Polyploidy Breeding of African Nightshade
(Solanum section Solanum)

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ABSTRACT

The perception of African nightshades (Solanum section Solanum) as “healthy” foods, supported by documentation of their high nutritional and medicinal benefits, has led to a sudden and steady upsurge in their consumption, demand and monetary value in Africa and most parts of South-East Asia. However, due to very low leaf yields, production of these vegetables remains on small-scales, resulting in acute shortages and escalating prices, especially in urban areas. Apart from local variants or landraces selected in some regions where these plants are utilized as food and/or medicinal plants, there are no improved cultivars developed through conventional plant breeding techniques. Species belonging to this section, generally referred to as “Solanum nigrum-complex” are predominantly autogamous, favouring production of many small fruits and seeds, which compete with leaves for photosynthates. S. nigrum-complex constitutes a polyploid series, with diploid (2n=2x=24), tetraploid (2n=4x=48), hexaploid (2n=6x=72) and rarely, octoploid (2n=8x=96) species, a trait that can be exploited for cultivar development. This review outlines the possible evolutionary mechanisms and modes of origin of the polyploids, breeding strategies to produce heteropolyploids such as triploid (2n=3x=36), pentaploid (2n=5x=60) and heptaploid (2n=7x=84) from existing or induced polyploids and envisaged advantageous properties of the novel polyploids or heteropolyploids over their wild-type progenitors. With few exceptions, heteroploidy is known to cause sterility or highly reduced fertility of both pollen and ovules. The African nightshade heteroploid series would putatively be male- and female-sterile, thus eliminating fruit- and seed-set and enhancing leaf productivity.

Keywords: breeding strategies, heteroploidy, leaf yield, Solanum nigrum-related species

INTRODUCTION

Winkler (1916), working on vegetative grafts and chimeras of Solanum nigrum, found that callus regenerating from cut surfaces of stem explants were triploid and introduced the term polyploidy. Today, polyploidy is recognized as an important process in the evolutionary history of plants (Wendel 2000). Polyploidization is the process of duplication of a whole genome occurring either within a species (autopolyploidy) or as a result of unreduced gametes or somatic doubling during hybridization (combination of two or more divergent genomes) between species (allopolyploidy) giving rise to organisms with multiple sets of chromosomes (Soltis and Soltis 1999; Mishiba and Mit 2006). Many important
crop plants such as wheat (Triticum aestivum), oat (Avena sativa), cotton (Gossypium hirsutum), and coffee (Coffeea arabica) are natural allopolyploids while others such as alfalfa (Medicago sativa) and potato (Solanum tuberosum) are natural autopolyploids (Hilu 1993). Other crops, such as maize (Gaut and Doebely 1997), soybean (Shoenaker et al. 1996) and cabbage (Lagerrantz and Lydiate 1996) appear to have undergone polyploidization in their ancestry (pseudo- polyploidy section). Solanum tuberosum constitutes a poly- ploidy series, with diploid (e.g. S. americanum, S. physali- folium, S. chenopodioides, and S. sarrachoides), tetraploid (e.g. S. reflexum, S. villosum, S. exisirihonbume, and S. interandinum) and hexaploid (e.g. S. nigrum, S. furtatum, S. scabrum, S. arequipense and S. macrotonum) species. Octoploid plants (2n=8x=96) have also been reported (Heiser 1963; Edmonds 1977, 1979). In this review we focus on aspects of polyploidy in Solanum section Solanum with em- phasis on (1) possible modes of origin of polyploidization (2) potential application of natural and induced polyploids (or haploids) in heteroploidy breeding schemes aimed at improving leaf productivity and (3) the envisaged advan- tages of polyploids and heteroploids over their wild-type parents.

