

# 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 heteroploids such as triploid ( $2x=3x=36$ ), pentaploid ( $2n=5x=60$ ) and heptaploid ( $2n=7x=84$ ) from existing or induced polyploids and envisaged advantageous properties of the novel polyploids or heteroploids 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

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## 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 (Wen-

del 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 Mii 2006). Many important

crop plants such as wheat (*Triticum aestivum*), oat (*Avena sativa*), cotton (*Gossypium hirsutum*), and coffee (*Coffea 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 Doebley 1997), soybean (Shoemaker *et al.* 1996) and cabbage (Lagercrantz and Lydiat 1996) appear to have undergone polyploidization in their ancestry (paleopolyploids). *Solanum* section *Solanum* constitutes a polyploid series, with diploid (e.g. *S. americanum*, *S. physalifolium*, *S. chenopodioides*, and *S. sarrachoides*), tetraploid (e.g. *S. retroflexum*, *S. villosum*, *S. excisiorhombeum*, and *S. interandinum*) and hexaploid (e.g. *S. nigrum*, *S. furcatum*, *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 emphasis 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 advantages 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 polyploidy 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 unreduced gametes was the primary mechanism for polyploidization 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 consequences 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 important 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 formation of gametes by mitosis instead of meiosis. The unreduced ( $2n$ ) gametes arise through first division restitution or second division restitution during meiosis. The first division restitution results from parallel spindle formation after normal first division. Cleavage furrows occur across the parallel spindles to form dyads and pollen with four sets of chromosomes ( $2 \times 2n$ ) instead of the formation of the normal tetrad and 4 haploid pollen. In the second division restitution, the first meiotic division is followed by cytokinesis but the second division is absent resulting in a dyad with  $2 \times 2n$ . The production of non-reduced gametes is common in *Solanum* spp. (Lewis 1980) and is probably an important mechanism by which *Solanum-nigrum*-related ploidy series evolved.

Somatic doubling may result from mitotic failure during somatic cell division, leading to a cell with doubled chromosome number. Somatic doubling has been reported in meristem tissue of juvenile sporophytes (Jørgensen 1928), non-meristematic plant tissues (d'Amato 1952) and polyembryonic seeds (Webber 1940). *Primula kewensis*, for example, originated from fertile tetraploid shoots of sterile diploid  $F_1$  progeny of *P. floribunda*  $\times$  *P. verticellata* (Newton 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 chromosome 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 exposed 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 increase of genome size commonly occurs during organogenesis of angiosperm species through a process termed endoreduplication, 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 mechanism that licences chromosomes to replicate only once each cycle, after an intervening mitosis. Endoreduplication occurs when cells re-replicate chromosomes in the absence of mitosis. Therefore, the key step in the switch to endoreduplication is to allow cells to start another round of DNA replication (synthesis or S-phase) while at the same time inhibiting mitosis (M-phase). The entire complement of chromosomes 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 replication and sister chromatids separate, resulting in polyploidy. Second, the chromosomes may replicate without 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 implies that the chromosomes undergo a condensation and division cycle as in mitosis; however, these processes take place inside the nuclear membrane without spindle formation or anaphase and telophase movements (Ramsey and Schemske 1998). Other mechanisms of polyploidy include nuclear fusion in binucleated cells and polyspermy (fertilization 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 increased chances of polyploidization following hybridization and mating between polyploids and their offspring with increasing lifespan (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-existent. 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 diploids 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 variability, natural hybridization and discordant variation have caused taxonomic difficulties in studying the origins and progenitors of various species in the section *Solanum* (Edmonds 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* (Dermen 1931). Autotriploids could generate small numbers of euploid ( $x$ ,  $2x$ ) 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 autotetraploid 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  $\times$  tetraploid species may undergo self-fertilization or backcrossing to the diploid parents to produce allotetraploids. Studies on spontaneous allotriploids of *S. nigrum*-related species and those obtained by diploid  $\times$  tetraploid crosses (Edmonds 1979) suggest that the production of diploid or non-reduced gametes can enable allotriploids to produce allotetraploids by selfing or backcrossing. Fourth, allotetraploids may be formed directly from diploid species in the  $F_1$  or  $F_2$  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  $F_1$  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 fertile 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  $F_1$  progeny of *S. nigrum* ( $6x$ ) and *S. sarachoides* ( $2x$ ) were sterile tetraploids, but when the genome was doubled using colchicine, fertile  $F_1$  and  $F_2$  progeny ( $8x$ ) were isolated (Edmonds 1979).

## PRODUCTION OF STERILE HETEROPLIOD SERIES

For success in the wild, semi-wild or as weeds of disturbed habitats, especially in agricultural areas (Rogers and Ogg 1981), *Solanum*-related taxa are primarily autogamous with

some occasional outcrossing (Edmonds 1979). With a fruit and seed production of over 3,600 per plant and over 40 seeds per fruit (Salisbury 1961), this autogamy favours a rapid increase in population and confers an evolutionary advantage on the plants in environments in which populations are frequently destroyed. It also results in high degree of homozygosity and concurrent genetic uniformity of plants both within a population and from generation to generation (Rogers and Ogg 1981). However, for small-scale farmers of taxa consumed as leafy vegetables, this autogamy poses a serious challenge. The respiratory costs associated with such high reproductive functions result in source-sink limitations after anthesis reducing leaf expansion and appearance of new leaves after anthesis. Dry matter partitioning at this stage is directed mainly towards pollen, fruit and seed formation and development, each demanding a high amount of energy. The leaf yields of these vegetables are, therefore, generally low.

Polyploidy is often accompanied with a loss of normal sexual reproduction in plants and animals, particularly in odd-ploidy individuals (such as triploids), a phenomenon known as heteroploidy. Production of heteroploids can be one important strategy to circumvent the problem of 'over-bearing' at the expense of leaf productivity. Heteroploids can be defined as organisms or cells having a chromosome number that is not an even multiple of the haploid chromosome number for that species. During the meiosis of heteroploids, heterovalents are formed (Cassani and Caton 1985). In the following anaphase I, the chromosomes are distributed onto the daughter cells, both of which have incomplete sets (aneuploidy), leading to lethality. Heteroploidy therefore, generally causes sterility (or strongly reduced fertility) of both pollen and ovules. Production of odd polyploid levels can be achieved by interploidy hybridization or apomixis (Rye 1979). The design of breeding schemes required to produce heteroploids may depend on the initial ploidy level of the wild-type parents. Heteroploidy breeding may include the following activities:

### Chromosome doubling

The discovery of colchicine's chromosome doubling effect (Blakeslee and Avery 1937) led to its use as an important tool for the experimental study of polyploidy. Presently, colchicine ( $C_{22}H_{25}O_6$ ) extracted from seeds or corms of autumn crocus (*Colchicum autumnale*) (Fig. 1), its synthetic analogue, colcemid and oryzalin are some of the agents used to achieve chromosome doubling.

For chromosome duplication, colchicine is generally applied to meristematic cells, usually the shoot primordia using cotton pads, or by dipping the tissues in the colchicine solution. Treatment of pre-soaked seeds is also effective. The concentration of colchicine solution and duration of application varies with plant species. Correct determination of colchicine dosages for use in chromosome doubling is important as high colchicine dosages can cause plant death due to its toxic effect (Navarro-Alvarez *et al.* 1994). To induce octoploidy in *S. villosum* (Ojiewo *et al.* 2006), spraying seedlings with a mixture of 0.01% colchicine, in 1 ml dimethyl sulphoxide (DMSO) and 0.1 ml polyoxyethylene

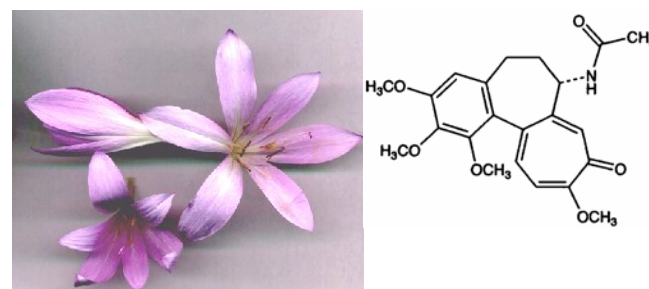


Fig. 1 Left: *Colchicum autumnale* flowers; Right: Structure of colchicine.

