Cytomorphological analysis of a novel hybrid from Solanum melongena 'Golden' x S. scabrum 'Scabrum' (Solanaceae)

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Abstract

Genotype manipulation through the introduction of novel genes for improved yield, agronomic qualities and a larger gene pool informed an experimental cross between diploid Solanum melongena 'Golden' (2n = 2x = 24) and tetraploid S. scabrum 'Scabrum' (2n = 4x = 48). The F1 fruit contained eight seeds which had a 100% germination and a chromosome number 2n = 6x = 72. Surviving hybrids were closer to the diploid parent in many characters. Leaves were fairly lobed, sparsely hairy and were 13.5 x 8.6 cm in the hybrid compared to the hairy, deeply lobed, 14.8 x 10.6 cm leaves in the diploid and glabrous, entire and 11.4 x 10.6 cm leaves in the tetraploid parent. The inflorescence in the hybrid was a raceme as in the diploid parent but was umbellate in the tetraploid. Pollen viability was 38.2% in the hybrid but was 71% and 97.4% in the diploid and tetraploid parents, respectively. Fruit was seedless in the F2; it was round and red, containing 384 seeds, globose-shaped, yellow seeds in the diploid, and 67 round, purple seeds in the tetraploid parent. Meiosis was regular in the hybrid with few univalents and impaired bivalents due to dissimilar parental genomes. Mitotic chromosomes were asymmetrical with various sizes. Epistasis and negative gene interaction mechanisms were implicated in the hybrids' low quality and breakdown. Backcross to the tetraploid parent may bring about gene recombination and allelic realignment for desirable phenotypes in the F2 and subsequent generations. Endoduplication of the triploid zygote might have produced an autoallohexaploid hybrid.

Additional key words: autopolyploidization, endoduplication, fruit set, hybrid breakdown, interspecific hybridization.

Resumen

Análisis citomorfológico de un nuevo híbrido de Solanum melongena 'Golden' × S. scabrum 'Scabrum' (Solanaceae)

La manipulación genotípica permite la introducción de genes nuevos para la mejora de la producción y otras cualidades agronómicas. Se llevó a cabo un cruce experimental entre Solanum melongena 'Golden' (diploide, 2n = 2x = 24) y S. scabrum 'Scabrum' (tetraploide, 2n = 4x = 48). Los frutos de la F1 contenían ocho semillas con un 100% de germinación y un n° de cromosomas 2n = 6x = 72. Los híbridos que sobrevivieron estaban más próximos, en muchos caracteres, al parental diploide. Las hojas del híbrido eran de 13,5 x 8,6 cm, algo lobuladas y tenían escasos pelos, mientras que las del diploide eran de 14,8 x 10,6 cm, con pelos y profundamente lobuladas, y las del parental tetraploide de 11,4 x 10,6 cm, sin pelos y sin lobular. Las inflorescencias del híbrido y del diploide eran en racimo, mientras que en el tetraploide eran umbeladas. La viabilidad del polen fue del 38,2%, 71% y 97,4% en el híbrido, diploide y tetraploide, respectivamente. El fruto de la F2 no tenía semilla, era rojo y púrpura; mientras que en el parental diploide contenía 384 semillas, era alargado y amarillo, y en el tetraploide tenía 67 semillas, y era rodeado y purpura. La meiosis en el híbrido fue regular, con algunos cromosomas sin aparear. Los cromosomas mitóticos fueron asimétricos y de varios tamaños. La baja calidad y fallo de los híbridos se explican por la epistasis y los mecanismos de interacción génica negativos. El retrocruza de generaciones. La endoduplicación del cigoto del tetraploide podría haber producido un híbrido autoallohexaploide.

Palabras clave adicionales: autopoliploidización, cuajado fruto, endoduplicación, fracaso de la hibridación, hibridación interspecífica.

