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INDUCTION AND APPLICATION OF DIHAPLOIDS OF POTATO (*SOLANUM TUBEROSUM* L.)

ABSTRACT

The breeding work with common potato as a tetraploid crop is complicated. Reducing the tetraploid chromosome number to the diploid one, makes the research and breeding simpler, because tetrasomic inheritance is replaced with disomic. Dihaploids of *S. tuberosum* crossable to various diploid *Solanum* species give also possibility for direct gene transfer from the wild and primitive cultivated *Solanum* species to the *S. tuberosum* background. In this way the gene pool of common potato is improved and enriched, but there are also disadvantages connected with using of dihaploids and it is necessary to change the ploidy level back to the tetraploid one in order to produce a cultivar. In spite of that, dihaploids were utilized in several potato breeding programmes conducted in Europe and USA. Dihaploids contributed to many modern potato cultivars, facilitating genetic works.

Key words: breeding, dihaploids, genetic research, potato

INTRODUCTION

The dihaploids of *Solanum tuberosum* subsp. *tuberosum* belong to haploid plants. The haploids are plants with gametophytic chromosome number of sporophyte. Haploids, which are euhaploids, are divided into two groups: 1) monoploids (monohaploids) with half of chromosomes number of diploid species; 2) polyhaploids with half of chromosomes number of polyploid species. For example potato dihaploids ($2n=2x=24$) are derived from tetraploid *Solanum tuberosum* species ($2n=4x=48$). Among polyhaploids five types of aneuhaploids are distinguished. Haploid plants can be used in genetic and taxonomic studies and breeding.

Common potato (*Solanum tuberosum* L. subsp. *tuberosum*) is one of the world's most important crops. This species is tetraploid and highly heterozygous, which complicates its breeding and research. By reducing the tetraploid chromosome number ($4x=48$) to the diploid number ($2x=24$) the problem of tetrasomic inheritance is omitted. Research and breeding become simpler in a disomic system (Chase 1963).

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Dihaploid potato plants were produced on a large scale *via* 4*x* x 2*x* crosses, where *S. phureja* clones were pollinators (Hougas and Peloquin 1958, Hermsen and Verdenius 1973). Pollination of tetraploid potatoes with pollen of specific diploid *S. phureja* clones induces the development of unfertilized ovules into a haploid embryo and seed by parthenogenesis (Hermsen and Verdenius 1973, Samitsu and Hosaka 2002). The development of fertilized ovules also can occur and then elimination of chromosomes belonging to the pollinator is observed (Clulow *et al.* 1991, Misoo *et al.* 1997). The second method of dihaploid production is an *in vitro* anther culture (Rokka *et al.* 1996a).

The first report on a haploid plant found was given by Blakeslee *et al.* (1922) describing a *Datura stramonium* plant, which had 12 chromosomes and was obtained after cold treatment of 24 chromosome diploid plants. In *S. tuberosum* the first dihaploid form produced was reported by Ivanovskaja (1939).

Peloquin with co-workers in 50s have initiated potato dihaploid production on a large scale by 4*x* × 2*x* interploid crosses with *S. phureja* pollination of tetraploid cultivars and have started using them widely in research (Hougas and Peloquin 1958). They initiated the application of dihaploids by breeders also in Europe. In developed schemes of potato analytical breeding dihaploids were taken into consideration in breeding cultivars. Dihaploids have given new possibilities for genetic research.

Apart from dihaploids of *S. tuberosum* subsp. *tuberosum* in the genus *Solanum* haploid plants were obtained from other tetraploid species like *S. polytrichon* (Marks 1955, Singsit and Hanneman Jr 1987), *S. tuberosum* subsp. *andigena* (Landeo and Hanneman Jr 1982), *S. fendleri*, *S. hjertingii*, *S. papita*, *S. stoloniferum* (Singsit and Hanneman Jr 1987), *S. acaule* (Rokka *et al.* 1998a) and from hexaploid *S. demissum* (Bains and Howard 1950, Dodds 1950).

METHODS OF PRODUCTION OF DIHAPLOIDS

The production of dihaploid plants from common potato is the first step in a breeding or research program, which exploits dihaploids. There are two methods for induction of dihaploids, the first *via* 4*x* x 2*x* matings and the second one *via* anther culture.