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 chiasmata (Cowan and Cande 2002) and impairs synaptonemal complex formation (Loidl 1989). Homologous chromosomes occupy separate nuclear territories in the premeiotic interphase (Maestra *et al.* 2002) and colchicine may affect some components of the mechanism that brings them together during leptotene. Thus, telomere convergence and centromere clustering are also possible targets of colchicine. Colchicine can also bind to spindle apparatus molecules such as tubuline and centrioles and prevent the assembly of microtubules or cause microtubule depolymerisation in cells (Dustin 1978). Other possible targets of colchicines include cell cycle control substances such as cyclins, ana-

phase promoting complex and substances indirectly involved in the cell cycle such as calmodulin, cellular or nuclear membrane (Attard *et al.* 2006).

## Chromosome halving

*Solanum nigrum* was proposed as a model plant in tissue and protoplast culture with valuable traits such as high *in vitro* multiplication rates, high regeneration capacity, amenable for different culture techniques, low frequency of somatic mutation, with potential marker genes such as herbicide or antibiotic resistance (Hassaneim and Soltan 2000). This proposal was based on the ease of callus, shoot and root formation from leaf, stem and shoot tip as explants. However, there are no reports on haploid plant production in the African nightshade. Chromosome halving or haploid production has been achieved in the closely related *S. melongena* through *in vitro* culture of gametic tissues including anthers (Rotino 1996) and isolated microspores (Miyoshi 1996). For anther culture, an incubation pretreatment of anthers under dark conditions for 8 days at high temperature ( $35\pm 2^\circ\text{C}$ ) in a medium containing a combination of both auxin (2,4-D) and cytokinin (kinetin), improved the efficiency of haploid plant formation (Dumas de Vault and Chambonnet 1982). For isolated microspore culture, the best results were obtained after a pretreatment period of 3 days at  $35^\circ\text{C}$ , 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-*

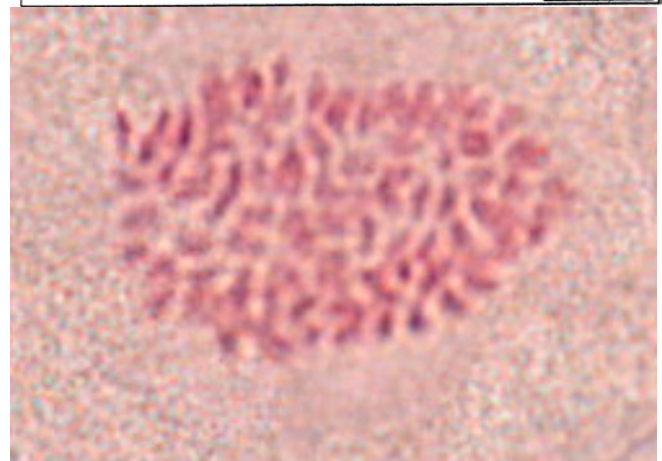
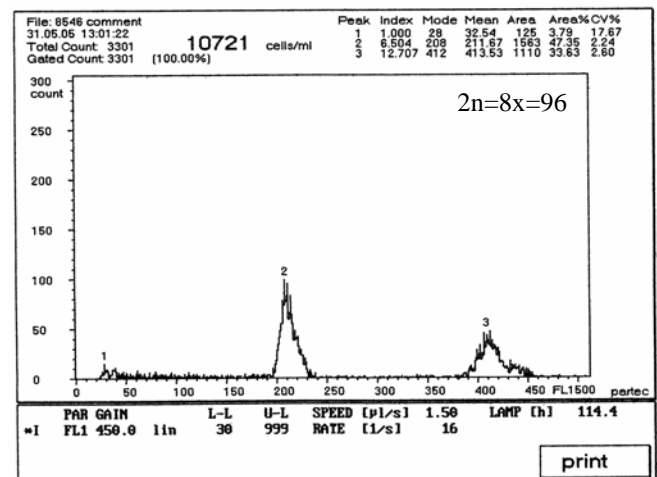
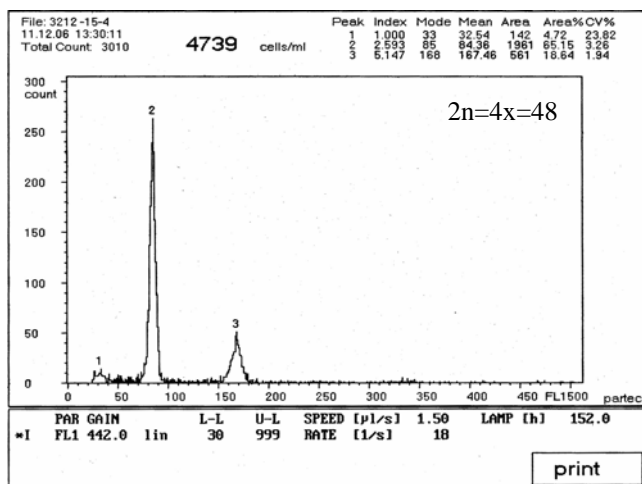


Fig. 2 Histograms (top) from flow cytometric analyses of nuclear DNA contents and photomicrographs (bottom) of root tip chromosomes of wild-type tetraploid (left) and octoploid (right) *S. villosum* plants.

*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<sub>6</sub> (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. villosum* flowers produced small pollen, with a mean area of 258.7  $\mu\text{m}^2$ , while pollen from octoploid flowers was about 1.5-times larger, with an average area of 390.9  $\mu\text{m}^2$  (Ojiewo *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. villosum* we found that although octoploids generally had larger pollen and larger stomata, not all plants with large-pollen and large-stomata were octoploids (Ojiewo *et al.* 2006). Further tests involving direct chromosome counts (in root tip cells) and flow cytometric analysis revealed that pollen and stomatal 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 pentaploid (5x) plants (Fig. 3). From a natural tetraploid parent, haploids obtained by chromosome halving and octoploids

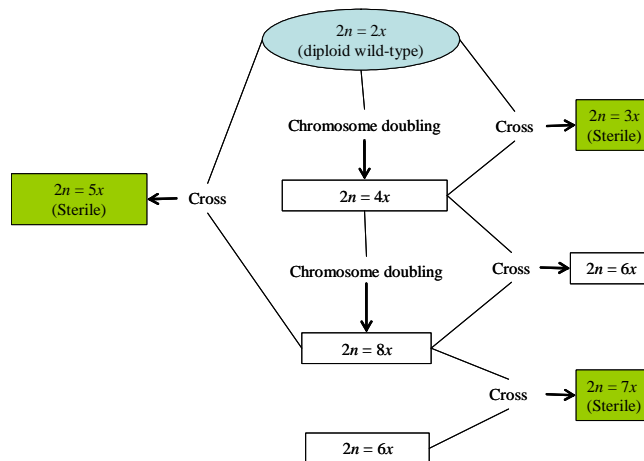


Fig. 3 Hypothetical breeding scheme for production of heteroploids from a natural diploid.