MECHANISMS OF POLYPLOIDIZATION IN ANGIOSPERMS

Various hypotheses regarding the origin of polyploids have been put forward. Two main modes of origin of polyploids are recognized as sexual by non-reduction in meiosis and asexual by somatic doubling in mitosis (Otto and Whitton 2000). Winge (1917) suggested that production of unre- duced gametes was the primary mechanism for polyploidi- zation in plants. Harlan and de Wet (1975) proposed that almost all polyploids arise by way of unreduced gametes and that all other mechanisms are negligible. Peloquin et al. (1989) made detailed studies on the occurrence and conse- quences of unreduced (2n) gametes on the development of polyploidy in Solanaceae. More recent theoretical modelling (Rodriguez 1996; Ramsey and Schemske 1998) and fieldwork (Husband 2000; Pikaard 2001) results agree that meiotic non-reduction (or nuclear restitution) is an impor- tant route for polyploid formation in angiosperms. Non- reduction can be caused by meiotic non-disjunction (failure of the chromosome to separate and subsequent reduction in chromosome number), failure of cell wall formation or for- mation of gametes by mitosis instead of meiosis. The unre- duced (2n) gametes arise through first division restitution or second division restitution during meiosis. The first divi- sion restitution results from parallel spindle formation at normal first division. Cleavage furrows occur across the parallel spindles to form dyads and pollen with four sets of chromosomes (2 × 2n) instead of the formation of the normal tetrad and 4 haploid pollen. In the second division residu- tion, the first meiotic division is followed by cytokinesis but the second division is absent resulting in a dyad with 2 × 2n. The production of non-reduced gametes is common in Solanum spp. (Webber 1940). Primula kewensis, for example, originated from fertile tetraploid shoots of sterile diploid F1 progeny of P. floribunda × P. verticellata (New- ton and Pellew 1929). Normal diploid Vicia faba contains tetraploid and octoploid cells in the stem cortex and pith, which could potentially produce new polyploid shoots (Coleman 1950). Spontaneous appearance of tetraploids in Oenothera lamarckiana and amphidiploid hybrids in Nicotiana have been shown to be a result of zygotic chromo- some doubling (Lewis 1980). The phenomenon of chromo- some doubling in the zygotes was best described from heat shock experiments in which young corn embryos briefly exp- osed to high temperatures (40°C), approximately 24 h after pollination, produced 1.8% tetraploid and 0.8% octoploid seedlings (Randolph 1932).

In addition to systemic polyploidization, somatic in- crease of genome size commonly occurs during organo- genesis of angiosperm species through a process termed endo- reduction, which involves DNA replication without nuclear and cell divisions, resulting in cells with nuclei that are larger than diploid nuclei (Edgar and Orr-Weaver 2001). During the normal mitotic cell cycle, cells have a mecha- nism that licences chromosomes to replicate only once each cycle, after an intervening mitosis. Endoreduplication oc- curs when cells re-replicate chromosomes in the absence of mitosis. Therefore, the key step in the switch to endoredup- lication is to allow cells to start another round of DNA repli- cation (synthesis or S-phase) while at the same time inhib- iting mitosis (M-phase). The entire complement of chro- mosomes is usually re-replicated during endoreduplication but, depending on the final configuration of chromosomes, there are two possibilities. First, the chromosomes may go through condensation and de-condensation stages after rep- lication and sister chromatids separate, resulting in poly- ploidy. Second, the chromosomes may replicate many times undergoing condensation and sister chromatids remain closely associated, resulting in polyteny (Nagl 1976). Most endoreduplicated chromosomes are likely to be polytene, as has been documented in wheat xylem cells (Martinez-Perez et al. 2001).

Another type of polyploidy is endomitosis, which im- plies that the chromosomes undergo a condensation and di- vision cycle as in mitosis; however, these processes take place inside the nuclear membrane without spindle forma- tion or anaphase and telophase movements (Ramsey and Schemske 1998). Other mechanisms of polyploidy include nuclear fusion in binucleated cells and polyspermy (ferti- lization of the egg by two male nuclei) (See review by Paterson et al. 2003).

POSSIBLE EVOLUTIONARY MODES OF ORIGIN OF AFRICAN NIGHTSHADE POLYPLOIDS

Cross fertilization and allogamy have been proposed to favour polyploidy and autogamy to restrict it (Stebbins 1950). In addition, perennial growth habit has been found to favour polyploidy more than annual growth habit due to in- creased chances of polyploidization following hybridization and mating between polyploids and their offspring with in- creasing lifespan from Otto and Whitton 2000). Contrary to these observations, polyploidy is highly developed in the largely autogamous annual herbs of the Solanum section Solanum. Information about the breeding history of African nightshade polyploid species is scanty or largely non-exist- ent. It is generally thought that the polyploid members of Solanum section Solanum are mostly allopolyploids, as most species show regular bivalent formation at meiosis. The main mode of origin of allopolyploids in annuals is by the fusion of unreduced gametes and autogamy favours this fusion (Grant 1981). For example, hybridization of maternal diplids with paternal tetraploids was observed to result in tetraploid progeny instead of triploids (Edmonds 1979). Therefore, it seems plausible to speculate that some of the African nightshade polyploids might have evolved through the fusion of unreduced gametes or somatic chromosome doubling.

