube containing 200 µl cold isopropanol and mixed. Tubes were stored a minimum of 4 h at -30°C. Thawed samples were centrifuged at 11,500 rpm for 10 min and the supernatant was removed. The DNA pellet was washed twice with 300 µl cold 75% ethanol and air dried overnight under a laminar flow hood. Dried DNA was suspended in 50 µl TE buffer (10 mM Tris HCl and 1mM EDTA) and 1 µl 1% RNAse-A was added. After incubation at room temperature for 2 h, DNA was stored at -20° to -30°C. DNA concentration

was measured with a DNA minifluorometer (Hoefer Scientific Instruments, model TKO 100) using Hoescht 33258 stain and a lambda DNA standard.

DNA amplification by polymerase chain reaction (PCR) was carried out in a DNA thermal cycler (Perkin Elmer Cetus, Model 480) with 10-mer oligonucleotide RAPD primers from Operon Technologies RAPD primer kits A, C, and G. Each reaction tube contained 2 µl DNA working solution (10 ng/µl) and 23 µl reaction mixture (14.3 µl sterile dH_2O, 2.5 µl $MgCl_2$, 2.5 µl PCR buffer, 2 µl 2.5 mM DNTPs, 1.5 µl RAPD primer, 0.2 µl *Taq* polymerase). PCR conditions were as specified by Sambrook et al. (1989). Following amplification 5 µl tracking dye (0.25% bromphenol blue, 0.25% xylene cyanol FF, and 15% type 400 pharmacia Ficoll in water) was added to each reaction tube. Amplification products were loaded into 15 µl wells in a 1.4% agarose (Sigma Type I-A, low EEO) gel (1.4% agarose in 1× TBE buffer). Electrophoresis buffer (1× TBE buffer) contained 10.8 g Trizma base, 5.48 g boric acid, and 4.0 ml 0.5 mM EDTA at pH = 8 per litre. Electrophoresis was conducted in a Horizontal Gel Electrophoresis System (Life Technologies, Inc., Model H4) at 70-80 V for 5 h.

Gels were stained with ethidium bromide, visualized under ultraviolet light (Fotodyne illuminator), and photographed with a Polaroid camera (Fotodyne 5-5344) (Sambrook et al. 1989). Only clearly visible bands that were present in the parent and not in the check lane were scored (Figure 2.5). All gels were scored separately by two individuals to confirm banding patterns.

A total of 30 RAPD primers was utilized. The genetic profile of 43 anther-derived monoploids of BARD1-3 was determined with RAPD data from 19 primers (53 informative

bands). RAPD data were analyzed with the RAPDistance program version 1.04 and dendrograms were constructed using version 2.0 of the NJTree program.

2.2.3 Results

ANOVA revealed date, date x treatment, replication within date and treatment (basal medium composition) as significant sources of variation (Table 2.2). Since the interaction between date and treatment was significant, the best treatment differed among the three dates (Figure 2.1). Basal medium composition had a significant effect upon mean embryo formation according to the Ryan-Einot-Gabriel-Welsch Multiple Range test (Table 2.2). Mean embryos per anther ranged from 0.61 with Gamborg B_5 medium to 0.42 with Nitsch and Nitsch (Table 2.3).

In the microwell study, ANOVA revealed significant differences only for replication (Table 2.4). Although treatment means differed by a factor greater than four, variation within treatments revealed by the high standard error prevented statistical separation of the treatment means. Although there was wide variation among the mean responses, none of the treatments applied to anthers in the Cell Wells experiment were significantly different at the $p<0.05$ level (Table 2.5).

The response of the three clones to anther culture varied widely, though all donor plants were grown under similar conditions and anther cultures were conducted in the same way (Table 2.6). In order of decreasing response the clones are: *S. phureja* 'BARD1-3', *S. phureja* 'PP5,' and *S. phureja* x *S. chaoense* 'CP2.' The percentage of regenerants that were monoploid ranged from 0% in 'CP2' to 48.7% in 'BARD1-3' (Table 2.7).

High variability for embryogenesis in anther culture could be seen both among cultures initiated on single days and on the average response of groups of cultures initiated on different

days. Generally, the response was relatively low at the beginning of the flowering period, reached a peak, then declined sharply (Figures 2.2, 2.3, and 2.4).

Of the 30 tested RAPD primers, 22 (73%) produced bands that were present in the parent, 'BARD1-3,' segregating in the progeny, and clearly scorable. A total of 62 informative bands was generated from the 22 useful primers resulting in a mean of 2.8 scorable bands per informative primer or two bands per tested primer. Analysis of 43 anther-derived monoploids of 'BARD1-3' with 19 primers, which produced 53 segregating bands, revealed that two of the genotypes were duplicated once in the population. The dendrogram generated shows that the monoploids 1-3-10 and 1-3-11 were identical as well as 1-3-51 and 1-3-52 (Figure 2.5). Compared with the anther-donor, the monoploids had on average 50% fewer bands. Veilleux et al. (1995) found that monoploids derived through anther culture from a *S. chacoense* × *S. phureja* hybrid had only 35-41% of the RAPD bands present in the anther donor. Anther- derived homozygous diploids had a distribution of RAPD bands that was similar to the monoploids.