During the development of the first technique many diploid species were tested as pollen donors to obtain dihaploids. The first dihaploid plant from *S. tuberosum* (cv. Aurora) was obtained by Ivanovskaja (1939) using pollination with *S. rybinii* as an inductor of the unfertilized ovule development. Hougas and Peloquin (1957) also used *S. rybinii* pollen to produce dihaploid plant from cv. Katahdin. Later, other diploid species were used as pollen donors with some success: *S. vernei* and *S. megistacrolobum* (Jakubiec 1964), *S. kesselbrenneri*, *S. stenotomum*, *S. macmillanii* and *S. phureja* (Budin 1969), *S. stenotomum* (Buketova 1970, Buketova and Yashina 1971), *S. goniocalyx* and *S. canasense* (Budin and Broksh 1972) and *S. tarijense* (Carroll and Low 1976).

The most efficient pollinators, with high ability of dihaploid induction, are diploid *S. phureja* clones. Well-known selected haploid-inductor clones are PI 225

682.1.1, PI 225 682.1.13, PI 225 682.1.22 (Peloquin and Hougas 1959, Frandsen 1967) and IvP 35, IvP 48, IvP 101 (Hermsen and Verdenius 1973, Hutten 1994).

Pollination of tetraploid potatoes with specific diploid *S. phureja* clones or with other diploid species can result in two different ways of dihaploid induction. Parthenogenesis is the first of mechanisms, based on haploid embryos and subsequently seeds arised from unfertilized egg cells of the tetraploid parent (Hermsen and Verdenius 1973, Samitsu and Hosaka 2002). In this way euhaploids and aneuhaploids can be formed (Hermsen *et al.* 1970, Samitsu and Hosaka 2002). Formation of aneuhaploids is caused by chromosomal irregularities during meiosis in autotetraploids (Hermsen 1969). The frequency of aneuploids produced depends on genotype (Hermsen *et al.* 1970). Study of Samitsu and Hosaka (2002) strongly supported the parthenogenesis method as a haploid induction mechanism. According to Hermsen and Verdenius (1973), Rowe (1974) and Samitsu and Hosaka (2002) the diploid pollinator did not contribute any genetic information to the dihaploid progeny developed by parthenogenesis.

Clulow *et al.* (1991, 1992) explained the alternative mechanism of dihaploid formation. Dihaploids could be developed from fertilized egg cells by a process of *S. phureja* chromosome elimination in developing zygotes. Dihaploid plants obtained in this way were aneusomatic. Most of the cells had 24 chromosomes of *S. tuberosum* parent, but some cells contained additional chromosomes (1, 2 or 12) from the *S. phureja* pollinator. Genes of *S. phureja* were expressed in the leaves and tubers of aneusomatic plants. Misoo *et al.* (1997) found *S. phureja* specific fragments with RAPD markers in euploid and aneusomatic clones of cv. Chijiwa obtained by pollination with *S. phureja*. Aneusomatic dihaploids with relatively small proportion (1-15%) of cells containing additional *S. phureja* chromosomes did not express leaf isozyme markers or patatin bands characteristic for the pollinator or showed its weak expression only. However, those dihaploids with a high proportion of cells containing additional chromosomes (50-55%) showed strong expression of *S. phureja* genes (Clulow *et al.* 1993). Clulow *et al.* (1993) found a dihaploid plant, which was euploid ($2n$=24) and expressed *S. phureja* markers, what was explained by occurrence of recombination or chromosome substitution between the genome of *S. phureja* and *S. tuberosum*. Straadt and Rasmussen (2003) analysed *S. phureja* DNA introgression into potato dihaploids obtained using three pollinators EC90, IVP48 and IVP101. They found *S. phureja* IVP101 as an excellent pollinator in the production of *S. tuberosum* dihaploids because no introgression of *S. phureja* DNA into the dihaploids was evidenced. The morphology of aneusomatic dihaploids, which expressed *S. phureja* markers, was much closer to *S. tuberosum* than to *S. phureja* (Allainguillaume *et al.* 1997).

In the progeny of $4x \times 2x$ crosses tetraploid, triploid and dihaploid individuals are formed. The tetraploid and triploid plants are hybrids resulting from the fertilization of egg cells by unreduced or reduced pollen grains from pollinator, respectively. Usually, the triploids occurred very rarely (van Suchtelen 1966, 1976, Hanneman and Peloquin 1967, Frandsen 1967). This phenomenon was described as semi-lethal "triploid block" (Marks 1966). The majority of the progeny from $4x \times 2x$ crosses were tetraploid hybrids. It was caused by the high number of *2n* pollen

grains formed by *S. phureja* (Höglund 1970). The number of produced dihaploids was usually very small (Nitzche and Wenzel 1977).