Origin of tetraploids

Complications relating to phenotypic plasticity, genetic vari- ability, natural hybridization and discordant variation have caused taxonomic difficulties in studying the origins and progenitors of various species in the section Solanum (Ed-monds and Chweya 1997). There are several possible routes through which the tetraploids of S. nigrum-related species
could have evolved. First, spontaneous formation of triploids (Rick 1945) in diploid populations and natural backcrossing or selfing could result in autotetraploids as observed in Petunia (Derme 1931). Autotriploids could generate small numbers of euploids (x, 2x) gametes (Lange and Wagenvoort 1973) and 3x gametes via non-reduction (Mok and Peloquin 1975), which results in tetraploids on selfing or backcrossing to diploids. Although S. nigrum-related species are known to be predominantly self-pollinating, some out-crossing occurs (Edmonds 1979) making both self-fertilization and back-crossing possible routes for autotetraploids formation via a triploid bridge. Second, the union of two unreduced (2n) gametes or somatic doubling of diploid chromosomes could result in autotetraploids as observed in Solanum tuberosum (Iwanaga and Peloquin 1982). Third, hybrid triploids resulting from interspecific crosses of diploid × tetraploid species may undergo self-fertilization or backcrossing to the diploid parents to produce allo-tetraploids. Studies on spontaneous allotriploids of S. nigrum-related species and those obtained by diploid × tetraploid crosses (Edmonds 1979) suggest that the production of diploid or non-reduced gametes can enable allotriploids to produce autotetraploids by selfing or backcrossing. Fourth, allotetraploids may be formed directly from diploid species in the F1 or F2 generation of interspecific crosses as observed in Digitalis spp. (Buxton and Newton 1928). Based on chromosomal counts of Chenopodium and Chrysanthemum, Winge (1917) proposed that chromosome doubling in sterile inter-specific hybrids is a means of converting them into fertile offspring. The allotetraploid *Raphanobrassica* (2n=4x=36), for example, originated by chromosome doubling of a sterile F1, intergeneric hybrid between *Raphanus* (radish, 2n=2x=18) and *Brassica* (cabbage, 2n=2x=18) (Karpechenko 1927). In Solanum, section Solanum, natural hybridization has been reported among different ploidy levels, such as S. scabrum (6x) and S. americanum (2x) (Henderson 1974), although Heiser (1976) observed that interbreeding between different ploidy levels is very rare in nature. Fifth, allotetraploids could result from interspecific crosses of autotetraploids species as observed in Lycopersicon spp. (Lindstrom and Humphrey 1933).

**Origin of hexaploids**

Within diploid and tetraploid populations, the union of reduced and unreduced gametes could generate higher ploidy levels such as hexaploids as observed in Beta vulgaris (Hornsey 1973). The hexaploid S. nigrum is thought to be derived from the tetraploid S. villosum and the diploid S. americanum through the amphiploidy of a sterile triploid (Soria and Heiser 1959). Edmonds (1979) obtained sterile triploids by crossing accessions of S. americanum and S. villosum and fertile branches of the triploid by application of 0.25% colchicine for 24 hours. The hexaploid derivatives were sterile and the seed progeny were morphologically similar to naturally occurring S. nigrum.

**Origin of octoploids**

Octoploids of S. nigrum-related species might have been produced by further duplication of the tetraploid genomes. This process may be extremely rare in nature, accounting for scarcity of natural octoploids (Heiser 1963; Edmonds 1977, 1979). The F1 progeny of S. nigrum (6x) and S. sarratoides (2x) were sterile tetraploids but when the genome was doubled using colchicine, fertile F1 and F2 progeny (8x) were isolated (Edmonds 1979).
sorbitan monolaurate (Tween-20) daily for 7 days was found to be effective. The treatment was initiated when the cotyledons of the germinating seedlings were well-developed, just before the first true leaves appeared. It is important to keep the seedlings continuously moist and the aqueous solution from drying out as this would increase its concentration resulting in cell death. This can be achieved by raising the relative humidity to approx. 100% by covering the seedlings with polyethylene sheets.

Colchicine prevents the formation of functional mitotic spindle, thus preventing the migration of daughter chromosomes to opposite poles, a process known as mitotic slippage which leads to a polyploid cell (Loidl 1990). A colchicine-binding protein was identified in the nuclear envelope of *Lilium*, suggesting that colchicine affects chromosome/nuclear envelope interactions or a prerequisite step (Stern and Hotta 1973). Molecules involved in chromatid attachment and separation and premeiotic chromosome arrangement such as presynaptic alignment, chromosome condensation, kinetochores, chromatid glue proteins and crossing over are possible colchicine targets (Loidl 1989). Colchicine causes a reduction of chiasma formation between homologous arms of conventional chromosomes. Treatment of meiotic tissues with colchicine reduces the frequency of both auxin (2,4-D) and cytokinin (kinetin), improved the efficiency of haploid plant formation (Dumas de Vaulx and Chambonnet 1982). For isolated microspore culture, the best results were obtained after a pretreatment period of 3 days at 35°C, in a medium lacking sucrose (Miyoshi 1996). Sucrose starvation was reported to suppress gametophytic development and DNA synthesis of cultured microspores. The efficiency of haploid regeneration was additionally improved by preculturing anthers or microspores to induce callus formation prior to inducing plantlet regeneration. A short period of high temperature during the initial culture was found to inhibit the normal development of the microspores, and then stimulated androgenesis.