2.2.4 Discussion

The three media types were originally developed for different applications. GB_5 was developed for *Glycine max* (soybean) root cell suspension cultures, NN for anther culture of several *Nicotiana* (tobacco) species, and LS was slightly modified from Murashige-Skoog medium which was originally developed for callus cultures of *Nicotiana* (tobacco) cultures (Gamborg et al. 1968; Nitsch and Nitsch 1969; Murashige and Skoog 1962). Gamborg's B_5 medium produced the highest mean embryos per anther, though it was not significantly different from the Linsmaier-Skoog medium. Since the most effective medium was different for the three dates on which the experiments were performed, there appears to be an interaction between

medium composition and factors contributing to the date effect. Perhaps the nutritional status of the anthers was different on the three dates. Clearly, none of the three media was generally superior; however, this does not imply that a consistently superior medium cannot be developed.

Neither isolated culture nor any form of anther injury had a statistically significant effect upon embryogenesis of BARD1-3. There was significant variation among replications within the same date for embryos per anther, with the quantity of embryos declining between replications. This was probably due to inadvertent selection of buds which were closer to the optimal size or were more normal in shape in the first and second replications. For breeding programs or genetic studies utilizing haploids or doubled haploids, it appears that it would be more efficient to culture anthers individually and discard muliple monoploid regenerants from single wells than to fingerprint all regenerants with molecular markers, particularly if molecular marker analysis is not being utilized for other purposes.

Anther culture results were as expected with the exception of the exclusive generation of diploids from CP2 anther cultures. In previous studies, a low percentage of monoploids was obtained from CP2, as well as diploids. The vast majority of diploid regenerants produced in other anther culture studies was found to be heterozygous with homozygous diploids occurring rarely (Cani 1998). It is likely that the heterozygous diploids were initiated from $2n$ pollen.

The general trend in embryos per anther in relation to date (flowering stage) was fairly consistent across 2 years and with two different clones. It appeared that embryogenesis was greatest as plants entered the peak of flowering and declined thereafter. Some physiological factor must change between the dates (i.e., phytohormone levels) to alter the percent of microspores capable of androgenesis. Further research is needed to characterize the date effect

more precisely. This could be accomplished by culturing all available buds over the entire flowering period on groups of plants grown under uniform environmental conditions. If the trend is consistent, it may be possible to elucidate the basis for the date effect by means of differential display of RNA or protein. Ideally, replications of anther culture experiments should be performed on plant material at the same physiological stage in order to minimize the date effect.

2.3 Gynogenesis

2.3.1 Materials and Methods

Seed parents *S. phureja* 'BARD1-3,' 'PP5,' and *S. phureja* × *S. chacoense* 'CP2' as well as the haploid-inducing pollinators *S. phureja* `IVP101' and `IVP035' were grown in ground beds from tubers or cuttings in a greenhouse with a 16 h photoperiod and temperature set at 24°C (day) and 15°C (night).

Pollinations were conducted three to four times a week. Only unopened buds with petal coloration were pollinated. Any open, unpollinated flowers were removed and all flowers were hand emasculated. Pollen was generally collected fresh each day and applied once to each stigma immediately after floral emasculation. All inflorescences were labeled and wrapped in cheese cloth during maturation to prevent loss or misidentification of fruit due to abscission.

After 5-6 wks of development, mature fruits were removed from the vines and ripened for 1-2 wks at room temperature. Fruit were sliced horizontally and seed were scraped onto paper towels to dry. Seed were collected into envelopes and counted using a Mettler-Toledo seed counter.

The haploid-inducing pollinator 'IVP101' is homozygous for a dominant gene encoding purple pigmentation at the base of the hypocotyl. Pigmentation is visible through the seed coat

as a purple spot. Seeds were viewed under a dissecting microscope at 10× magnification to separate the spotted diploid seed from the potentially monoploid spotless seed. Spotless seeds were surface sterilized for 5 sec in 80% ethanol and 20 min in 30% bleach followed by two rinses in ddH_2O. Sterilized mesh filters were used to transfer seeds between beakers. Sterile seeds were then soaked in 1,500 ppm GA_3 for 12 h to induce uniform germination. Seeds were transferred through two ddH_2O rinses and placed in square petri dishes containing 15 ml embryo regeneration medium. Seeds were germinated in a Percival incubator at 21°C under fluorescent lighting with 16 h daylength. Germinated seedlings were transferred to MS basal medium. Seedlings with purple pigmentation at nodal regions (escapes from prior sorting) were discarded. The ploidy of resulting seedlings was determined by flow cytometry as described above for anther-derived regenerants.