Generally, the influence of the pollinator and tetraploid parents on the frequency of dihaploid progeny from interploid crosses was found by several authors (Hougas *et al.* 1964, Jakubiec 1964, 1967, Frandsen 1967, Werner 1970, Hermsen and Verdenius 1973, Hutten 1994). However, von Breukelen (1981) showed lack of the interaction between pollinator and seed parent on the frequency of dihaploids produced.

Growing conditions of parental plants can affect indirectly dihaploid production. Wöhrmann (1964) and von Breukelen (1981) found that low temperature had a positive influence on dihaploid formation. Peloquin and Hougas (1959) tried to increase the efficiency of producing dihaploids in $4x \times 2x$ crosses by decapitation of the pistillate *S. tuberosum* parent. This method increased fruit setting 5 to 10 times.

An alternative way to produce dihaploids is the anther culture (androgenesis). The first description of this technique for the production of embryos from anthers and then plantlets was done by Guha and Maheshwari (1964) for *Datura innoxia*. This method was developed for different important crops, among them for potato. First potato dihaploids obtained *via* anther culture were reported by Dunwell and Suderland (1973) and Irikura (1975).

The combination of genotypes and anther age was more important for successful androgenesis, than the composition of the medium and culture conditions (Mix 1983). However Johansson (1986) presented that the embryogenesis in anther cultures of *S. tuberosum* could be increased by treatment of donor plants (N,N-dimethylsuccinamic acid), pre-treatment of buds (low temperature) or manipulation of the culture media (addition of L-cystein-HCL). Irikura (1975) found an important role of the interaction between the exogenous growth regulators and the type of basal media played in this technique. Rokka *et al.* (1996a) first estimated androgenic capacity for many commercially important cultivars.

The method of haploid production by anther culture is more laborious than $4x$ x $2x$ crosses (Ross 1986), but very efficient for tuber-bearing *Solanum* species (Irikura 1975, Veilleux 1999). With this method dihaploid clones producing *2n* gametes in combination with good tuber yield and tuber appearance were obtained from tetraploids (Wang and Ran 2000).

IDENTIFICATION OF DIHAPLOID PLANTS

Among the progeny derived from pollination of tetraploid form with a haploid-inductor identification of dihaploid plants is necessary since tetraploids and triploids also occur. Some pollinators possess embryo spot, which is a useful seed-marker (Peloquin and Hougas 1959, Hermsen and Verdenius 1973). Embryo spot is a deep purple or reddish coloration at the base of the cotyledons of the embryo, visible through the seed coat. Spotted seeds germinate into plants showing coloured rings or bands at the base of plant organs like: leaves and leaflets, scale leaves of stolons, tuber eye-brows and floral abscission layers. The genes involved in red embryo spot determination, have a pleiotropic effect.

According to Dodds (1955) the anthocyanins of diploid potato are controlled by at least two independent, complementary, dominant genes with a pleiotropic action. *P* gene controls anthocyanin formation, B^d gene causes the distribution of pigment at the plant parts. Anthocyanin spots on the embryo help to identify in early stage and on large scale, seeds giving dihaploid plants. Seeds without embryo spot are selected as containing haploid embryos. Dihaploids are morphologically diverse and have some characteristic morphological features, for example narrow leaves, which can be employed to identify them. Number of chloroplasts per guard cell of stomata and size of pollen grains can be also used to distinguish dihaploid plants among tested progeny (Frandsen 1967, 1968). Size of periderm cells can be a good ploidy indicator as well (De Maine 1998). Clones with mean size of periderm cells not greater than 60% of the parent's cell size could be classified as dihaploids. The final proof of dihaploidity is chromosome count in cells of root tips.

DESCRIPTION OF THE *S. TUBEROSUM* DIHAPLOIDS

Dihaploid plants differ in some traits from their tetraploid parents. Generally, primary dihaploids had reduced vigour and tuber yield (Hermundstand and Peloquin 1985, Tiemann and Peter 1998), smaller leaves and more leaves per stem (Frandsen 1967), smaller number of chloroplasts in the guard cells of the stomata (Rothacker *et al.* 1966) in comparison to their tetraploid parents. Some dihaploid plants were weak, chlorotic and did not initiate tubers (Peloquin and Hougas 1960, van Suchtelen 1966, Hutten 1994). Variability among dihaploids derived from the same tetraploid form was observed. Observations were made for dihaploids of cv. Katahdin (Peloquin and Hougas 1960) and cv. Chippewa (Matsubayashi 1979). Many workers described variability in respect of some characters among dihaploid plants produced from different tetraploid parents (Jakubiec 1964, 1967, van Suchtelen 1966, Frandsen 1967, Yerk and Peloquin 1989, Tiemann and Peter 1998, Hutten 1994).