We have initiated steps towards obtaining diploid *S. vil-

Fig. 2 Histograms (top) from flow cytometric analyses of nuclear DNA contents and photomicrographs (bottom) of root tip chromosomes of wild-type tetraploid (left) and octoploid (right) *S. villosum* plants.
losum from the wild-type tetraploid plants. However, a number of factors and conditions still need to be optimized to come up with a recommendation on the best protocol. First, internal and environmental factors affecting plant growth such as age, light intensity, temperature and nutritional status may influence anther and pollen response to in vitro culture. Second, the stage at which microspores can be diverted into embryogenesis may vary with the species (Sunderland and Dunwell 1977). Third, the nutrient requirements may differ for induction and growth of callus. Success of haploid plant regeneration in S. melongena was shown to be dependent on the stage of anther development, genotype, as well as culture conditions, such as temperature and growth regulators in the culture medium (Rotino et al. 1987). Media based on N_6 (Chu 1978), MS (Murashige and Skoog 1962), B5 (Gamborg et al. 1968) and Nitsch and Nitsch (1969) are in wide use. Modifications involve addition of auxins, cytokinins, and organic substances. Addition of glutamine, proline, serine and ficoll into the culture medium has shown beneficial effects in microspore culture. Miyoshi (1996) cultured isolated microspores of S. melongena on Nitsch Lichter Nitsch (Litcher 1982) medium supplemented with NAA (0.5 mg/l) and BA (0.5 mg/l) initially for 4 weeks. The calli obtained were transferred to MS medium supplemented with zeatin (4 mg/l) and IAA (0.2 mg/l) for plant regeneration. We will continue to use these reports as a basis to develop a working protocol for haploid plant regeneration in the African nightshade.

Detection and selection of polyploids

Generally, the cotyledons and new leaves of doubled plantlets are darker green in colour, thicker and heavier in texture than unaffected seedlings. Also, the first true leaves may be abnormal or ragged in growth. Under the microscope, the cells of the polyploids are larger in volume and surface area when compared with undoubled cells. For example, wild-type S. viloillum flowers produced small pollen, with a mean area of 258.7 μm², while pollen from octoploid flowers was about 1.5-times larger, with an average area of 390.9 μm² (Ojewo et al. 2006). In Portulaca grandiflora, the mature pollen size of all tetraploid plants was found to be about two times larger than that of diploid plants, though the flow cytometric analysis revealed that the diploid plants were more polysomatic than the tetraploids (Mishiba and Mii 2000). However, in S. viloillum we found that although octoploids generally had larger pollen and larger stamata, not all plants with large-pollen and large-stamata were octoploids (Ojewo et al. 2006). Further tests involving direct chromosome counts (in root tip cells) and flow cytometric analysis revealed that pollen and stamatal cell size may not correlate accurately with ploidy level (Fig. 2). Flow cytometric analysis revealed that polysomaty, especially in mature leaf tissues may cause variability in cell size. Confirmatory tests, involving direct chromosome scoring in root tip cells and flow cytometry in young leaves are necessary after initial isolation of polyploids on the basis of cell or organ size.

Making back-crosses

Crossing the induced polyploids or haploids back to their wild-type parents is the next step to obtain heteroploids. To obtain heteroploids from a natural diploid parent, a number of back-crossing operations would be necessary. A back-cross of the induced tetraploids (4x) to their diploid (2x) wild-type parent would yield sterile triploid (3x) plants. A back-cross of octoploids (8x; obtained from further duplication of the tetraploid genome) to their tetraploid parent would result in a hexaploid (6x) plants. Backcrossing the hexaploid to the octoploid parent would yield sterile heptaploid (7x) plants. Further, backcrossing octoploids to the wild-type diploid grandparents would yield sterile penta- ploids (5x) plants (Fig. 3). From a natural tetraploid parent, haploids obtained by chromosome halving and octoploids obtained by chromosome duplication are employed in the backcrosses as shown (Fig. 4). The number of heteroploids that can be obtained from natural hexaploids is limited to triploids by direct chromosome halving or nanoploid (9x) by backcrossing the picroploid (12x) derivative to its wild-type parent (Fig. 5). From natural octoploids, tetraploids and diploids obtained by two consecutive chromosome halving procedures are employed (Fig. 6). Given cross-compatibility across the various ploidy levels or if cross pollination

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**Fig. 3 Hypothetical breeding scheme for production of heteroploids from a natural diploid.**

**Fig. 4 Hypothetical breeding scheme for production of heteroploids from a natural tetraploid.**

**Fig. 5 Hypothetical breeding scheme for production of heteroploids from a natural hexaploid.**
and fertilization barriers are overcome, interspecific hybrids can also be obtained by crossing natural interpolyploids without the need for chromosome doubling or halving (Fig. 7). Besides being less time-consuming, this latter method may be more advantageous in improving leaf yields as the progeny will have greater vigour due to heterosis in addition to reduced competition from the reproductive function. For success of this scheme, induction of male-sterility may be useful to enable crossing with limited chances of contamination with unwanted pollen. Conditional male-sterility may be advantageous to allow a two line breeding system that is even more economical in terms of space, time and cost of hybrid seed production. Details of mutagenic induction of male-sterility have been discussed elsewhere (Ojiewo et al. unpublished).