2.3.2 Results

The total number of pollinations performed on each clone is reported in Table 2.8. Percent fruit set ranged from 10.8 with 'CP2' to 87.8 with 'PP5.' Seeds per fruit ranged from six to 94 in 'CP2,' from 12 to 513 in 'BARD1-3,' and from 13 to 443 in 'PP5.' Mean number of seeds per fruit was 32.2, 249.1, and 156.7 for 'CP2,' 'BARD1-3,' and 'PP5,' respectively (Table 2.8).

Percent spotless seed, spotless seed per berry, % germination, and % monoploids are reported in Table 2.9. Less than 0.62% of all seed from the three clones was potentially gynogenic, with the majority of the seed being diploid hybrids carrying the purple embryo spot. 'CP2' had the greatest percent of potentially monoploid (spotless) seed; however, the mean number of seeds per fruit was much lower than for the other clones (Table 2.8). The germination

rate was quite low in general with an average for the three clones of 49.8%. Haploids per 100 berries were 8.5 for 'BARD1-3,' 18.2 for 'PP5,' and 0 for 'CP2' (Table 2.9).

2.3.3 Discussion

In general, the seed parents and the haploid-inducing pollinator 'IVP101' grew well. During the summer of 1996, *S. phureja* "BARD1-3' was infected with several viruses which resulted in low fruit set. When replaced with *in vitro* grown virus-free plants in the fall of 1996, 'BARD1-3' performed much better (Table 2.10). Although it was possible to transfer the haploid-inducing pollinator *S. phureja* 'IVP35' from *in vitro* culture to the greenhouse, the resulting plants grew poorly. Plants of 'IVP35' were stunted, infertile, and displayed thickened, curled leaves. No viruses, viroids, or other pests were identified on the plants; however, they cannot be eliminated as a plausible explanation for the distorted growth. Alternative causes of the poor growth are epigenetic tissue culture effects or somaclonal variation. Liu and Douches (1993) also reported low pollen production from this clone in East Lansing, Michigan. The poor growth of this clone in multiple locations is indicative of a non-pest problem, i.e., methylation changes, ploidy alteration, or mutation in a critical biosynthetic pathway such as growth regulation.

The influence of the seed parent genotype upon gynogenesis in monoploid derivation is evident from the wide variation in response among the three clones pollinated with the same pollinator in similar environments. It appears that the seed parent may influence this trait to some degree through seeds per berry. As hybrids per 100 berries (hy/100b) increased from 0-100, haploids per 100 berries (h/100b) similarly increased. As hy/100b continued to increase from 100-1000, h/100b decreased with a negatively correlation (r=-0.35 and slope=-0.11; r=-0.19 and

slope=-0.12) with 100b. Continued increase of hy/100b, had little effect on h/100b (van Bruekelen 1981). This was demonstrated by the lack of haploids in CP2 and higher numbers of haploids per 100 berries in PP5 than in BARD1-3, though PP5 had a lower number of seeds per fruit. It is also evidenced by the low number of haploids per 100 berries (2.5 h/100b) when BARD1-3 yielded 86.9 seeds per fruit in the summer of 1996 as opposed to 9.22 haploids per 100 berries with 274.8 seeds per fruit in the fall and early winter of 1996 (Table 2.10). The frequency of h/100b achieved in this study compares favorably with the results of van Breukelen (1981). Using the same family of haploid inducing pollinators, they produced 0-17 h/100b from *S. tuberosum* dihaploids.

One possible explanation for the low germination rate (Table 2.9) was the occurrence of malformed and under-developed seeds among the putatively gynogenic ones, since they also may lack or possess an ambiguous embryo spot. A small fraction of the seedlings from all clones was severely deformed. If transferred several times, these malformed seedling structures often regenerated into diploid plants with purple markings and a normal *in vitro* phenotype. Perhaps these are underdeveloped seeds which possessed competence for regeneration in an *in vitro* environment. It is also possible that some of the monoploid seeds did not germinate due to weakness or lethal alleles.

Although gynogenesis was fairly efficient for BARD1-3 and PP5, it was not feasible to develop a gynogenic population from CP2 in this study. The clones utilized had been previously selected based upon known anther culture competence; therefore, generalizations regarding success of the two alternative mechanisms of haploid derivation for unselected potato clones cannot be made. Though the gynogenic competence of the parental clones was not known,

gynogenic monoploids were obtained from all clones that produced androgenic monoploids in these experiments.

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