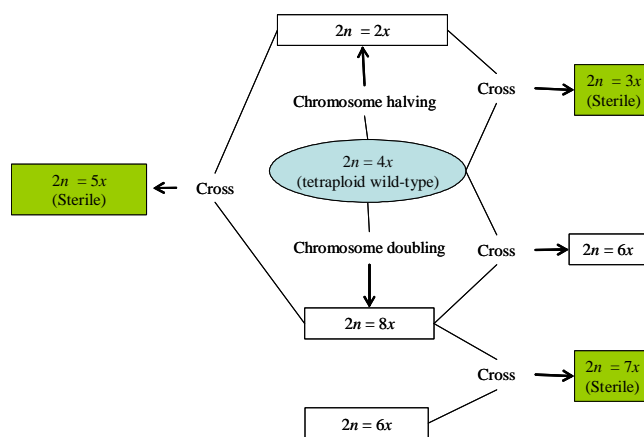


Fig. 4 Hypothetical breeding scheme for production of heteroploids from a natural tetraploid.

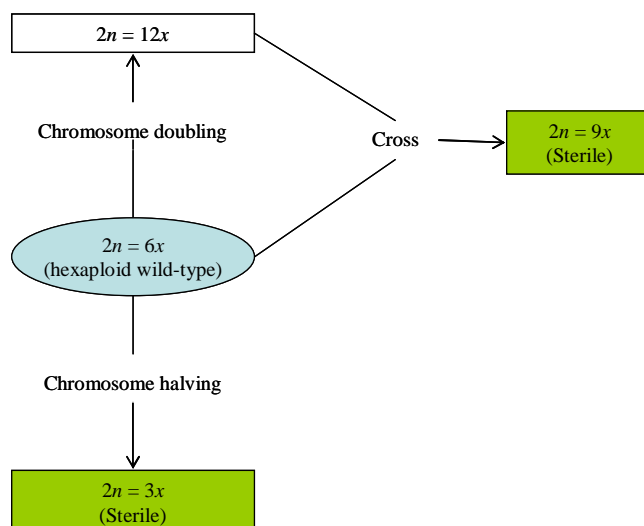


Fig. 5 Hypothetical breeding scheme for production of heteroploids from a natural hexaploid.

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 nonaploid (9x) by backcrossing the picoploid (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

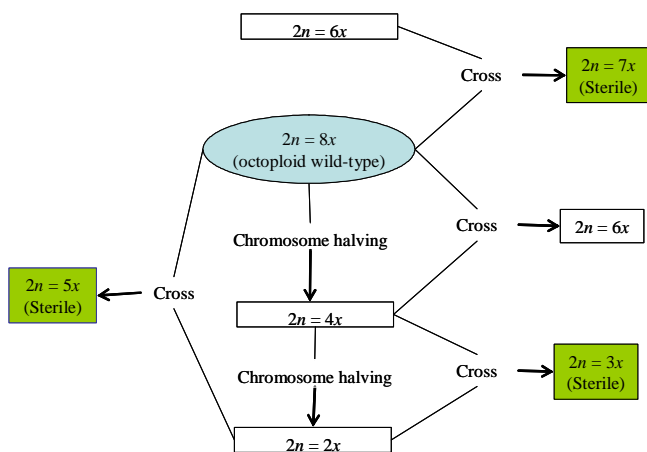


Fig. 6 Hypothetical breeding scheme for production of heteroploids from a natural octoploid.

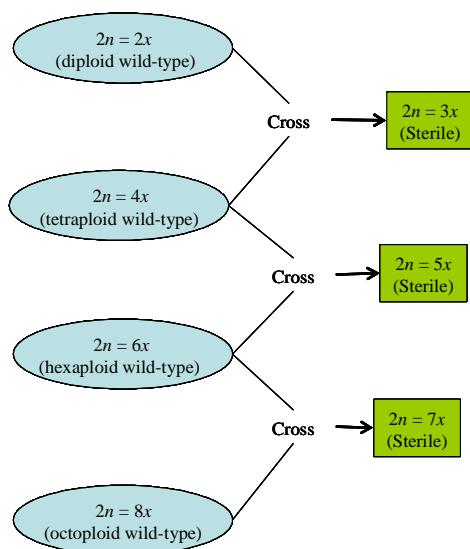


Fig. 7 Hypothetical breeding scheme for production of heteroploids by interploidy crosses between naturally occurring polyplods.

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 (Iarmarcovai *et al.* 2006). Numerous mechanisms could consequentially 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 (Iarmarcovai *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).

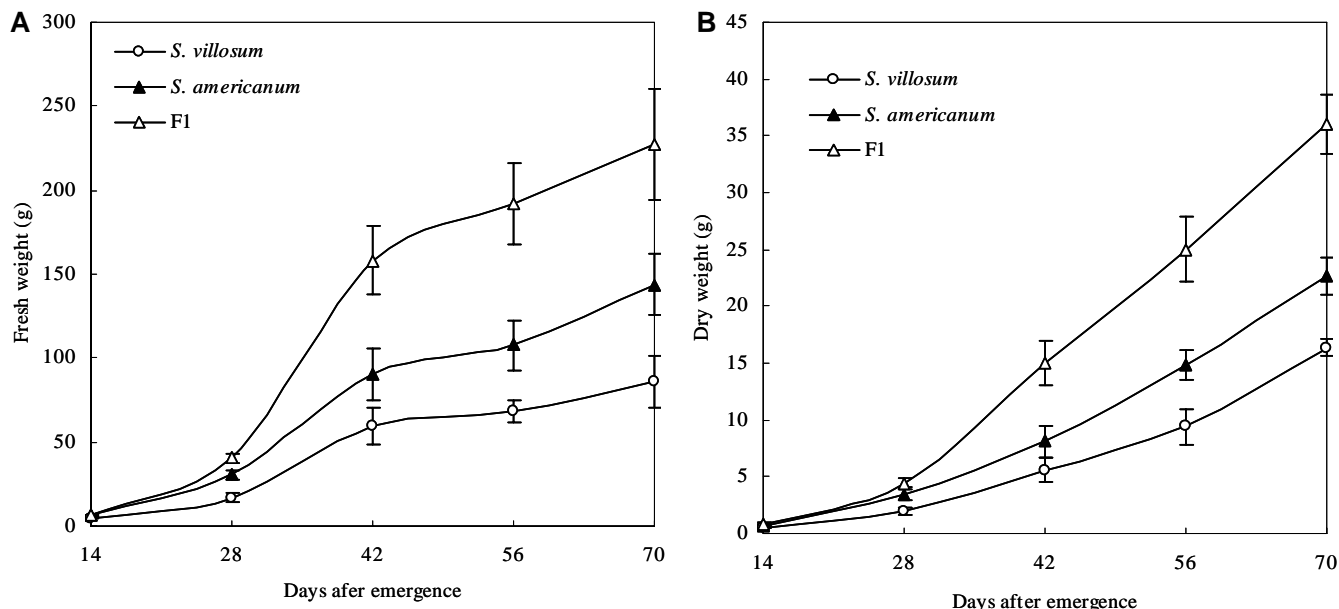
Colchicine has been widely used for chromosome doubling in various plants for several decades but side effects of chromosomal aberrations including chromosome losses, rearrangements and gene mutations have been reported (Lucet 1989). Chimerical plants have been detected by root tip analysis of colchicine 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 (Iarmarcovai *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 chromosome are other possible causes.

