STERILITY IN A SWEET POTATO CLONE[1] /

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Resumen

En una población de camote derivada del cruce entre clones de flores blancas y rosadas fue identificada una planta que mostró extensibilidad tanto en la fase masculina como también femenina. En ella se estudió la microsporogénesis, la megaesporogénesis y la distribución de almidón de la antera y en los óvulos. Los resultados fueron comparados con datos similares obtenidos del progenitor fértil de flores rosadas en contraste con el masculino fértil. En la planta estéril el tepetum es persistente y presenta almidón en su parte externa, el cual aumenta conforme la entera madura. La falta de nutrimentos para el polen en formación resulta en su esterilidad, similarmente el saco embrionario se encuentra en estado de inanición debido al comportamiento aberrante del endotelio. Se llegó a la conclusión que tanto el tepetum como el endotelio han perdido su actividad fisiológica, fallando en la transferencia de nutrimentos hacia el polen en desarrollo y al saco embrionario respectivamente, lo que resulta en la total esterilidad de las fases tanto masculina como femenina.

Introduction

Ipomoea batatas (Linn.) popularly known as sweet potato is an important tuber crop of tropics and sub-tropics. It is a polyploid, but the clones generally show high pollen fertility. Though the incompatibility system prevalent in this crop has been widely studied (11, 16) the factors reducing male and female fertility have been attributed to polyploidy (12, 20). While studying a population of sweet potato raised from a cross between white flowered and normal pink coloured clones, a plant was found to have rather small anthers and also without any seed set; further studies revealed it to be completely male and female sterile. The present communication describes the sequence of microsporogenesis and megasporogenesis resulting in sterility of the hybrid.

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Materials and methods

The buds of different stages and the flowers of sterile clone identified in the present study and normal fertile parent with pink flowers, were fixed in 3:1 alcohol acetic acid mixture. The shrivelled ovaries of sterile plant and the developing capsules of the fertile parent were also fixed. The usual infiltration and embedding procedures were followed. Sections were cut at 8-12 μ thickness and stained with Heidenhains hematoxylin. The starch distribution was studied with hematoxylin and counter stained with 1.0% iodine solution. However, iodine masked the staining of hematoxylin, hence two sets of slides were studied for micro-and megasporogenesis and starch distribution, separately. The pollen were stained with 1% acetocarmine.

Results

Microsporogenesis. Male Fertile (MF)

The young anthers at pre-meiotic stage show an epidermis, a prominent sub-epidermal layer, two or three middle layers, a tapetum and pollen mother cells (Figure 1). As the PMCs enlarge, the middle layers get crushed. The tapetum becomes binucleate and exhibit maximum development as the PMCs enter meiosis and up to microspore tetrad stages (Figure 2). Though in the early stages, the starch is almost absent in the anther tissue, at the tetrad stage the wall layers and connective tissue show the presence of starch, but tapetum is devoid of any starch. The tapetum gradually passes into a phase of decline and senescence and remnants may be noticed for some more time and some starch may be noticed around the septum (Figure 3). Later, the tapetum completely disappears while the microspores continue growth and differentiation. The mature anthers are devoid of any starch and the single layered endothecium has well developed fibrillar thickenings (Figure 4). The pollen grains are globular and fertility is as high as 95% (Figure 5)

Male Sterile (MS)

The sequence of differentiation and development of different layers are normal and comparable with MF up to the microspore tetrad stage. In the young anthers, the tissues are devoid of starch as in MF, but in a few anthers intense starch deposit has been noticed outside the tapetum even at the late PMC stage approaching meiosis (Figure 6). The starch deposits are more intense at tetrad stage (Figure 7) and the intensity increases as the microspores separate (Figure 8). The tapetum remains intact even after the development of exine ornamentation on the pollen (Figure 9). The tapetum is found to develop vacuoles but never found to increase in size. Occasionally, the tapetal cells coalesce and form a periplasmodium and in contrast to MF plants, the starch deposits increase outside the tapetum as the anthers mature (Figure 10). Even in the fully mature anthers, dense deposits of starch are noticed in the connective tissue, septum and wall layers and tapetum is still discernible (Figure 11). The endothecium is poorly differentiated and does not show any fibrillar thickenings and consequently the pollen are not liberated. The pollen grains are globular and much smaller than the fertile pollen and the sterility is total (Figure 12)

Megasporogenesis. Female fertile

The female gametophyte is monosporic, 8-nucleate and the antipodals are ephemeral. The synergids are pointed towards the micropylar end and one of the synergids is destroyed by the entrance of the pollen tube. The fertilization of the egg is followed by secondary fertilization