Most of dihaploids had reduced fertility or were sterile particularly on the male side and their flowering was poor or even lacking (van Suchtelen 1966, Carroll and Low 1975, 1976, Hutten 1994). Gorea (1970) examined both male and female fertility of dihaploids and found that female fertility was good. He also noticed good abundant flowering of those dihaploids. The usual low fertility of primary dihaploids can increase after dihaploid intercrosses (De Maine 1997). Capo *et al.* (2001) found that male sterility depended on post meiotic abnormalities or on desynapsis. In the desynaptic clone only *2n* pollen grains were fertile. Two dihaploids produced *2n* pollen grains and three clones produced *2n* egg cells in Capo's *et al.* (2001) studies.

In dihaploids following meiotic abnormalities were found: univalents at metaphase I, anomalous orientation of spindles at metaphase II, lagging chromosomes, fragments and bridges at anaphase I and metaphase II, dyads and triads (Dinu *et al.* 1999) and different shapes of bivalents (Sosa and Hernandez De Sosa 1971).

ADVANTAGES AND DISADVANTAGES OF USING DIHAPLOIDS

The breeding of the modern potato cultivars is very difficult because of necessity of combining a lot of traits in one genotype. Using of dihaploids of *S. tuberosum* offer a method, which can make breeding and research work easier, and many researches point it out (Hougas and Peloquin 1958, 1960a, Chase 1963, Rowe 1967, Howard 1970, Świeżyński 1980, Świeżyński *et al.* 1985, Zimnoch-Guzowska 2003).

Hougas and Peloquin (1958) emphasized two major advantages of *S. tuberosum* dihaploids use: 1. direct gene transfer from wild and primitive cultivated diploid *Solanum* species to background of *S. tuberosum*, 2. disomic instead of tetrasomic inheritance of characters. Świeżyński (1980) and Świeżyński *et al.* (1985) pointed out that obtaining homozygous forms is easier with use of dihaploids. The frequency of individuals with desired allele combination is higher in comparison with the frequency of such hybrids in tetraploid progeny.

Most tuber-bearing wild *Solanum* species are diploid. They are a big gene reservoir of traits desirable in the cultivated tetraploid germplasm. Most of them can be crossed relatively easy with dihaploids (Hougas and Peloquin 1960b) giving progeny with high flowering intensity and good male fertility (Werner 1970, Tiemann 1993) able to produce *2n* gametes (Hermundstad and Peloquin 1985). Hybrids obtained this way had good vigour and normal growth (Hougas and Peloquin 1960b, Sawicka 1976).

The combination and selection of desirable characters at the tetraploid level are much more laborious and require larger size of progeny than at the diploid level. So, the reduction of tetraploid level to diploid one facilitates potato breeding and research.

Part of dihaploids were of great importance in breeding, however Zimnoch-Guzowska (2003) listed disadvantages connected with utilizing them in breeding. Haploidization can cause inbreeding, which is manifested in some undesired traits. The germination rate of dihaploid seeds was low, at about 50% (Neele and Louwes 1986) and some seedlings were weak and deformed (Caligari *et al.* 1988). On average about 60% of dihaploids formed tubers (Hutten 1994) and most of them had reduced flowering and fertility (van Suchtelen 1966, Corroll and Low 1975, 1976).

APPLYING OF DIHAPLOIDS IN POTATO BREEDING

Using of dihaploids is one of possible method in potato breeding (Chase 1963). He outlined a hypothetical breeding scheme to show how manipulation of the ploidy level may be useful in potato breeding programme. This scheme, called analytic breeding, contains three following steps: 1. reduction from the tetraploid potato to diploid lines *via* parthenogenesis, 2. intensive breeding and selection at the diploid level, 3. recovery of the tetraploid level and further selection.

Dihaploids reflect the gametic sample of their tetraploid parent, so they can be helpful in estimating the breeding potential of that parent. However, Neele (1991) found that this method had some limitations.

Primary dihaploids (obtained directly from tetraploids) can possess resistance and qualitative desirable traits of tetraploid initial material. The dihaploid clones were donors of resistance to *Synchytrium endobioticum* (Werner 1970), to *Phytophthora infestans* and *Globodera pallida* (De Maine 1978). Dihaploids selected by Tiemann (1996) were good for chips production and French fries processing. Pietilä *et al.* (1996) selected dihaploids with high tuber yield and with the high starch content.