Abbreviations used: FORIG (Forestry Research Institute of Ghana), ICRAF (International Centre for Research in Agroforestry, Nairobi, Kenya), MI (Metaphase I), NIHORT (National Horticultural Research Institute, Nigeria), PROSEA (Plant Resources of South-East Asia Foundation).
Introduction

Diploid *Solanum* species are common leafy and/or fruit vegetables in many tropical countries (Daunay and Chadha, 2004; Fontem and Schippers, 2004). Some species bear tubers as in *S. tuberosum*, which is the most important vegetable in the United States and the fourth most important world crop after rice (*Oryza sativa* L.), wheat (*Triticum aestivum* L.) and maize (*Zea mays* L.) (FAO, 2008; USDA, 2008). The species large size and widespread use make them popular food crops (Omidiji, 1983; Gbile, 1985), tubers (Edmonds, 1986; Knapp, 1991) and weeds (Mwai and Schippers, 2004; Schippers, 2004). The species are shrubs to small trees, annual and rarely perennial (Lester and Seck, 2004). Leaves are simple, variously lobed, and lobing characteristics are often species specific. Fruits are round, globose to sub-globose and red, yellow or purple when ripe.

The genus is diverse morphologically and cytologically (Okoli, 1988; Oyelana and Ugborogho, 1997) and members express variation in growth habit, ecogeographical distribution, adaptation and ploidy. The basic chromosome numbers of \(x=12, 24\) (Sangowawa, 1986; Oyelana, 2005) are readily encountered in many of the domesticated and a few wild species. There are also reports of mixed populations in which aneuploid, aneusomatic and mixoploid species (Hawkes, 1990; Gavrilenko et al., 1999) grow in close proximity.

Genetic improvement of member species focuses on improving fruit quality (Behera and Narendra, 2002), harvestable leaves and resistance to pests and diseases (Dorrance et al., 2001; Lynch et al., 2003; Lebecka et al., 2005) for consumer acceptability. Interspecific crosses (Omidiji, 1983; Ugborogho and Oyelana, 1999; Musuelli et al., 2006) have been used as a strategy to improve quality of member species. Swarms of hybrids have been developed and a few have been stabilized through repeated backcrosses (Ugborogho and Oyelana, 1999) to either or both parents. There are also reports of past inter-series (Khvedynich and Podgaetskii, 1993; Behera and Narendra, 2002) and intergeneric (Stoeva et al., 1990; Ji and Chetelat, 2003) crosses aimed at introducing novel genes on which further improvements could be predicated. Tuber quality and soft rot resistance have been developed in hybrids of *S. tuberosum* and an incongruent wild relative *S. commersonii* (Carputo et al., 2002). A number of hybridization attempts involving species at different ploidy levels have also been effected. Beamish (1955) carried out crosses involving the hexaploid *S. demissum* with four diploid *Solanum* species.

Polyploidization through mutation breeding (Oyelana and Ogunwenmo, 2005; Stupar et al., 2007) and mutant screening has also been explored as a breeding strategy to produce cultivars with desirable phenotypes for cyto-genetic and agronomic purposes. This is a practice commonly reported in angiosperms (Blanc and Wolfe, 2004; Peterson et al., 2004; Yu et al., 2005; Cui et al., 2006) and may confer enhanced vigour and other advantages (Adams et al., 2003; Osborn et al., 2003). A number of haploid mutants were successfully generated from regular tetraploids (Ercolano et al., 2004) and doubling of the chromosome numbers to generate \(2n\) gametes (Hayes et al., 2005; Oyelana and Ogunwenmo, 2005) has been achieved in some cultivars. A number of well-known polyploid plants of agricultural importance are classical allopolyploids including important crops such as bread wheat (*T. aestivum* L.) (\(2n = 6x = 42\)) and cotton (*Gossypium* sp.) (\(2n = 4x = 56\)) and other synthetized allopolyploids (Wang et al., 2004; Madlung et al., 2005; Albertin et al., 2006).

Species tolerance of ploidy manipulation and the ease by which \(2n\) pollen and eggs are generated through mutation breeding (Peloquin et al., 1999; Oyelana and Ogunwenmo, 2005) explains the number of repeated efforts in this direction. A number of research institutes including the Forestry Research Institute of Ghana (FORIG), the World Agroforestry Centre (ICRAF) in Nairobi, the Prosea Foundation (PROSEA) in Indonesia, and the National Horticultural Research Institute (NIHORT) in Ibadan, Nigeria, to mention a few, were set up to collect, preserve and improve the existing germplasms of some vegetables and arable crops and coordinate outreach and extension services for immediate and remote farming communities. The development of phenotypes that ensure better quality yields and increased farm revenue are the major goals of these different organizations.