**COLCHICINE-INDUCED CHROMOSOMAL ABERRATION AND ANEUPLOIDY**

The molecular mechanisms ensuring accurate chromosome segregation during mitosis and meiosis are critical to the conservation of euploidy in eukaryotic cells (Iarmarcovi et al. 2006). Numerous mechanisms could consequently destabilize chromosomes, including loss of mitotic checkpoint function, abnormal amplification of centrosome, defects in the kinetochore-microtubule attachment, and movement of the chromosome relative to the pole (Iarmarcovi et al. 2006). Errors in DNA metabolism, repair, recombination or other rearrangements of the DNA sequence, misregulation of the cell cycle, disruption of the mitotic spindle apparatus, and centrosomal duplication result in unequal segregation of the chromosomes at cell division, in numerical chromosomal changes, and in the production of aneuploid cells (Attard et al. 2006). Aneuploidy, defined as the possession of chromosome numbers either greater or less than an exact multiple of the base chromosome number, x, is common in flowering plants (Grant 1981).

Colchicine has been widely used for chromosome doubling in various plants for several decades but side effects of chromosomal aberrations including chromosome losses, rearrangements and gene mutations have been reported (Lucet 1989). Chromosomal plants have been detected by root tip analysis of colchicine treated plants (Cohen and Yao 1996). One possible cause of polyploids with a reduced number of chromosomes is an accompanying aneuploidy in the process of polyploidization. Aneuploidy corresponds to a change in the number of chromosomes in a cell resulting from the gain or loss of one or more chromosomes during cell division. Aneuploidy can be caused by chromosome non-disjunction which occurs when an aberrant segregation produces progeny cells in which one cell gains a chromosome (e.g. trisomic 2n + 1 during mitosis or disomic n + 1 during meiosis) and the other loses a chromosome (e.g. monosomic 2n-1, during mitosis or nullisomic n-1 during meiosis). The monosomies may also result from chromosome loss by events such as chromosome lagging during anaphase separation (Iarmarcovi et al. 2006). For example, spindle fibres may fail to attach to a chromosome, which consequently remains behind the metaphase plate. Non-conjunction (when homologous chromosomes fail to establish a paired state), defective centromere division (wrongful separation of sister chromatids at first meiotic division) or extra replication of chromosomes are other possible causes.

Colchicine can cause aneuploidy through any of the above processes and result in reduced fertility (Iarmarcovi et al. 2006). It is difficult to establish the exact number of chromosomes in higher ploidy levels such as octoploids (8x). Octoploids of *S. villosum* have reduced fruit and seed production (Masinde, unpublished data), and possible contribution of aneuploidy to this trait may not be ruled out.

**POTENTIAL CONSEQUENCES AND ADVANTAGES OF POLYPLOID OR HETEROPLOID (ANEUPLOIDY) IN AFRICAN NIGHTSHADE**

**Vegetative-reproductive balance**

Lower fertility rates observed in most polyploids and sterility expected in heteroploids is disadvantageous in fruit and grain crops, potentially lowering production. However, in leafy vegetables or flowers where fruiting or seed development may be undesirable, reduced fertility or ultimate sterility may be desirable for providing vegetative-reproductive balance (Ojiewo et al. 2006). As fruits are the major sinks of the plant, a reduction in fruit load could favor the redistribution of dry matter to the vegetative parts. *Phaselia* and Buiskool (1995) observed that whereas total tomato dry matter production was not influenced by sink-source ratio, dry matter distribution between fruits and vegetative parts was greatly affected. Subsequently, changes in dry matter distribution were highly correlated with leaf area, such that when sink-source ratio was reduced (less fruits), plant leaf area and dry matter increased. In *S. nigrum*, manual de-flowering increased yield by 40% (Mwai Luta 1992). Although there have not been any deliberate attempts to improve yields of leafy vegetables through induced sterility, Eckhart (1992) reported that in gynoecious *Phaselia linearis*, male-sterile plants attained a greater shoot biomass compared to their hermaphrodite counterparts. Poot (1997) also reported that in natural populations of *Plantago lanceolata*, male-sterile plants attained a higher total plant biomass than their semi-sterile and hermaphrodite counterparts after five weeks of flowering. We have observed that the com-
Altered gene expression