Many hybrids with desirable, outstanding traits obtained by crossing dihaploids with diploid species were introduced into breeding materials. Hybrids with good agronomic and processing traits were selected from crosses with *S. sparsipilum*, *S. berthaultii*, *S. bukasovii*, *S. phureja*, *S. vernei*, *S. kurtzianum*, *S. gourlayi* and *S. famatinae* (Tiemann 1993, 1996, Serquen and Peloquin 1996). From *S. sanctae-rosae* the tolerance to frost was transferred to *S. tuberosum* background (Tucci *et al.* 1996). *S. canasense*, *S. multidissectum* and *S. tarijense* species gave the resistance to black leg and tuber soft rot (Frusciante *et al.* 1996, Carputo *et al.* 1997, 1999). Werner (1970) reported clones resistant to *G. rostochiensis* obtained from crossing dihaploid with *S. vernei*. Barchend and Peter (1998) described two dihaploid transgenic potato lines carrying the resistance gene to *PVY*, which had very low rates of infection in the field conditions.

Primary dihaploids or hybrids between dihaploids and diploid species produced *2n* gametes were crossed with tetraploids. Obtained tetraploid genotypes had good tuber characteristics and high tuber yield (Carputo *et al.* 1999, Concilio and Peloquin 1987). Kleinhempel and Tiemann (1987) selected tetraploids combining high tuber yield and low discolouration of tuber flesh after cooking with the resistance to viruses and to *G. rostochiensis.*

Some potato cultivars have dihaploid clones in their pedigree. Tiemann and Peter (1998) reported on four cultivars Tewadi, Agave, Rasant and Livera developed by utilization of dihaploids. Two cultivars Yukon Gold (Johnston and Rowberry 1981) and Krantz (Lauer *et al.* 1988) were derived from dihaploids of cv. Katahdin.

At the beginning of 70s in the Plant Breeding Station, Mielno, Poland dihaploid and diploid forms were used in a breeding programme (Dudek 1976). Among hybrids obtained from mating dihaploids with diploids, clones tolerant to drought and highly yielding on sandy soils were selected. Table cultivar Ibis (registered in 1987) was the first Polish cultivar having in pedigree dihaploid of *S. tuberosum,* which was a bridge clone for wild species *S. spegazzini* (Dudek 1988). Two table cultivars Lena (from The Plant Breeding Station, Mielno, registered in 1991) and Cykada (from The Plant Breeding Station, Płochocin, registered in 1998) were the next Polish cultivars with dihaploids in their origin, being a bridge form for wild species *S. polyadenium.*

In the Institute of Genetics and Cytology in Mińsk, Belarus a breeding programme using dihaploid clones has been developed (Yermishin 2000). They have intended to establish a collection of dihaploids of cultivars as donors of qualitative traits.

In the Młochów Research Center of IHAR (former Institute for Potato Research) breeding on diploid level has been conducted since 1970 and it was modified