Basic genotype manipulation through introduction of rare genes for expansion of a species genome and mutation breeding are the basis of a number of attempt made to improve the quality of many of these species. This informed our choice of crossing a tetraploid (\(2n = 48\)) and a diploid (\(2n = 24\)) species from two *Solanum* subgenera (*Leptostemonum* and *Solanum*) with the expectation of developing hybrids for cyto-genetic and agronomic purposes and possibly to produce new genome combinations upon which further improvements could be made.
**Material and methods**

**Description of parent species**

*Solanum melongena* 'Golden'. Is an annual shrub and is rarely perennial. The stem is erect and woody and branches are profuse and spreading. Height ranges from 149 to 180 cm. Leaves are simple, hairy, ovate, deeply lobed and acute at apices and attenuate to oblique bases (Fig. 1A). Stomata are anomocytic. The inflorescence is a raceme and subtends 3-4 flowers. Petals are five and pink. Fruits are globose and yellow (Fig. 2A). It is a diploid with a gametic chromosome number of n = 12 (Fig. 3A). Chromosomes are symmetrical and mostly metacentric with a few being sub-metacentric.

*Solanum scabrum* 'Scabrum'. Is an annual herb. Stem is erect or procumbent up to 60 cm tall, herbaceous and angular. Leaves are simple, glabrous on both surfaces, ovate, entire, acute to acuminate at the apices and truncate at base (Fig. 1C). Stomata were anomocytic. The inflorescence is simple umbellate to sub-umbellate, and has 3-6 flowers. Petals are five and white. Fruits are round, purple and berry-like (Fig. 2D). It is a tetraploid with a gametic chromosome number of n = 24 (Fig. 3B). Chromosomes were symmetrical and mostly metacentric.

**Hybridization, emasculation and flower pollination**

Reciprocal crosses were made between *S. melongena* 'Golden' and *S. scabrum* 'Scabrum' from 12 weeks of growth. The F₁ hybrids were derived from the cross: ♀ *S. melongena* 'Golden' x ♂ *S. scabrum* 'Scabrum'.

Twenty-five unopened flowers were tagged and bagged 48 h prior to emasculation to exclude any insects. Flower emasculation was carried out 18 h prior to anthesis. Flowers were bagged and pollinated with pollen from freshly dehisced anthers from the designated male parent. Pollen was rubbed onto the stigmatic surface with a hand brush. The process was repeated two hourly and was discontinued 1 h before the flowers closed. Pollinated flowers remained in bags until their corolla had withered. The procedure was repeated for the reciprocal crosses.

**Germination and seedling screening**

Parental and F₁ seed were sown in planting trays in a greenhouse for the emerging seedlings to form roots. At 3 weeks they were transferred into planting bags. The two surviving F₁ seedlings were placed under shade, in the nursery for 2 weeks before exposure to field conditions.

**Field cultivation**

Six beds 6 x 4 m, 1 m apart, were made in a 20 x 10 m plot. Two beds were designated for each parent species and one each for their respective F₁ hybrids. Plants were placed in 18 cm deep holes following removal of the bottom of the polythene bags. They were watered twice daily, early and at sunset.
Morphometric and cytological analysis

Detailed observations on morphological and floral features were carried out using a hand lens and stereomicroscope. Measurement of plant parts was done with a metre rule.

The techniques of Ugborogho and Oyelana (1992), and Ogunwunmo (1999) were used for stomata, pollen, and mitotic and meiotic chromosome studies.

Results

The two parental species were rarely interfertile, and the only F1 fruit obtained was poorly developed and contained only 8 seeds. The only F1 fruit obtained was when Solanum scabrum ‘Scabrum’ was the pollen donor. All other crossing attempts, including reciprocal crosses, were unsuccessful.

The F1 seeds had a 100% germination. However only 2 seedlings (25%) reached maturity and produced F2 fruits.