Merger of two distinct genomes can be followed by genomic changes such as sequence elimination, sequence homogenization, and repeat inversion and epigenetic changes resulting in gene silencing, novel gene expression, and transposable element depression (Adams and Wendel 2005). Studies in maize indicate that the expression of many genes exhibit up to two-fold increase or decrease in gene expression (see review by Osborn et al. 2003). Studies in synthetic polyploids of *Brassica* (Song et al. 1995) and allotetraploid wheat (Ozekan et al. 2001; Shaked et al. 2001) demonstrate that extensive genomic changes can occur within a few generations. Differential expression among gene duplicates can occur immediately following polyploid formation as reported from studies of cDNA-single-stranded conformation polymorphisms in homeologous gene pairs of natural and synthetic *Gossypium* tetraploids (Adams et al. 2003), where 10 out of 40 homeologous gene pairs exhibited variable expression levels and silencing patterns in 10 different floral organs. Gene expression as well as epistatic and pleiotropic interactions within the genomes of each progenitor species confer their phenotypes (Liu and Wendel 2002). The unique gene product combinations resulting from the merger of two genomes may be due to the permanent heterozygosity and increased genetic variation caused by the inheritance of one allele set from each progenitor lineages, particularly if duplicate gene copies confer similar function (Pikaard 2001). Studies in *Gossypium* (Liu et al. 2001; Pikaard 2001), *Spartina anglica* (Baumel et al. 2002) and *Clarkia gracilis* (Ford and Gottlieb 1999) suggest that aspects of genome maintenance can vary among polyploid plant lineages, are not necessarily deleterious, and may lead to increased genetic diversity. High levels of segregating genetic variation can be maintained in polyploid species due to the merging of diversity from diploid progenitor species and subsequent segregation and recombination following polyploidization (Soltis and Soltis 1999). The resulting vigour of polyploid plants is, therefore, associated with changes in genome organization and gene expression (Osbourn et al. 2003).

By having a different number of alleles at a locus, polyploid species often display new traits and genetic variability that differ from their diploid ancestors in overall gene expression levels (Ramsey and Schemske 1998). Duplicated genes may undergo functional redundancy, subfunctionalization, neofunctionalization, or pseudogene formation (Hughes 2002; Prince and Picket 2002). Functional redundancy occurs if both gene copies retain their original function and are equally maintained such that one gene copy can substitute for another (Lynch and Conery 2000). Redundant duplicate genes in the floral developmental pathway of the model plant *Arabidopsis thaliana* are found at all stages during floral development, from meristem initiation to the specification of organ identity and seed shattering (see review by Briggs et al. 2006).

Subfunctionalization occurs if both gene copies accumulate compensatory mutations such that the combined activities of both genes are required for the original function, i.e. the copies retain different subsets of the functionality of the ancestral gene (Force et al. 1999; Lynch and Force 2000). For example, in the developing maize flower, C-class floral homeotic function results from non-overlapping expression of two duplicate maize genes (zag1 and zmm2), indicating that C-class floral homeotic function in maize has been partitioned between these two gene duplicates (Lawton-Rauh et al. 2000). Subfunctionalization of duplicated genes may be a transition state to neofunctionalization (Ras-togi and Liberles 2005). Neofunctionalization occurs when one gene copy retains the original function and the second gene copy accumulates mutations such that it is no longer involved in the original function. The second gene copy may effectively change function, leading either to incorporation of the gene into a different pathway or to a gain of novel function (Walsh 1995, 2003). Pseudogene formation occurs if mutational accumulation or epigenetic gene silencing renders the second gene copy non-functional (Hughes 2002).

Altered gene expression in polyploids may also be due to epigenetic changes, which do not involve alterations in DNA sequences, but affect gene expression through interrelated modifications, such as DNA methylation, histone modification and chromatin packaging (Wolffle and Matzke 1999). Epigenetic changes in new polyploids might lead to repression of gene expression or expression (derepression) of sequences that were repressed in the diploid (Jenewein et al. 2006).
and Allis 2001). Ploidy-dependent, epigenetically altered gene expression has been reported among diploid A. thaliana (2n = 2x = 10), diploid Cardaminopsis arenosa (2n = 4x = 32) and their natural allotetraploid Arabidopsis suecica (2n = 4x = 26) plants (Lee and Chen 2001). Twenty-five (22.7%) of the 110 cDNA fragments that were sequenced exhibited differential expression patterns of parental genes in the allotetraploid A. suecica. For example, sequence results showed a base transition (G → A) among the orthologous alleles of TCP3 from A. thaliana (AtTCP3), A. suecica (AsTCP3), and C. arenosa (CaTCP3).

Preliminary results from chromosome duplication of a tetraploid temperature-sensitive S. villosum mutant with abnormal floral organs have shown that the octoploid has normal floral organs (Ojiewo et al. unpublished observation). Further confirmatory tests to establish a strong link between polyploidization and complete floral organ and fertility restoration in this mutant are currently underway. However, effect of functional divergence among duplicated genes in controlling the floral structure and fertility restoration will be difficult to quantify because flower developmental genes exert their biological roles in a variety of different ways (see review by Ojiewo et al. 2007). Some gene products are part of subcellular structures, some are involved in protein-protein interactions or interactions with DNA or RNA, while others catalyze the transformation of small molecules. Furthermore, genes with the same biochemical functions may be expressed at different times or in different places (Galitski et al. 1999).