Colchicine can cause aneuploidy through any of the above processes and result in reduced fertility (Iarmarcovai *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 POLYPLOIDY OR HETEROPLDITY (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. Heuvelink 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 deflowering increased yield by 40% (Mwafusi 1992). Although there have not been any deliberate attempts to improve yields of leafy vegetables through induced sterility, Eckhart (1992) reported that in gynodioecious *Phaseolia 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-



**Fig. 8** Shoot tip fresh (A) and dry (B) weights of field-grown *S. americanum* (2x), *S. villosum* (4x) and their F<sub>1</sub> (3x) progeny plants. Data are means of 5 plants. Bars indicate standard errors.

pletely sterile triploid F<sub>1</sub> progeny of *S. villosum* (4x) and *S. americanum* (2x) has greater leaf yields on fresh and dry weight basis than either parent or the mean of both parents throughout the growing season (Fig. 8; Ojiewo *et al.* unpublished data).

### Altered gene expression

Merger of two distinct genomes can be followed by genomic changes such as sequence elimination, sequence homogenization, and repeat invasion 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 (Ozkan *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 (Os-

born *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 Pickett 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 (Rastogi 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 inter-related modifications, such as DNA methylation, histone modification and chromatin packaging (Wolffe 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 (Jenuwein

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, sequencing gel results showed a base transition (G to 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 associated with an increase in cell volume, with the highest ploidy levels up to  $2n = 640$  (approx. 80X) in angiosperms e.g. the stonecrop *Sedum suaveolens* (Leitch and Bennett 1997; Otto and Whitton 2000), and up to  $2n = 1260$  (approx. 84X) in land plants e.g. the fern *Ophioglossum pycnostichum* (Otto and Whitton 2000).

Polyploidy affects genetic mechanisms that control the size of plants, plant organs and plant cells (Kondorosi *et al.* 2000). The relationship between ploidy and cell size was first described in floral apices of *Datura stramonium*, different cell layers have different ploidies, showing a clear relationship between nuclear DNA content, nuclear volume and cell size (Satina and Blakeslee 1941). The periclinal chimeras were induced by colchicine treatment and had 2C and 8C nuclei. This gave rise to the 'karyoplasmic ratio' or 'nuclear-cytoplasmic ratio' theory which suggests that there is a control mechanism that regulates cytoplasmic volume, adjusting it with respect to the DNA content of the nucleus (Wilson 1925). Such a correlation is found in various natural forms including multinucleate syncytia, such as Characean algae. In an extreme example, embryo suspensors of *Tropaolum majus* have a DNA content of 2048C (Nagl 1976). Presumably, endoreduplication has evolved as a developmental means of providing differential gene expression in species with a small genome (Mizukami 2001). Cell size can influence growth rates and metabolic rates as surface: volume and DNA: cytoplasm ratios change. Polyploidization is also associated with longer and wider leaves in *Bromus inermis* (Tann and Dunn 1975) and *S. villosum* (Ojiewo *et al.* unpublished data).

Larger seed sizes may result in higher developmental rates for polyploidy plants than for their diploid progenitors and might improve seedling establishment in resource-limited environments. Large leaf size may translate directly to greater yields in the case of leafy vegetables such as African nightshade. Many crop products have high-ploidy varieties, which have a relatively greater green mass and

produce larger flowers, seeds, or fruits than those of diploid counterparts. This type of enlargement is of potential application in ornamental flowers where polyploid petals are thicker and leafy vegetables where the leaves are wider, thicker and more succulent. Tetraploid waxflowers (*Chamaelanium*) were reported to have an average flower diameter of 27.0 mm, triploids, 24.7 mm, and diploids 13.6 mm (Yan 2001). In *Dactylis glomerata* L. subsp. *lusitanica* the mean seed dry weight was significantly higher in tetraploid (48.40 g/plant) than in diploid (28.8 g/plant) plants (Bretagnolle and Lumaret 1995). Synthetic octoploid *Solanum villosum* have larger leaves (>2x) and heavier seeds (>1.5x) than wild-type tetraploid plants (Ojiewo *et al.*, unpublished data).

### Heterosis

Combination of different genomes (or the increase in heterozygosity) in heteroploids can lead to hybrid vigour (heterosis). Heterosis is a quantitative effect that results from crossing two different inbred lines such that the  $F_1$  generation is superior to the better parent (Birchler *et al.* 2003). The phenomenon of progressive heterosis (Groose *et al.* 1989) suggests that increased allelic diversity creates a more robust heterotic response. Progressive heterosis refers to the fact that double cross hybrids typically show greater vigour than single cross hybrids. In other words, when four different alleles are present in the genome, there is greater heterosis than when only two alleles are present. Differences in phenotype between polyploids and their diploid progenitors can be caused by increased variation in dosage-regulated gene expression (Guo *et al.* 1996). For genes having allele dosage effects, polyploidy increases the potential variation in expression levels (Osborn *et al.* 2003). For instance, changes in enzyme levels can affect many aspects of plant physiology, morphology and life history (Thompson *et al.* 1997).

Hybridization provides a mechanism for large and rapid adaptive transitions, made possible by the genetic variation of genes in a single generation (Rieseberg *et al.* 2003). Significant differences between some polyploid species and their diploid progenitors in vegetative traits such as growth rate and tolerance to biotic and abiotic stresses (de Bodt *et al.* 2005) and reproductive traits such as the initiation and duration of flowering time, fertility, apomixis, self-compatibility and germination and organ size have been reported (Lumaret 1988; Segraves and Thompson 1999; Schranz *et al.* 2002). Autopolyploid *Dactylis glomerata* L. subsp. *lusitanica* showed an increase in overall vigour, with broader and thicker, leaves (Bretagnolle and Lumaret 1995). Side-by-side trials of 13 elite *Gossypium hirsutum* genotypes and 21 *G. arboreum* diploids (AA) adapted to a common production region (India) show average seed cotton yield of 1135 ( $\pm 90$ ) kg/ha for the tetraploids, a 30% advantage over the 903 ( $\pm 78$ ) kg/ha of the diploids, at similar quality levels (Paterson 2005). Yield increase resulting from hybrid vigor is positively correlated with increasing heterozygosity or diversity of the contributing genomes in autotetraploid alfalfa (Bingham *et al.* 1994), potato (Peloquin 1995) and maize (Fasoula 2002).

### Increased photosynthesis

Polyploidy often produces immediate and dramatic changes in photosynthetic rates of plants, some of which have been summarised in **Table 1**. *Phlox drummondii* tetraploids, particularly in the eleventh generation (C11), were reported to show better photosynthetic performance (**Table 1**; Vyas *et al.* 2004). Photosynthetic rates have also been observed to increase with ploidy level in naturally occurring allopolyploid *Festuca arundinaceae* (Joseph *et al.* 1981), naturally occurring autopolyploid *Agropyron cristatum* (Frank 1980) and in synthetic autopolyploid *Lolium perenne* (Rathnam and Chollet 1980) *Medicago sativa* (Bingham 1998) and spontaneous autotetraploid of *Pennisetum americanum* (Warner and Edwards 1988). Polyploidy has interrelated effects on