The F1 hybrids were erect with woody stem as in the female diploid parent. Leaves were simple, fairly lobed, ovate, and sparsely hairy on both surfaces with oblique base as in the diploid parent (Fig. 1A-C). Branches were profuse and spreading as in the diploid parent. Stomata were anomocytic as in both parents and evenly distributed on both leaf surfaces. A summary of morphological characters and variation among parents and the hybrid are shown in Table 1.

The inflorescence in the hybrids was a raceme with five pink flowers, like the diploid parent. Flowers were few as many dropped as buds. Petals were intermediate in size to both parents. Pollen was regular with a 38.2% viability (Table 1). Ovaries appeared normal.

Fruit was round, red and seedless and closer in shape and size to the male parent (Table 1). They contained little or no mesocarp as the pericarps were almost empty (Fig. 2).

Chromosomes of the parental lines segregated normally into n = 12 (Fig. 3A) and n = 24 (Fig. 3B) gametes. However, the hybrid produced an n = 36 gamete (Fig. 3C). Meiosis was regular but few univalents and impaired bivalents were encountered in the hybrid. Anaphase was normal and equal chromosomes were distributed to the poles. Tetrads appeared regular.

The diploid chromosome number of 2n = 72 (Fig. 3D) was obtained for the hybrid. Chromosomes were asymmetrical in the hybrid, 1.18 to 2.42 µm long and mostly sub-metacentric.

Discussion

Plant hybridization is a means of producing new genomes through introduction of new genes, expansion of existing genomes and the range of genotypic and phenotypic variation beyond that expressed in natural species (Rieseberg et al., 2000; Oyelana and Ugborogho, 2008). Interspecific hybridization has been used as a strategy to introduce new genes to re-invigorate and/or stabilize existing genomes as beneficial alleles from both parents are often merged in the heterozygote hybrids (Carputo et al., 2003; Troyer, 2006; Springer and Stupar, 2007).

Whereas, a triploid hybrid (2n = 3x = 36) was expected from the fertilization of diploid and tetraploid parents’ gametes (n = 12 + 24), a hexaploid was produced. This might have arisen from endoduplication of the resulting triploid zygote giving an autoallohexaploid. A triploid hybrid resulted from a tetraploid by diploid cross in ryegrass (Lolium perenne L.) (Ahloowalia, 1975).

Midparent values of leaves, internode length, petal and sepal sizes and petiole length characterized the two surviving hybrids. Similar observations were reported by Okada and Clausen (1985) which they
attributed to scores of heterozygote alleles in the hybrids’ genome. On the other hand, these hybrids ($S. \text{melongena} \times S. \text{scabrum}$) were products of unequal-sized gametes and