Chase's scheme of analytic breeding (Jakuczun *et al.* 1997, Sawicka *et al.* 1977, Świeżyński 1980, Zimnoch-Guzowska *et al.*1997). Obtaining of dihaploids from cultivars and tetraploid clones was the first step of this work and was followed by the preselection. Then, selected dihaploids were mated with preselected hybrids obtained among or within wild diploid species. The best tetraploid clones from the Laboratory of Genetics with high starch content, resistant to PVX, PVY, PVA, PVS and to *P. infestans* were chosen for obtaining dihaploids (Sawicka *et al.* 1977). In breeding at the diploid level dihaploids of cultivars and tetraploid clones were used as donors of following characters: earliness, tuber yield, tuber quality, cooking quality, resistance to PVX (*Rx* gene), PVY, PVS (*Ns* gene), PLRV, and resistance to eelworm, wart and *P. infestans*. Incorporation of wild germplasm from different diploid *Solanum* species into *S. tuberosum* background was done *via* crosses of wild species to dihaploids (Świeżyński *et al.* 1985, Zimnoch-Guzowska and Dziewońska 1989, Wasilewicz-Flis and Dziewońska 1997). Sawicka (1976, 1984) transferred high starch content from *S. chacoense* and resistance to *P. infestans* from *S. verrucosum* on *S. tuberosum* background using dihaploids of starch cv. Wulkan. Directly after first crosses between dihaploids of *Solanum tuberosum* with wild species an increase of tuber yield was observed. The first visible improving of quality traits on diploid level was found as an effect of using in cross program dihaploids from Młochów and from Groß Lüsewitz (Zimnoch-Guzowska *et al.* 1985). Diploid clones obtained at Młochów are composite hybrids of *Solanum* species. In their pedigree there are dihaploids of tetraploid stocks and cultivars with theoretical contribution to the composition of diploid clones varying from 50% to 90% (Jakuczun *et al.* 1995). Dihaploids of tetraploid stock FM-1/17 obtained at Młochów have the large contribution in the origin of complex hybrids bred at Młochów. The pedigree of diploid clones contains also dihaploids of cultivars Certa, Pola, Prosna, Leda, Azalia and Pierwiosnek and dihaploids from the Netherlands and Germany. The main idea of utilisation of dihaploids in breeding programmes at Młochów was using them as bridge forms between wild diploid *Solanum* species being sources of mention traits and *S. tuberosum* background and inducing tuber yield heterosis. Domański *et al.* (2000, 2004) selected tetraploid progeny from 4*x* x 2*x* crosses performed agronomic traits and cold chipping ability exceeding level of these traits in progeny from 4*x* x 4*x* crosses. Diploid (dihaploid) germplasm contribute approximately in 70% of tetraploid parental lines obtained in breeding programme at Młochów Research Center (Jakuczun 2005). In 2002 the crossing program was conducted to produce new pool of dihaploids in diploid program at Młochów.

APPLYING DIHAPLOIDS IN BREEDING RESEARCH

Dihaploids do not only simplify breeding procedure, they are also useful in breeding research. The populations derived from crosses with dihaploid clones were valuable experimental material to study inheritance of different traits, for example agronomical traits (De Maine 1984a), resistance to dry rot (Suska 1985), distribution of anthocyanin in tuber (De Jong 1987), or tuber shape (De Jong and Burns 1993).

Induced trisomic lines from dihaploids were used for cytogenetic analysis by Hermsen *et al.* (1973), who located a gene causing chlorophyll deficiency and by Wagenvoort (1982), who found *ym* gene (yellow margin) on chromosome XII. Dihaploids were used to analyse the nature of polyploidy and to conduct genome analyses in relation to the origin of the common potato (Yeh *et al.* 1964, Matsubayashi 1979). Hougas and Peloquin (1958) used dihaploids to study the influence of ploidy level on tuber yield and vigour and Landeo and Hanneman Jr (1982) for estimation the heritability of quantitative traits. Another sector of knowledge to which dihaploids can contribute, is the evolutionary studies of species (Howard 1973). From dihaploid clones monohaploids could be induced for easy production of homozygotic lines (Hougas and Peloquin 1958, Hermsen 1984). Van Harten and Bouter (1973) used dihaploids in mutation studies to evaluate the dose rates of irradiation. Dihaploids were also used in molecular studies to construct a genetic map of potato with molecular markers (Bonierbale *et al.* 1988, Gebhardt 1994, Gebhardt *et al.* 1989) and to locate genes. Resistance genes acting against *P. infestans* (Leonards-Schippers *et al.* 1992, El-Kharbotly *et al.* 1994, 1996, Meksem *et al.* 1995), against *G. rostochiensis* (Barone *et al.* 1990, Ballvora *et al.* 1995, Jacobs *et al.* 1996), against *G. pallida* (van der Voort *et al.* 2000) and against PVX (Tommiska *et al.* 1998) were located using dihaploid clones.

In Młochów and other departments of IHAR diploid clones with dihaploids in the pedigree are used in many research aspects. Przetakiewicz (2003) elaborated a method of production of somatic hybrids with clones originated from Młochów. Diploid clones took a part in the construction of potato genome map (Zimnoch-Guzowska *et al.* 2000). They were used for the identification and mapping molecular markers linked to the resistance genes: PLRV (Marczewski *et al.* 2001, 2004), PVS (Marczewski *et al.* 2002) and to *P. infestans* (Śliwka *et al.* 2004). The study of inheritance of quality traits, like starch content (Zimnoch-Guzowska 1986), glucose level in tubers (Jakuczun and Zimnoch-Guzowska 2004), tuber greening (Jakuczun 2001), blackening of tuber flesh (Jakuczun and Eising 2003) and inheritance of resistance to *Erwinia* spp. (Zimnoch-Guzowska and Łojkowska 1993, Lebecka and Zimnoch-Guzowska 2004), to PLRV (Flis and Wasilewicz-Flis 1998) and to *P. infestans* (Świeżyński *et al.* 1991, 1997a,b) were conducted with diploid clones having dihaploids in their pedigree.