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

Trait changed	Species	Ploidy	Increase rate	Reference
Chlorophyll content	<i>Phlox drummondii</i>	4x>2x; C <sub>0</sub> , C <sub>11</sub>	4x (auto), 18%, 110%	Vyas <i>et al.</i> 2004
	<i>Medicago sativa</i>	4x>2x; 8x>4x	2-fold	Bingham 1998
	<i>Lolium perenne</i>	4x>2x	47%	Rathnam and Chollet 1980
	<i>Pennisetum americanum</i>	4x>2x	2-fold	Warner and Edwards 1988
Net photosynthesis	<i>Phlox drummondii</i>	4x>2x; C <sub>0</sub> , C <sub>11</sub>	43%, >2-fold	Vyas <i>et al.</i> 2004
	<i>Atriplex confertifolia</i>	4x>2x	2-fold per cell	Warner and Edwards 1989
	<i>Panicum virgatum</i>	8x>4x	40%	Warner <i>et al.</i> 1987
	<i>Medicago sativa</i>	4x>2x; 8x>4x	2-fold; 67%	Bingham 1998
	<i>Triticum</i> spp.	6x>2x	>2-fold	Austin 1990
Stomatal conductance	<i>Phlox drummondii</i>	4x>2x, C <sub>11</sub>	2.75-fold	Vyas <i>et al.</i> 2004
Activity and amount of RuBisCo per leaf (cell)	<i>Medicago sativa</i>	4x>2x; 8x>4x	2-fold; 50%	Bingham 1998
	<i>Triticum</i> spp.	6x>2x	>2-fold	Austin 1990
Amount of leaf DNA	<i>Panicum virgatum</i>	8x>4x	2-fold	Warner <i>et al.</i> 1987
	<i>Pennisetum americanum</i>	4x>2x	2-fold	Warner and Edwards 1988
	<i>Medicago sativa</i>	4x>2x; 8x>4x	2-fold	Bingham 1998
	<i>Triticum</i> spp.	6x>2x	>2-fold	Austin 1990
	<i>Panicum virgatum</i>	8x>4x	2-fold	Warner <i>et al.</i> 1987
	<i>Pennisetum americanum</i>	4x>2x	2-fold	Warner and Edwards 1988
Number of chloroplasts per cell	<i>Medicago sativa</i>	4x>2x; 8x>4x	2-fold	Bingham 1998
	<i>Triticum</i> spp.	6x>2x	>2-fold	Austin 1990
	<i>Panicum virgatum</i>	8x>4x	15~20%	Warner <i>et al.</i> 1987
Cell volume mesophyll; bundle sheath	<i>Medicago sativa</i>	4x>2x	2-fold	Sharma-Natu and Ghildiyal 2005
	<i>Pennisetum americanum</i>	4x>2x	2-fold	Warner and Edwards 1988
	<i>Panicum virgatum</i>	8x>4x	2-fold	Warner <i>et al.</i> 1987

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  $\mu\text{m}$  longer 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 tetraploids. 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).

Enhanced gene expression observed in photosynthetically active parenchyma cells of some polyploid plants can result in increased net protein synthesis (Edgar and Orr-Weaver 2001). Multiplication of the genome has been proposed to increase metabolic activity, rRNA synthesis and transcriptional activity (Baluska and Kubika 1992). Increase in ploidy level significantly increased CO<sub>2</sub> exchange rate (Bryne *et al.* 1981), photosystem I electron transport activity (Krueger and Miles 1981), total carboxylase protein (Nelson *et al.* 1975), and total Rubulose-1,5-Bisphosphate Carboxylase/Oxygenase (RuBisCO) activity (Joseph *et al.* 1981) with the overall effect of increased net photosynthesis. Under normal field conditions, photosynthetic rates are limited by the activity of RuBisCO in addition to physical resistance to CO<sub>2</sub> diffusion in the leaf (Wareing *et al.* 1968). Increased gene expression with increasing ploidy level results in increased RuBisCO synthesis. Photosynthetic rates per cell, contents of DNA per cell, and activities of the bundle sheath enzymes RuBisCO and NAD-malic enzyme per cell were correlated with ploidal level at 99% or 95% confidence levels in *Atriplex confertifolia* (Warner and Edwards 1989). The authors concluded that there is a pro-

portional relationship between gene dosage and gene products. Since RuBisCO is the primary site for carboxylation in C<sub>3</sub> plants, the increased amount of RuBisCO whose synthesis is under nuclear control (Sharma-Natu and Ghildiyal 2005), could in turn increase photosynthetic capacity and photosynthetic performance in leaves with an overall effect of increased net photosynthesis. This increase may coincide with a possible increase in the number of chloroplasts associated with large cell size.

Photosynthetic 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 perenne* 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 satigrum* (Bjurman 1959), *Brassica oleracea* var. *gonglylodes* (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; Ojiewo *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 F<sub>2</sub> 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. americanum* (2x) and *S. scabrum* (6x) (Ojiewo *et al.* unpublished data).

Failure to obtain fertile F<sub>1</sub> 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 1960). 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 *Solanum*.