The starch deposition occurs in the integument only after early 8-nucleate stage and by the time the egg apparatus is organised, the egg is found filled with starch but the synergids are completely devoid of any starch (Figure 13). The endothelium remains undifferentiated even after fertilization and the development of embryo and the distribution of starch in the integument gradually diminishes as the seed matures

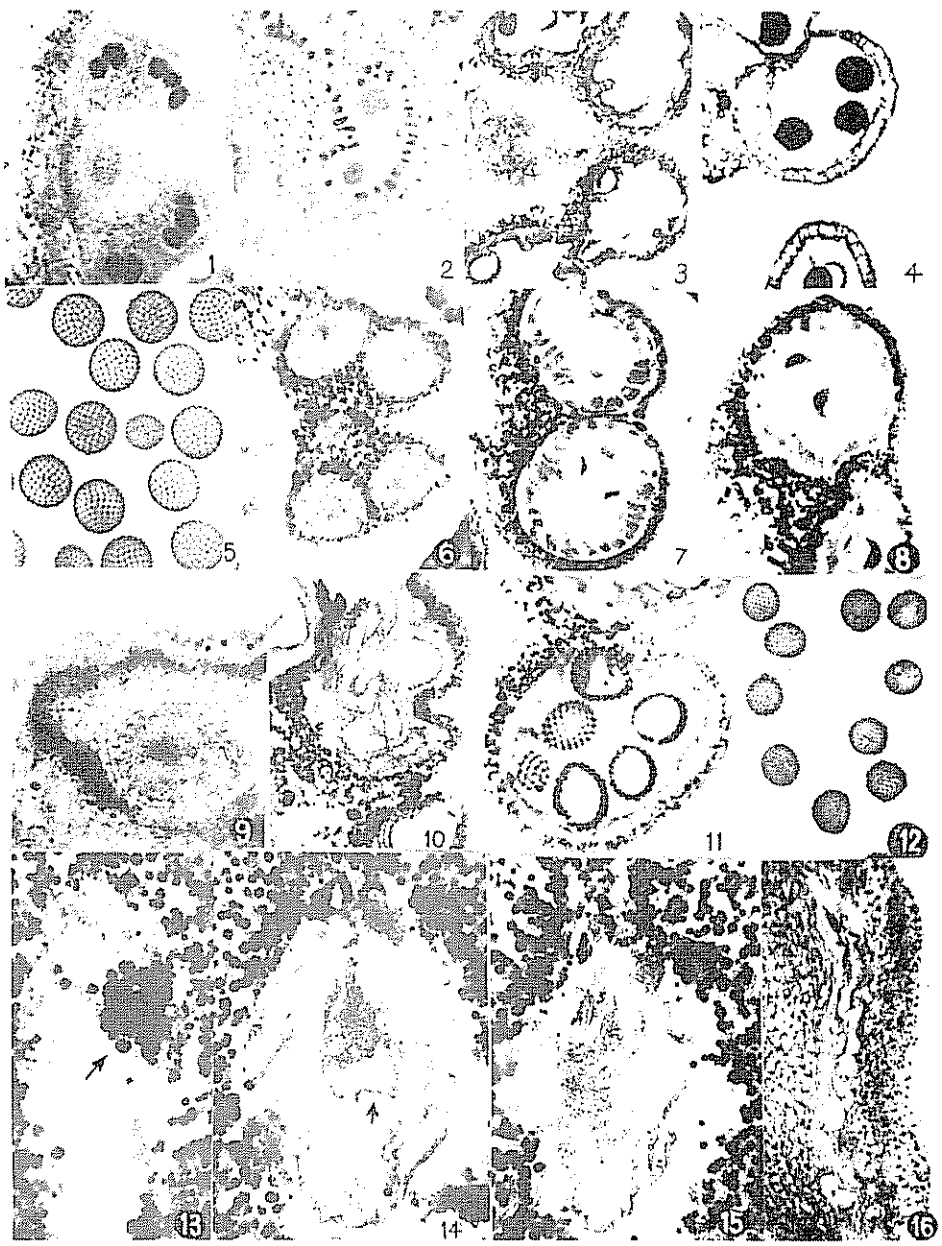
Female sterile

The early megasporogenesis is normal but when the egg apparatus is organised, the egg is found devoid of any starch (Figure 14), while plenty of starch is noticed in the integuments. The intensity of starch concentration outside the embryo sac is found to increase and the distribution becomes more and more pronounced than that of fertile parent. Both synergids remain intact indicating the absence of fertilization, when degeneration sets in the embryo sac (Figure 15). The endothelial cells gradually become prominent and later embryo sac is found completely crushed by these bloated cells (Figure 16) resulting in the collapse of the ovules

Discussion

Though sterility is of significant interest to the plant breeder the phenomenon has wide cytogenetical implications. Since the sterility is noticed both in male and female phases, the sequences are considered separately for pollen sterility and ovule sterility. The factors operating in male sterility have been widely studied in a number of plant species. Besides meiotic abnormalities, disorganization of PMCs (21), absorption of microspores (5, 14), prematures degeneration of tapetum (13), coalescence and persisting tapetum (4, 9) and non-dehiscence of anthers (6) are known to cause male sterility. Among them, the aberrant behaviour of tapetum is the most important and wide spread in crop plants.