<table>
<thead>
<tr>
<th>Character</th>
<th>$S. \text{melongena} \ 'Golden'$</th>
<th>$F_1$ hybrid</th>
<th>$S. \text{scabrum} \ 'Scabrum'$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plant habit</td>
<td>Erect 1.5 m high, many spreading branches</td>
<td>Erect 1.2 m high, spreading branches</td>
<td>Prostrate 0.6 m high, many branches</td>
</tr>
<tr>
<td>Leaf length (cm)</td>
<td>12.5 - 15.6 a</td>
<td>8.4 - 14.0</td>
<td>9.5 - 13.3</td>
</tr>
<tr>
<td>Leaf width (cm)</td>
<td>14.75 ± 1.2</td>
<td>13.5 ± 0.3</td>
<td>11.4 ± 1.3</td>
</tr>
<tr>
<td>Stipule length (cm)</td>
<td>8.5 ± 11.4</td>
<td>6.4 - 10.0</td>
<td>9.3 - 12.8</td>
</tr>
<tr>
<td>Stipule width (cm)</td>
<td>10.6 ± 1.4</td>
<td>8.6 ± 1.2</td>
<td>10.8 ± 1.1</td>
</tr>
<tr>
<td>Internode number</td>
<td>3.2 - 4.0</td>
<td>2.9 - 3.4</td>
<td>2.8 - 3.8</td>
</tr>
<tr>
<td>Internode length (cm)</td>
<td>3.5 ± 0.2</td>
<td>3.1 ± 0.2</td>
<td>3.7 ± 1.0</td>
</tr>
<tr>
<td>Length of petiole (cm)</td>
<td>2.4 - 3.2</td>
<td>2.6 - 3.8</td>
<td>3.3 - 4.1</td>
</tr>
<tr>
<td>Inflorescence type</td>
<td>2.6 ± 1.8</td>
<td>3.0 ± 0.3</td>
<td>3.8 ± 0.3</td>
</tr>
<tr>
<td>Number of buds</td>
<td>16 - 24</td>
<td>14 - 22</td>
<td>8 - 14</td>
</tr>
<tr>
<td>Petal length (mm)</td>
<td>3.4 - 4.7</td>
<td>3.9 - 5.2</td>
<td>3.2 - 4.3</td>
</tr>
<tr>
<td>Petal width (mm)</td>
<td>4.4 ± 1.9</td>
<td>4.4 ± 1.4</td>
<td>3.6 ± 0.4</td>
</tr>
<tr>
<td>Sepal length (mm)</td>
<td>5.3 - 6.4</td>
<td>3.4 - 4.4</td>
<td>4.2 - 5.6</td>
</tr>
<tr>
<td>Sepal width (mm)</td>
<td>5.6 ± 1.6</td>
<td>3.9 ± 0.4</td>
<td>4.8 ± 1.3</td>
</tr>
<tr>
<td>Pedicel length (mm)</td>
<td>Raceme</td>
<td>Raceme</td>
<td>Umbellate</td>
</tr>
<tr>
<td>Fruit colour</td>
<td>5.0 - 6.5</td>
<td>4.0 - 5.0</td>
<td>3.0 - 4.4</td>
</tr>
<tr>
<td>Fruit diameter (mm)</td>
<td>6.0 - 69.0</td>
<td>15.5 - 16.0</td>
<td>6.8 - 8.3</td>
</tr>
<tr>
<td>Flower colour</td>
<td>63.0 ± 2.5</td>
<td>15.7 ± 0.2</td>
<td>7.5 ± 0.5</td>
</tr>
<tr>
<td>Open flower diameter (mm)</td>
<td>33.0 - 34.5</td>
<td>26.5 - 28.5</td>
<td>5.0 - 6.5</td>
</tr>
<tr>
<td>Abaxial stomata length (µm)</td>
<td>33.5 ± 0.5</td>
<td>27.5 ± 1.0</td>
<td>5.5 ± 0.5</td>
</tr>
<tr>
<td>Abaxial stomata width (µm)</td>
<td>34.5 ± 1.0</td>
<td>33.5 ± 0.3</td>
<td>46.0 - 47.5</td>
</tr>
<tr>
<td>Pollen size (µm)</td>
<td>25.5 - 28.0</td>
<td>27.0 - 30.0</td>
<td>31.0 - 33.5</td>
</tr>
<tr>
<td>Pollen viability (%)</td>
<td>27.0 ± 1.5</td>
<td>28.5 ± 1.0</td>
<td>31.5 ± 0.5</td>
</tr>
<tr>
<td>Germination (%)</td>
<td>36.0 - 37.0</td>
<td>36.0 - 37.0</td>
<td>31.5 ± 0.5</td>
</tr>
<tr>
<td>Number of seeds per fruit</td>
<td>36.5 ± 0.5</td>
<td>36.5 ± 0.0</td>
<td>31.5 ± 0.5</td>
</tr>
<tr>
<td>Chromosome number</td>
<td>71</td>
<td>38.2</td>
<td>97.4</td>
</tr>
<tr>
<td>Germination (%)</td>
<td>85</td>
<td>100</td>
<td>44.8</td>
</tr>
<tr>
<td>Number of seeds per fruit</td>
<td>327 - 451</td>
<td>8 (F1), 0 (F2)</td>
<td>57 - 83</td>
</tr>
<tr>
<td>Chromosome number</td>
<td>384 ± 3.0</td>
<td>67 ± 3.0</td>
<td>44.8</td>
</tr>
</tbody>
</table>

Table 1. Morphological and growth characteristics of $S. \text{melongena} \ 'Golden'$, $S. \text{scabrum} \ 'Scabrum'$ and the $F_1$ hybrids. Mean ± SE

\(a\) Range
genomes, hence their midparent values may either suggest a higher ratio of dominant alleles in the diploid parent genotype or the masking of numerous alleles in the tetraploid parent by epistasis or other form of negative gene interaction. Investigation of triploid hybrids of *Lolium* species (Thomas *et al.*, 1988) revealed that the orientation and respective position of chromosomes could have a significant effect on the incorporation of specific alleles in gametes and future genotypic interaction.