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## REFERENCES

- Adams KL, Cronn R, Percifield R, Wendel JF (2003) Genes duplicated by polyploidy show unequal contributions to the transcriptome and organ-specific reciprocal silencing. *Proceedings of the National Academy of Sciences USA* **100**, 4649-4654
- Adams KL, Wendel JF (2005) Polyploidy and genome evolution in plants. *Current Opinion in Plant Biology* **8**, 135-141
- Allard RW (1960) General features of polyploidy. In: Allard RW (Ed) *Principles of Plant Breeding*, John Wiley and Sons, New York, pp 370-376
- Attard G, Greystoke A, Kaye S, de Bono J (2006) Update on tubulin-binding agents. *Pathologie Biologie* **54**, 72-84
- Austin RB (1990) Prospects for genetically increasing the photosynthetic capacity of crops. In: Zelitch I (Ed) *Perspectives in Biochemical and Genetic Regulation of Photosynthesis*, Alan R Liss, Inc, New York, pp 395-409
- Baluska F, Kubica S (1992) Relationship between the content of basic nuclear proteins, chromatin structure, rDNA transcription and cell size in different tissues of the maize root apex. *Journal of Experimental Botany* **252**, 991-996
- Baumel A, Ainouche A, Kalendar R, Schulman AH (2002) Retrotransposons and genomic stability in populations of the young allopolyploid species *Spartina anglica* C.E. Hubbard (Poaceae). *Molecular Biology and Evolution* **19**, 1218-1227
- Bingham ET (1998) Role of chromosome blocks in heterosis and estimates of dominance and over-dominance. In: Lamkey KR, Staub JE (Eds) *Concepts and Breeding of Heterosis in Crop Plants*, Publication 25. CSSA, Madison, WI, USA, pp 71-87
- Bingham ET, Groose RW, Woodfield DR, Kidwell KK (1994) Complementary gene interactions in alfalfa are greater in autotetraploids than diploids. *Crop Science* **34**, 823-829
- Birchler JA, Auger DL, Riddle NC (2003) In search of a molecular basis of heterosis. *Plant Cell* **15**, 2236-2239
- Blakeslee AF, Avery AG (1937) Methods of inducing doubling of chromosomes in plants. *The Journal of Heredity* **28**, 393-411
- Bjurman B (1959) Photosynthesis in diploid and tetraploid *Ribes sativum*. *Physiologia Plantarum* **12**, 183-187
- Bretagnolle F, Lumaret R (1995) Bilateral polyploidization in *Dactylis glomerata* L. subsp. *lusitanica*: occurrence, morphological and genetic characteristics of first polyploids. *Euphytica* **84**, 197-207
- Briggs GC, Osmont KS, Shindo R, Sibout R, Hardtke CS (2006) Unequal genetic redundancies in *Arabidopsis* – a neglected phenomenon? *Trends in Plant Science* **11**, 492-496
- Bryne MC, Nelson CJ, Randall DD (1981) Ploidy effects on anatomy and gas exchange of tall fescue leaves. *Plant Physiology* **68**, 891-893
- Buxton BH, Newton WCF (1928) Hybrids of *Digitalis ambigua* and *Digitalis purpurea*, their fertility and cytology. *Journal of Genetics* **19**, 1269-1279
- Cassani JR, Caton WE (1985) Induced triploidy in grass carp, *Ctenopharyngodon ideila*. *Aquaculture* **46**, 31-44
- Chu CC (1978) The N<sub>6</sub> medium and its application to anther culture of cereal crops. In: *Proceedings of Symposium on Plant Tissue Culture*, Science Press, Beijing, China, pp 45-50
- Cohen D, Yao JL (1996) *In vitro* chromosome doubling of nine *Zantedeschia* cultivars. *Plant Cell, Tissue and Organ Culture* **47**, 43-49
- Coleman L (1950) Nuclear conditions in normal stem tissue of *Vicia faba*. *Canadian Journal of Research* **28**, 382-391
- Cowan CR, Cande WZ (2002) Meiotic telomere clustering is inhibited by colchicine but does not require cytoplasmic microtubules. *Journal of Cell Science* **115**, 3747-3756
- D'Amato F (1952) Polyploidy in the differentiation and function of tissues and cells in plants. *Caryologia* **4**, 311-358
- de Bodd S, Maere S, van de Peer Y (2005) Genome duplication and the origin of angiosperms. *Trends in Ecology and Evolution* **20**, 591-597
- Dermen H (1931) Polyploidy in *Petunia*. *American Journal of Botany* **18**, 250-261
- Dumas de Vaulx R, Chambonnet D (1982) Culture *in vitro* d' anther d' aubergine (*Solanum melongena* L.): stimulation de la production de plantes au moyen de traitements a +35°C associés à de faibles teneurs en substances de croissance. *Agronomie* **2**, 983-988 (in French with English summary)
- Dustin P (1978) *Microtubules*, Springer Verlag, Berlin, 452 pp
- Eckhart VM (1992) Resource compensation and evolution of gynodioecy in *Phaselia linearis* (Hydrophyllaceae). *Evolution* **46**, 1313-28
- Edgar BA, Orr-Weaver TL (2001) Endoreduplication cell cycles: more for less. *Cell* **105**, 297-306
- Edmonds JM (1977) Taxonomic studies on *Solanum* L. Section *Solanum* (Maurella). *Botanical Journal of Linnaeus Society* **75**, 141-178
- Edmonds JM (1979) Biosystematics of *Solanum* L. section *Solanum* (Maurella). In: Hawkes JG, Lester RN, Skelding AD (Eds) *The Biology and Taxonomy of the Solanaceae*, Academic Press, London, UK, pp 529-548
- Edmonds JM, Chweya JA (1997) *Black Nightshades. Solanum nigrum* L. and related species. Promoting the conservation and use of underutilised and neglected crops. 15. Institute of Plant Genetics and Crop Plant Research, Gatersleben/International Plant Genetic Resources Institute, Rome, Italy, pp 56-67
- Fasoula DA (2002) The effects of clonal propagation on the genetic improvement of potato. *Acta Horticulture* **579**, 71-75
- Force A, Lynch M, Pickett FB, Amores A, Yan YL, Postlethwait J (1999) Preservation of duplicate genes by complementary, degenerative mutations. *Genetics* **151**, 1531-1545
- Ford VS, Gottlieb LD (1999) Molecular characterization of PgiC in a tetraploid plant and its diploid relatives. *Evolution* **53**, 1060-1067
- Frank AB (1980) Photosynthesis, transpiration, and ribulose bisphosphate carboxylase of selected crested wheatgrass plants. *Agronomy Journal* **72**, 313-316
- Frydrych J (1970) Photosynthetic characteristics of diploid and tetraploid forms of *Brassica oleracea* var. *gonglyodes* grown under different irradiance. *Photosynthetica* **4**, 139-145
- Galitski T, Saldanha AJ, Styles CA, Lander ES, Fink GR (1999) Ploidy regulation of gene expression. *Science* **285**, 251-254
- Gamborg OL, Miller RA, Ojima K (1968) Nutrient requirements of suspension cultures of soybean root cells. *Experimental Cell Research* **50**, 151-158
- Gaut BS, Doebley JF (1997) DNA sequence evidence for the segmental allotetraploid origin of maize. *Proceedings of the National Academy of Sciences USA* **94**, 6809-6814
- Grant VP (1981) *Polyploidy*, Columbia University Press, New York, pp 283-352
- Groose RW, Talbert LE, Kojis WP, Bingham ET (1989) Progressive heterosis in autotetraploid alfalfa: Studies using two types of inbreds. *Crop Science* **29**, 1173-1177
- Guo M, Davis D, Birchler JA (1996) Dosage effects on gene expression in a maize ploidy series. *Genetics* **142**, 1349-1355
- Harlan JR, de Wet JMJ (1975) On Ö. Winge and a prayer: the origins of polyploidy. *Botanical Reviews* **41**, 361-390
- Hassaneim AM, Soltan DM (2000) *Solanum nigrum* is a model system in plant tissue and protoplast cultures. *Biologia Plantarum* **43**, 501-509
- Hawkes JG (1958) Potatoes: Taxonomy, Cytology and Crossability. In: Kappert H, Rudolf W (Eds) *Handbuch der Pflanzenzüchtung* (2<sup>nd</sup> Edn, Vol III), pp 1-43
- Heiser CB (1963) Estudio biosistemático de *Solanum* (Morella), en el Ecuador. *Ciencia y Naturaleza* **6**, 50-58
- Heiser CB Jr. (1976) *The Sunflower*, University of Oklahoma Press, Oklahoma, USA, 198 pp
- Henderson RJF (1974) *Solanum nigrum* L. (Solanaceae) and related species in Australia. *Contributions from the Queensland Herbarium* **16**, 1-78
- Heuvelink E, Buiskool RPM (1995) Influence of sink-source interaction on dry matter production in tomato. *Annals of Botany* **75**, 381-89
- Hilu KW (1993) Polyploidy and the evolution of domesticated plants. *American Journal of Botany* **80**, 1494-1499
- Hornsey KG (1973) The occurrence of hexaploid plants among autotetraploid populations of sugar beet (*Beta vulgaris* L.), and the production of tetraploid progeny using a diploid pollinator. *Caryologia* **26**, 225-28
- Hughes AL (2002) Adaptive evolution after gene duplication. *Trends in Genetics* **18**, 433-434
- Husband B (2000) Constraints on polyploid evolution: a test of the minority cytotype exclusion principle. *Proceedings of the Royal Society of London B: Biological Sciences* **267**, 217-223