RETETRAPLOIDIZATION OF DIHAPLOIDS

According to Chase's scheme retetraploidization is the third step of the analytic breeding. This step is necessary to make dihaploids accessible for practical breeding. Dihaploid clones and other diploids do not reach the yield level of the tetraploid parents, as 4*x* level is the optimum for potato tuberization. Four methods are used for retetraploidization potato dihaploid clones: (1) generative *via* unreduced gametes (*2n*) in 4*x* x 2*x* crosses, (2) vegetative doubling *via* colchicine application, (3) protoplast fusion or (4) tissue cultures of tuber discs or leaves.

Colchicine treatment cause undesirable effects such as sterility, abnormal growth and morphology or chimeric plants (De Maine and Fantes 1983). Obtaining clones with all three germ layers doubled was difficult (Frandsen 1967, Langton 1974).

Chimeras and their identification which takes a lot of time are the main disadvantages of the colchicine treatment method. Production of unreduced gametes (*2n*) occurs only in certain diploid genotypes. Their frequency is often low. In result, obtaining greater number of seeds from interploid crosses is difficult and laborious. The doubling number of chromosomes *via* protoplast fusion and tissue cultures requires specialised laboratory. Apart from some difficulties colchicine treatment and unreduced gametes are the most frequently used methods of retetraploidization in breeding research.

Frandsen (1967) treated seeds of dihaploids with colchicine and obtained 50% of totally doubled plants, but also 23% of chimeras. Colchicine was applied also on nodal, auxiliary meristems using Dionne's method (van Suchtelen 1966, Ross *et al.* 1967, Langton 1973, 1974, De Maine and Fantes 1983, De Maine 1985). Comparing dihaploids with their colchicine chromosome-doubled derivatives, De Maine observed that tuber yield and rate of photosynthesis per unit leaf area (1984b) and total leaf areas per plant (1985) were similar.

A widely advocated method to return to the tetraploid level is sexual polyploidization by unreduced gametes. Sexual polyploidization can be either unilateral ($4x \times 2x$ and $2x \times 4x$ crosses) or bilateral ($2x \times 2x$ crosses), depending on frequency of type of unreduced gametes in breeding material. In IHAR Młochów unilateral sexual polyploidization by $4x$ x $2x$ crosses is usually used, because selected diploids are characterized by much more frequent production of *2n* pollen grains than *2n* egg cells (Strzelczyk-Żyta *et al.* 1997). There are many reports on using sexual polyploidization to recover tetraploid level of dihaploid clones (van Suchtelen 1966, Concilio and Peloquin 1987, Kleinhempel and Tiemann 1987, Hofferbert and Wenzel 1994, Tiemann 1993, Tiemann and Peter 1998, Carputo *et al.* 2000).

The retetraploidization is also achieved with somatic hybridization (Przetakiewicz *et al.* 2002, Orczyk *et al.* 2003). Somatic hybrids were obtained between dihaploids (Rokka *et al.* 1996b) or between dihaploids and *Solanum* species to transfer desirable characters on common potato background. From *S. brevidens* the resistance to PLRV, PVY and *P. infestans* (Austin *et al.* 1985, Horvath *et al.* 1993, Rokka *et al.* 1994), from *S. commersonii* the tolerance to frost (Frusciante *et al.* 1993, Nyman and Waara 1997), from *S. etuberosum* the resistance to PVY (Novy and Helgeson 1994), from *S. pinnatisectum* the resistance to *P. infestans* and *Erwinia* spp. (Menke *et al.* 1996), from *S. acaule* the tolerance to frost (Rokka *et al.* 1998b) and from *S. verrucosum* the resistance to PLRV (Carrasco *et al.* 2000) were transferred by this method.

The next method of retetraploidization is spontaneous doubling of chromosome number by tissue culture. Some authors applied leaf culture (Jacobsen 1978, 1981, Karp *et al.* 1984) or tuber disc culture (Mozafari *et al.* 1997). Their results showed that this method was potentially a good alternative or additional method to the colchicine treatment. Regeneration from dihaploid leaf pieces gave a high proportion (60%) of doubled genotypes and a very small proportion of mixoploids, aneuploids and no chimeras (Karp *et al.* 1984).