Increased plant size

The most immediate effect of polyploidy is often seen in changes of plant morphological characteristics which are visible in increased organ size. By increasing their ploidy level through successive rounds of DNA replication, plant cells commonly enlarge to hundreds or even thousands of times their original size. Increase in nuclear ploidy has been observed to influence growth rates and metabolic rates as sur-
structural, biochemical, and physiological elements, and this may influence photosynthetic rates. The number of nuclear chromosomes determines, to some extent, the size of leaves (Mizukami 2001), the size of cells (Sugimoto-Shirasu and Roberts 2003), the number of chloroplasts per cell (Bryne et al. 1981), and amounts of photosynthetic enzymes (Warner and Edwards 1993) and pigments (Vyas et al. 2004) in cells of polyploid plants. In tetraploid *Pennisetum americanum* plants, both mesophyll and bundle-sheath cells were 16 μM larger than in diploids (Warner and Edwards 1988). The same authors reported that changes in other dimensions resulted in a doubling (two times) of bundle-sheath cell volume, and a 45% increase in mesophyll cell volume in tetraploid cells. Also, the number of chloroplasts per cell in the tetraploids was doubled (Table 1). Nuclear genome duplication may be involved in the alteration of some photosynthetic and photorespiratory parameters of cells and organisms (Rathnam and Chollet 1980). Altered nuclear genomic constitution has been correlated with alterations in the efficiency and/or turnover of the photosynthetic light reactions (Leto et al. 1979).

**Table 1** Summary of some increment in photosynthetic parameters observed in various plant species after chromosome duplication.

<table>
<thead>
<tr>
<th>Trait changed</th>
<th>Species</th>
<th>Ploidy</th>
<th>Increase rate</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorophyll content</td>
<td><em>Phlox drummondii</em></td>
<td>4x&gt;2x, C₀₁</td>
<td>4x (auto), 18%, 110%</td>
<td>Vyas et al. 2004</td>
</tr>
<tr>
<td></td>
<td><em>Medicago sativa</em></td>
<td>4x&gt;2x; 8x&gt;4x</td>
<td>2-fold</td>
<td>Bingham 1998</td>
</tr>
<tr>
<td></td>
<td><em>Lolium perrenne</em></td>
<td>4x&gt;2x</td>
<td>47%</td>
<td>Rathnam and Chollet 1980</td>
</tr>
<tr>
<td></td>
<td><em>Pennisetum americanum</em></td>
<td>4x&gt;2x</td>
<td>2-fold</td>
<td>Warner and Edwards 1988</td>
</tr>
<tr>
<td>Net photosynthesis</td>
<td><em>Phlox drummondii</em></td>
<td>4x&gt;2x; C₀₁</td>
<td>43%, &gt;2-fold</td>
<td>Vyas et al. 2004</td>
</tr>
<tr>
<td></td>
<td><em>Atriplex confertifolia</em></td>
<td>4x&gt;2x</td>
<td>2-fold per cell</td>
<td>Warner and Edwards 1989</td>
</tr>
<tr>
<td></td>
<td><em>Panicum virgatum</em></td>
<td>8x&gt;4x</td>
<td>40%</td>
<td>Warner et al. 1987</td>
</tr>
<tr>
<td></td>
<td><em>Medicago sativa</em></td>
<td>4x&gt;2x;8x&gt;4x</td>
<td>2-fold; 67%</td>
<td>Bingham 1998</td>
</tr>
<tr>
<td></td>
<td><em>Triticum spp.</em></td>
<td>6x&gt;2x</td>
<td>&gt;2-fold</td>
<td>Austin 1990</td>
</tr>
<tr>
<td>Stomatal conductance</td>
<td><em>Phlox drummondii</em></td>
<td>4x&gt;2x; C₀₁</td>
<td>2.75-fold</td>
<td>Vyas et al. 2004</td>
</tr>
<tr>
<td>Activity and amount of Rubisco per leaf (cell)</td>
<td><em>Medicago sativa</em></td>
<td>4x&gt;2x;8x&gt;4x</td>
<td>2-fold; 50%</td>
<td>Bingham 1998</td>
</tr>
<tr>
<td>Amount of leaf DNA</td>
<td><em>Panicum virgatum</em></td>
<td>8x&gt;4x</td>
<td>2-fold</td>
<td>Warner et al. 1987</td>
</tr>
<tr>
<td></td>
<td><em>Pennisetum americanum</em></td>
<td>4x&gt;2x</td>
<td>2-fold</td>
<td>Warner and Edwards 1988</td>
</tr>
<tr>
<td></td>
<td><em>Medicago sativa</em></td>
<td>4x&gt;2x; 8x&gt;4x</td>
<td>2-fold</td>
<td>Warner and Edwards 1988</td>
</tr>
<tr>
<td></td>
<td><em>Triticum spp.</em></td>
<td>6x&gt;2x</td>
<td>&gt;2-fold</td>
<td>Austin 1990</td>
</tr>
<tr>
<td></td>
<td><em>Panicum virgatum</em></td>
<td>8x&gt;4x</td>
<td>2-fold</td>
<td>Warner et al. 1987</td>
</tr>
<tr>
<td>Number of chloroplasts per cell</td>
<td><em>Medicago sativa</em></td>
<td>4x&gt;2x; 8x&gt;4x</td>
<td>2-fold</td>
<td>Warner and Edwards 1988</td>
</tr>
<tr>
<td></td>
<td><em>Triticum spp.</em></td>
<td>6x&gt;2x</td>
<td>&gt;2-fold</td>
<td>Austin 1990</td>
</tr>
<tr>
<td></td>
<td><em>Panicum virgatum</em></td>
<td>8x&gt;4x</td>
<td>2-fold</td>
<td>Warner et al. 1987</td>
</tr>
<tr>
<td>Cell volume mesophyll; bundle sheath</td>
<td><em>Medicago sativa</em></td>
<td>4x&gt;2x</td>
<td>2-fold</td>
<td>Sharma-Natu and Ghildiyal 2005</td>
</tr>
<tr>
<td></td>
<td><em>Pennisetum americanum</em></td>
<td>4x&gt;2x</td>
<td>2-fold</td>
<td>Warner and Edwards 1988</td>
</tr>
<tr>
<td></td>
<td><em>Panicum virgatum</em></td>
<td>8x&gt;4x</td>
<td>2-fold</td>
<td>Warner et al. 1987</td>
</tr>
</tbody>
</table>