- Iarmarcovai G, Botta A, Orsière T (2006) Number of centromeric signals in micronuclei and mechanisms of aneuploidy. *Toxicology Letters* **166**, 1-10
- Iwanaga M, Peloquin SJ (1982) Origin and evolution of cultivated tetraploid potatoes via 2n gametes. *Theoretical and Applied Genetics* **61**, 161-169
- Jenuwein T, Allis CD (2001) Translating the histone code. *Science* **293**, 1074-1080
- Jørgensen CA (1928) The experimental formation of heteroploid plants in the genus *Solanum*. *Journal of Genetics* **11**, 133-210
- Joseph MC, Randall DD, Nelson CJ (1981) Photosynthesis in polyploid tall fescue II. Photosynthesis and ribulose-1,5-bisphosphate carboxylase of polyploid tall fescue. *Plant Physiology* **68**, 894-898
- Karpechenko GD (1927) Polyploid hybrids of *Raphanus sativus* X *Brassica oleracea* L. *Bulletin of Applied Botany* **17**, 305-408
- Kondorosi E, Roudier F, Gendreau E (2000) Plant cell-size control: growing by ploidy? *Current Opinion in Plant Biology* **3**, 488-492
- Krueger RW, Miles D (1981) The effects of ploidy on electron transport and photophosphorylation in isolated chloroplasts of fescue. *Plant Physiology* **65**, S-9
- Lagercrantz U, Lydiate DJ (1996) Comparative genome mapping in *Brassica*. *Genetics* **144**, 1903-1910
- Lange W, Wagenvoort M (1973) Meiosis in triploid *Solanum tuberosum* L. *Euphytica* **22**, 8-18
- Lawton-Rauh A, Alvarez-Buylla ER, Purugganan MD (2000) Molecular evolution of flower development. *Trends in Ecology and Evolution* **15**, 144-149
- Lee HS, Chen ZJ (2001) Protein-coding genes are epigenetically regulated in *Arabidopsis* polyploids. *Proceedings of the National Academy of Sciences USA* **98**, 6753-6758
- Leitch I, Bennett M (1997) Polyploidy in angiosperms. *Trends in Plant Science* **2**, 470-476
- Leto K, Beckett J, Arntzen CJ (1979) Effect of nuclear gene dosage on photosynthetic light reactions. *Plant Physiology* **63** (Suppl), 160
- Lewis WH (1980) Polyploidy in species populations. In Lewis WH (Ed) *Polyploidy: Biological Relevance*. Plenum, New York, pp 103-44
- Lindstrom EW, Humphrey LM (1933) Comparative cytogenetic studies of tetraploid tomatoes from different origins. *Genetics* **18**, 193-209
- Litcher R (1982) Induction of haploid plants from isolated pollen *Brassica napus* rape. *Zeitschrift für Pflanzenphysiologie* **105**, 427-434
- Liu B, Brubaker, CL, Mergeai G, Cronn RC, Wendel JF (2001) Polyploid formation in cotton is not accompanied by rapid genomic changes. *Genome* **44**, 321-330
- Liu B, Wendel JF (2002) Non-Mendelian phenomena in allopolyploid genome evolution. *Current Genomics* **3**, 489-505
- Loidl J (1989) Colchicine action at meiotic prophase revealed by SC spreading. *Genetica* **78**, 195-203
- Loidl J (1990) The initiation of meiotic chromosome pairing: the cytological view. *Genome* **33**, 759-78
- Lockett DJ (1989) Colchicine mutagenesis is associated with substantial heritable variation in cotton. *Euphytica* **42**, 177-182
- Lumaret R (1988) Adaptive strategies and ploidy levels. *Acta Aecologica* **9**, 83-93
- Lynch M, Conery JS (2000) The evolutionary fate and consequences of duplicate genes. *Science* **290**, 1151-1155
- Lynch M, Force A (2000) The probability of duplicate gene preservation by subfunctionalization. *Genetics* **154**, 459-473
- Maestra B, de Jong JH, Shepherd K, Naranjo T (2002) Chromosome arrangement and behaviour of two rye telosomes at the onset of meiosis in disomic wheat-5RL addition lines with and without the *Ph1* locus. *Chromosome Research* **10**, 655-667
- Martinez-Perez E, Shaw P, Moore G (2001) The *Ph1* locus is needed to ensure specific somatic and meiotic centromere association. *Nature* **411**, 204-207
- Mishiba K, Mii M (2000) Polysomaty analysis in diploid and tetraploid *Portulaca grandiflora* *Plant Science* **156**, 213-219
- Mishiba K, Mii M (2006) Polysomaty and somaclonal variation in plants. In: Teixeira da Silva JA (Ed) *Floriculture, Ornamental and Plant Biotechnology: Advances and Topical Issues* (1<sup>st</sup> Edn, Vol II), Global Science Books, London, pp 262-270
- Miyoshi K (1996) Callus induction and plantlet formation through culture of isolated icropores of eggplant (*Solanum melongena* L.). *Plant Cell Reports* **15**, 391-395
- Mizukami Y (2001) A matter of size: developmental control of organ size in plants. *Current Opinion in Plant Biology* **4**, 533-539
- Mok DWS, Peloquin SJ, Tarn TR (1975) Cytology of potato triploids producing 2n pollen. *American Potato Journal* **52**, 171-174
- Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue culture. *Physiologia Plantarum* **15**, 473-497
- Mwafusi CN (1992) Effects of propagation method and deflowering on vegetative growth, leaf yield; phenolic and glycoalkaloid contents of three black nightshade selections used as vegetables in Kenya. MSc. Thesis, University of Nairobi, Kenya, 85 pp
- Nagl W (1976) DNA endoreduplication and polytene understood as evolutionary strategies. *Nature* **261**, 614-615
- Navarro-Alvarez W, Baenziger PS, Eskridge KM, Hugo M, Gustafson VD (1994) Addition of colchicine to wheat anther culture media to increase doubled haploid plant production. *Plant Breeding* **112**, 192-198
- Nelson CJ, Asayk H, Patton LD (1975) Photosynthetic responses of tall fescue plant tissue to selection for longevity below the CO<sub>2</sub> compensation point. *Crop Science* **15**, 629-633
- Newton WCF, Pellew C (1929) *Primula kewensis* and its derivatives. *Journal of Genetics* **20**, 405-467
- Nistch JP, Nistch C (1969) Haploid plants from pollen grains. *Science* **163**, 85-87
- Ojiewo CO, Agong SG, Murakami K, Masuda M (2006) Chromosome duplication and ploidy level determination in African nightshade [*Solanum nigrum* L. ssp. *villosum* (L) Ehrh]. *Journal of Horticultural Science and Biotechnology* **81**, 183-188
- Ojiewo CO, Murakami K, Masinde PW, Agong SG (2007) Floral genetics of African nightshade (*Solanum* section *Solanum*). *International Journal of Plant Developmental Biology* **1**, 75-81
- Osborn TC, Pires JC, Birchler JA, Auger DL, Chen ZJ, Lee HS, Comai L, Madlung A, Doerge RW, Colot V, Martienssen RA (2003) Understanding mechanisms of novel gene expression in polyploids. *Trends in Genetics* **19**, 141-147
- Otto SP, Whitton J (2000) Polyploid incidence and evolution. *Annual Review of Genetics* **34**, 401-437
- Ozkan H, Levy A, Feldman M (2001) Allopolyploidy-induced rapid genome evolution in the wheat (*Aegilops-Triticum*) group. *Plant Cell* **13**, 1735-1747
- Paterson AH, Bowers JE, Peterson DG, Estill JC, Chapman BA (2003) Structure and evolution of cereal genomes. *Current Opinion in Genetics and Development* **13**, 644-650
- Paterson AH (2005) Polyploidy, evolutionary opportunity, and crop adaptation. *Genetica* **123**, 191-196
- Peloquin SK, Yerk GL, Wesnov JE, Dasmo E (1989) Potato breeding with haploid and 2n gametes. *Genome* **31**, 1000-1004
- Peloquin S (1995) Genetic mechanisms of genome evolution and speciation in autotetraploid plants-the potato model. In: Raupp J, Gill BS (Eds) *Classical and Molecular Cytogenetic Analysis*, Proceedings of US-Japan Symposium Kansas State University, Manhattan, March 21-23, 1994, pp 276-282
- Pikaard CS (2001) Genomic change and gene silencing in polyploids. *Trends in Genetics* **17**, 675-677
- Poot P (1997) Reproductive allocation and resource compensation in male sterile and hermaphroditic plants of *Plantago lanceolata* (Plantaginaceae). *American Journal of Botany* **84**, 1256-1265
- Prince VE, Pickett FB (2002) Splitting pairs: the diverging fates of duplicated genes. *Nature Reviews. Genetics* **3**, 827-837
- Ramsey J, Schemske DW (1998) Pathways, mechanisms and rates of polyploidy formation in flowering plants. *Annual Reviews of Ecological Systems* **29**, 467-501
- Randolph LF (1932) Some effects of high temperature on polyploidy and other variations in maize. *Proceedings of the National Academy of Sciences USA* **18**, 222-229
- Rastogi S and Liberles DA (2005) Subfunctionalization of duplicated genes as a transition state to neofunctionalization *Evolutionary Biology* **5**, 28
- Rathnam CKM, Chollet R (1980) Photosynthetic and photorespiratory carbon metabolism in mesophyll protoplasts and chloroplasts isolated from isogenic diploid and tetraploid cultivars of perennial ryegrass (*Lolium perenne* L.). *Plant Physiology* **65**, 489-494
- Rick CM (1945) A survey of cytogenetic causes of unfruitfulness in the tomato. *Genetics* **30**, 347-362
- Rieseberg LH, Raymond O, Rosenthal DM, Lai Z, Livingstone K, Nakazato T, Durphy JL, Schwarzbach AE, Donovan LA, Lexer C (2003) Major ecological transitions in wild sunflowers facilitated by hybridization. *Science* **301**, 1211-1216
- Rodriguez DJ (1996) A model for the establishment of polyploidy in plants. *The American Naturalist* **147**, 33-46
- Rogers BS, Ogg AG (1981) Biology of weeds of the *Solanum nigrum* complex (*Solanum* Section *Solanum*) in North America. *Agricultural Reviews and Manuals, Western Series* **23**, 1-30
- Romero-Aranda R, Bondada BR, Syvertsen JP, Grosser JW (1997) Leaf characteristics and net gas exchange of diploid and autotetraploid citrus. *Annals of Botany* **79**, 153-160
- Rotino GL (1996) Haploidy in eggplant. In: Jain SM, Sopory SK, Veilleux RE (Eds) *In Vitro Production in Higher Plants* (Vol 3), Kluwer Academic Publishers, Dordrecht, Netherlands, pp 115-141
- Rotino GL, Falavigna A, Restaino F (1987) Production of anther-derived plantlets of eggplant. *Capsicum Newsletter* **6**, 89-90
- Rye BL (1979) Chromosome number variation in the Myrtaceae and its taxonomic implications. *Australian Journal of Botany* **27**, 547-573
- Salisbury E (1961) *Weeds and Aliens*, Collins, London, UK, pp 187-188
- Sapra VT, Hughes JL, Sharma GC (1975) Frequency, size and distribution of stomata in triticales leaves. *Crop Science* **15**, 356-358
- Satina S, Blakeslee AF (1941) Periclinal chimeras in *Datura stramonium* in relation to development of leaf and flower. *American Journal of Botany* **28**, 862-871
- Schranz ME, Quijada P, Sung SB, Lukens L, Amasino R, Osborn TC (2002)