Hybrid vigour (Romagnoli *et al.*, 1990; Springer and Stupar, 2007) was absent in the hybrids as they were closer to the female diploid parent in their morphology and growth habit. A few genes, from the diploid parent, responsible for flower colour and inflorescence type expressed dominance but the expected fitness advantage such as vigorous growth, “gigas type” and special adaptability, often associated with higher ploidy (Comai, 2005) was suppressed. Conversely, the expression of some genes in a triploid maize plant increased with ploidy level (Guo *et al.*, 1996). Frascaroli *et al.* (2007) established a positive relationship between the level of heterozygosity and phenotypic performance in other maize hybrids. However, a few triploid maize hybrids expressed negative correlation values with increased ploidy level (Auger *et al.*, 2005).

The hybrid fruits were seedless and had little or no mesocarp content (empty pericarp). This could be linked to allelic incompatibility at fertilization and the development of an abnormal embryo arising from unequal ploidy levels in the parents (Brandvain and Haig, 2005). Analysis of hybrids from distantly related species (*S. pinnalisectum, S. vermeosum* and *S. tuberosum*) revealed ovule abortion at different development stages as a result of endosperm and embryo collapse. Thus, seed production in interspecific *Solanum* hybrids may be dependent on the compatibility of the parental genomes in the embryo and endosperm, and genetic and biochemical interactions among embryo, endosperm and maternal sporophytic tissue (Chen *et al.*, 2004).

In spite of the high divergence between the two constitutive genomes, meiosis was regular in the allopolyploids resulting in fertile F<sub>1</sub> hybrids. The pollen were regular in shape and fairly fertile (38.2%). The hybrids exhibited relatively normal microsporogenesis without diads or triads suggesting an existing mechanism within the genome that helped eliminate irregularities before chromosomes were distributed into gametes. The few univalents and incomplete pairing of bivalents in these hybrids expressed a degree of genetic incompatibility between the parental genomes (homoeologues). The absence of multivalents at Metaphase I (MI) may further indicate considerable differentiation between the genomes of the parental species or the presence of an effective homoeologous pairing suppressor genetic system. Lashermes *et al.* (2000) observed diploid-like meiotic behaviour in *Coffea arabica* L. in spite of its tetraploid genome constitution.

The relative asymmetric configuration of the somatic chromosomes in the hybrids and their size variation were evidence of the combination of two different genomes and genetic differences. According to Rieseberg *et al.* (2000), ploidy dissimilarity often creates constraints for interspecific gene flow. This was evident in the hybrid breakdown resulting from the development of seedless fruits.

The success of hybrids, especially from crosses involving species of different ploidy levels depends on the direction of gene flow (Le Pierres, 1995; Herrera *et al.*, 2002). Crosses were unsuccessful when *S. scabrum* 'Scabrum' (2n = 4x = 48) was the maternal parent. Fertilization failed and consequently, all pollinated flowers withered. In contrast, the reciprocal crosses produced an eight-seeded fruit which eventually produced two F<sub>1</sub> plants. Successful crosses were obtained with a large number of diploid species when *Coffea arabica* was the maternal parent (Herrera *et al.*, 2002). However, no hybrid resulted when *C. arboidea* was the pollen donor (Le Pierres, 1995).

The potential high level of male fertility (97.4%) in the tetraploid parent and its regular meiosis (Oyelana, 2005) can be harnessed. However, epistasis and other forms of negative gene interactions might have masked these effects in the hybrids. Therefore, backcrosses to the tetraploid parent may bring about allelic re-shuffling, gene realignment towards producing desirable phenotypes in future crosses.

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