Photonsynthetic carbon uptake increases in higher ploidy levels on a leaf area basis in *Agropyron cristatum* L. (Frank 1980), *Festuca arundinacea* Schreb (Joseph et al. 1981), and *Panicum virgatum* (Warner et al. 1987). It increases on a chlorophyll basis in *Lolium perrenne* L. (Rathnam and Chollet 1980), and per cell in *Medicago sativa* L. (Bingham 1998), *Panicum virgatum* (Warner et al. 1987), and *Pennisetum americanum* (Warner and Edwards 1988). Although increases in ploidy level was reported to be associated with a decreased stomatal density in a number of species including *Ribes satigum* (Bjurman 1959), *Brassica oleracea* var. *gonglyloides* (Frydrych 1970), *Bromus inermis* Leyss (Tann and Dunn 1975), *Medicago sativa* (Setter et al. 1978) and triticale (Sapra et al. 1975), recent studies show that this decrease in stomatal number is generally accompanied by an increase in stomatal size (Romero-Aranda 1997; Yan 2001; Ojewo et al. 2006). Rates of photosynthesis and transpiration often closely follow changes in stomatal aperture (Wong et al. 1979). Therefore, it is envisaged that heteroploidy may improve the agronomic performance of African nightshade through such changes.

**CONCLUSIONS AND PROSPECTS**

The occurrence of polyploidy in the section *Solanum* is probably the most efficient barrier to natural hybridization between these species. Successful crosses are more difficult between taxa of differing ploidy levels than of the same chromosome numbers, with interploidy crosses leading to intermediate but sterile progeny (Heiser 1976). The natural tetraploid and hexaploid species are more genetically isolated from one another, with genetic breakdown occurring at various stages from pollination to the maturation of the F₂ progeny (Edmonds 1977). We have also observed that col-
chicine-induced octoploids of *S. villosum* are cross-incompatible with the wild-type tetraploid plants and with other taxa such as *S. americana* (2x) and *S. scabrum* (6x) (Ojiewo et al. unpublished data).

Failure to obtain fertile F$_1$ hybrids may be caused by genetic or cytoplasmic incompatibilities that are expressed either in failure of fertilization or in death of the zygote at any stage between early cleavage divisions and maturity (Allard 1966). Hybridization ability in the genus *Solanum* may be reduced by mechanisms such as gametic sterility, self-incompatibility, one-way incompatibility or endosperm and embryo abortion (Hawkes 1958). For the success of heteroploidy breeding strategy, pollen-pistil interaction and embryonic studies as well as ways to circumvent cross incompatibility in natural and synthetic polyploids are indispensable subjects for further research. Besides, cytological and molecular analyses are necessary to determine the role of unreduced gametes, apomixis and spontaneous somatic doubling as processes leading to ploidy series in *Solanum* section *Solomon*.

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