- Characterization and effects of the replicated flowering time gene FLC in *Brassica rapa*. *Genetics* **162**, 1457-1468
- Segraves K, Thompson J** (1999) Plant polyploidy and pollination: floral traits and insect visits to diploid and tetraploid *Heuchera grossulariifolia*. *Evolution* **53**, 1114-1127
- Setter TL, Schrader LE, Bingham ET** (1978) Carbon dioxide exchange rates, transpiration and leaf characteristics in genetically equivalent ploidy levels in alfalfa. *Crop Science* **18**, 327-333
- Shaked H, Kashkush K, Ozkan H, Feldman M, Levy AA** (2001) Sequence elimination and cytosine methylation are rapid and reproducible responses of the genome to wide hybridization and allopolyploidy in wheat. *Plant Cell* **13**, 1749-1759
- Sharma-Natu P, Ghildiyal MC** (2005) Potential targets for improving photosynthesis and crop yield. *Current Science* **88**, 1918-1928
- Shoemaker RC, Polzin K, Labate J, Specht J, Brummer EC, Olson T, Young N, Concibido V, Wilcox J, Tamulonis JP, Kochert G, Boerma HR** (1996) Genome duplication in soybean (*Glycine* subgenus *soja*) *Genetics* **144**, 329-338
- Soltis DE, Soltis PS** (1999) Polyploidy: recurrent formation and genome evolution. *Trends in Ecology and Evolution* **14**, 348-352
- Song K, Lu P, Osborn T** (1995) Rapid genome change in synthetic polyploids of *Brassica* and its implications for polyploid evolution. *Proceedings of the National Academy of Sciences USA* **92**, 8089-8091
- Soria J, Heiser CB Jr.** (1959) The garden huckleberry and the sunberry. *Baileya* **7**, 33-35
- Stebbins GL** (1950) *Variation and Evolution in Plants*, Oxford University Press, London, UK, 643 pp
- Stern H, Hotta Y** (1973) Biochemical controls of meiosis. *Annual Reviews of Genetics* **7**, 37-66
- Sugimoto-Shirasu K, Roberts K** (2003) Big it up: endoreduplication and cell-size control in plants. *Current Opinion in Plant Biology* **6**, 544-553
- Sunderland N, Dunwell JM** (1977) Anther and pollen culture. In: Street HE (Ed) *Plant Tissue and Cell Culture*, Blackwell, Oxford, pp 223-265
- Tann G, Dunn GM** (1975) Stomatal length, frequency and distribution in *Bromus inermis* Leyss. *Crop Science* **15**, 283-286
- Thompson JN, Cunningham BM, Segraves KA, Althoff DM, Wagner D** (1997) Plant polyploidy and insect/plant interactions. *The American Naturalist* **150**, 730-743
- Vyas P, Funayama-Noguchi S, Yano S, Hanba Y, Terashima I** (2004) Effects of polyploidy on photosynthetic performance in leaves of *Phlox drummondii*. *13<sup>th</sup> International Congress on Photosynthesis Montreal*, Quebec, Canada, Abstract 519
- Walsh JB** (1995) How often do duplicated genes evolve new functions? *Genetics* **139**, 421-428
- Walsh JB** (2003) Population-genetic models of the fates of duplicate genes. *Genetica* **118**, 279-294
- Wareing PF, Khalifa MM, Treharne KJ** (1968) Rate-limiting processes in photosynthesis at saturating light intensities. *Nature* **220**, 453-457
- Warner DA, Ku MSB, Edwards GE** (1987) Photosynthesis, leaf anatomy, and cellular constituents in the polyploid C4 grass *Panicum virgatum*. *Plant Physiology* **84**, 461-466
- Warner DA, Edwards GE** (1988) C4 photosynthesis and leaf anatomy in diploid and autotetraploid *Pennisetum americanum* (pearl millet). *Plant Science* **56**, 85-92
- Wareing DA, Edwards GE** (1989) Effects of polyploidy on photosynthesis rates, photosynthetic enzymes, contents of DNA, chlorophyll, and sizes and numbers of photosynthetic cells in the C<sub>4</sub> dicot *Atriplex confertifolia*. *Plant Physiology* **91**, 1143-1151
- Warner DA, Edwards GE** (1993) Effects of ploidy on photosynthesis. *Photosynthesis Research* **35**, 135-147
- Webber JM** (1940) Polyembryony. *Botanical Reviews* **6**, 575-98
- Wendel JF** (2000) Genome evolution in polyploids. *Plant Molecular Biology* **42**, 225-249
- Wilcox J, Tamulonis JP, Kochert G, Boerma HR** (1996) Genome duplication in soybean (*Glycine* subgenus *soja*). *Genetics* **144**, 329-338
- Wilson EB** (1925) *The Cell in Development and Heredity*, Macmillan, New York, 1232 pp
- Winge Ö** (1917) The chromosomes. Their numbers and general importance. *Comptes Rendus des Travaux du Laboratoire Carlsberg* **13**, 131-175
- Winkler H** (1916) Ueber die experimentelle erzeugung von pflanzen mit abweichenden chromosomenzahlen. *Zeitschrift für Botanik* **8**, 417-531
- Wolfe AP, Matzke MA** (1999) Epigenetics: regulation through repression. *Science* **286**, 81-486
- Wong SC, Cowan IR, Farquhar GD** (1979) Stomatal conductance correlates with photosynthetic capacity. *Nature* **282**, 424-426
- Yan G** (2001) A report for the Rural Industries Research and Development Corporation RIRDC Publication No 01, RIRDC Project No UWA 35A, 52 pp