HAPLOID PLANTS FROM OTHER *SOLANUM* SPECIES

Haploid plants were obtained not only from *S. tuberosum* subsp. *tuberosum*, but also from other *Solanum* species.

The wild Mexican hexaploid (2*n*=6*x*=72) species *S. demissum* resistant to *P. infestans* is widely used in potato breeding. The polyhaploid plants (2*n*=3*x*=36) were obtained from crosses between *S. demissum* and diploid species *S. rybinii* or *S. toralapanum* (Bains and Howard 1950) and *S. stenotomum* or *S. ascasabii* (Dodds 1950). The size of polyhaploid plants was reduced, they had smaller leaves and flowers in comparison with hexaploid *S. demissum* (Bains and Howard 1950, Dodds 1950). Each polyhaploid had the same resistance to *P. infestans,* as the particular line of *S. demissum* from which it originated (Dodds 1950).

Marks (1955) derived a dihaploid plant from tetraploid tuber bearing *S. polytrichon* using tetraploid *S. stoloniferum* as inductor of parthenogenesis. The plant was chlorotic, smaller and slower growing, than the plant of *S. polytrichon* and had narrow leaflets. Also narrow leaflets were a morphological character to distinguish dihaploid. This plant did not flower, but produced tubers. In the dihaploid there was a considerable reduction in bivalent formation of meiotic chromosomes.

Rokka *et al.* (1998a) obtained *via* androgenesis over 250 dihaploids from allotetraploid species of *S. acaule* subsp. *acaule* Bitt. Generally this species has a lot of valuable traits as resistance to many potato pests (*PVX*, *PVY*, *PLRV*, *PSTVd*, nematodes) and tolerance to abiotic stress (Hawkes 1994). *S. acaule* does not readily cross with *S. tuberosum*, because of differences in the endosperm balance number (EBN) (Johnston *et al.* 1980), so it is useful to have its dihaploids. Obtained by Rokka *et al.* (1998a) dihaploid plants were normal-looking, similar to each other and to the tetraploid anther donor *S. acaule*. The male fertility was low and none of the dihaploids produced berries.

Yamada *et al.* (1998) obtained 11 somatic hybrids (2*n*=68 to 74) between *S. tuberosum* and dihaploid of *S. acaule*. Somatic hybridisation facilitates transferring of useful traits from *S. acaule* to the *S. tuberosum* background.

Also from other tetraploid species like *S. tuberosum* subsp. *andigena* (Landeo and Hanneman Jr 1982), *S. fendleri*, *S. hjertingii, S. papita* and *S. stoloniferum* (Singsit and Hanneman Jr 1987) dihaploid plants were obtained.

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P L A N T B R E E D I N G A N D S E E D S C I E N C E
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COLLECTING MISSIONS IN POLAND IN 1999 (SHORT COMMUNICATION)

ABSTRACT

Three collecting missions were organised by National Centre for Plant Genetic Resources of the Plant Breeding and Acclimatisation Institute at Radzików in 1999. The aim was collecting of old varieties and local populations of crop plants, cultivated in gardens and fields, old varieties of fruit-trees and wild growing fodder, medicine and ornamental species. A total of 339 accessions of 41 genera of crop plants were collected during these expeditions. Although genetic erosion of crop plants is still being continued and old varieties occurring in the recent past yet are replaced by new ones, there can be still found old, valuable varieties and local populations in many region of Poland.

Key words: crop plants, genetic resources, landraces, old varieties

DESCRIPTION OF EXPEDITIONS

Systematic collecting of plant genetic resources has been realized in Poland since 1971 (Podyma 1997). Every year expeditions are organized to the regions that are thought as the most rich in landraces and old varieties of crop plants areas (Nowosielska, Podyma, 1998, 2001).

In 1999 three regions of Poland were explored during collecting missions. The places of explorations were: the valley of lower Narew river (53°12' N, 22°46' E), Ponidzie (Kielce region, 50°53' N, 20°37' E) and Przedkarpacie (suroundings of Przemyśl, 49°48' N, 22°47' E).

All expeditions were organised by National Centre for Plant Genetic Resources of the Plant Breeding and Acclimatisation Institute at Radzików. The participants of these missions were dr. W. Podyma (NCPGR), D. Nowosielska (NCPGR), A. Kwiecień (Institute of Vegetables), G. Hodun (Research Institute of Pomology and Floriculture), T. Gałecka (Warsaw Agricultural University) and Melania Masarykova (Research Institute of Plant Production Piestany, Slovak Republic).

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