The observations made in the present study on the microsporogenesis and pollen development in the MF and MS plants indicate that the failure of the tapetum to disintegrate at the appropriate time and its persistent nature resulted in the starving of the developing pollen grains and consequent degeneration. Maheswari (10) proposed that the tapetum serves as a reservoir of food material and the nutrients are made available to the developing pollen grains by the degeneration of tapetum at the appropriate time. Though the nutritive role of tapetum is well recognised, Echlin (3) established that the sensitive phases of microsporogenesis are dependent on tapetum through which the nutritive materials either pass or to be metabolised. Sterility of pollen can be caused by the disturbed or retarded activity of tapetum resulting in the decrease or total prevention of transfer of carbohydrates, fats, proteins and nucleic acids (8). The failure



Figs 1-5. Microsporogenesis in fertile clone.
Fig 1 Young anther (350 X) Fig 2 Well defined tapetum and without starch anywhere (350 X) Fig. 3. Young polen with remants of tapetum and a few scattered starch around septum (80 X) Fig. 4 Anther with fertile pollen and endothecium with fibrillar trickennings (80 X) Hematoxylin Fig 5 Fertile pollen (80 X). Acetocarmine or Hematoxylin-iodine.

Figs. 6-12 Microsporogenesis in steril plant
Fig 6. PM stage with starch outside the tapetum (350 X) Fig 7. Intense distribution of starch in microspore tetrad stage (350 X) Fig. 8 Distribution of starch in young microspore stage (350 X). Fig 9 Persisting tapetum even when the pollen have developed ornamentation (700 X) Hematoxylin Fig 10 Starch deposit increases as anthers mature (350 X). Fig 11 Mature anther with poorly developed endothecium, remnants of tapetum and starch in the septum, and connective tissue and wall layers (350 X) Fig 12 Steril pollen (80 X) Acetocarmine or hematoxylin-iodine

Fig 13 Megasporogenesis in fertile plant – egg (→) filled with starch (700 X)

Figs 14-16 Megasporogenesis in sterile plant

Fig 14 Egg (→) without starch (700 X) Fig 15 Both synergids are intact (700 X) Fig 16 Collapsed embryo sac surrounded by prominent endothelium (700 X) of tapetum during the development of microspores is widely reported in *Sorghum* (18), *Aglaonema* (4) and cassava (9)

The occurrence of more starch outside the tapetum and the increase in the intensity of the starch deposit as the anther matures in the MS in contrast to be MF indicate the tapetum has lost the ability to transfer the nutrients to the developing pollen grains. It is reported that the reduced enzymatic activity of tapetum result in the insufficient supply of carbohydrates and other nutrients from the tapetum to the developing microspores (1, 7). The ability of tapetum to produce and transfer nucleic acids and proteins to developing microspores and pollen is reported to have been depressed in the MS plants of *Allium cepa* (15), *Beta vulgaris* (2) and Sorghum (19). When the increased intensity of starch deposits outside the tapetum and the persistent nature of tapetum are considered together in the present material, it may be concluded that the tapetum might have lost its enzymatic activity; furthermore, by its persisting nature it has become a physiological barrier in preventing supply of nutrients to the developing pollen grains resulting in total pollen sterility

Similarly in the ovule, the endothelium otherwise known as 'integumentary tapetum' surrounding the embryo sac has a critical role in transferring the nutrients to the developing embryo sac The egg is found filled with starch in MF, but the distribution of starch in the integument gradually diminishes as the seed matures and the endothelium remains undifferentiated In contrast, the MS plant shows that egg is completely devoid of starch while the distribution of starch in the cells outside the integumentary tapetum becomes more and more pronounced underlining the fact that the nutrients are prevented from entering the embryo sac, thereby accumulating in the intergument Later the endothelial cells also become bloated and further crush the starving embryo sac

While studying the sterile florets in sunflower, Savchenko (17) concluded that the nutrients are not transferred to the embryo sac but accumulated in the cells of the integumentary tapetum stimulating it to grow further. However, Satina *et al.* (16) observed the endothelium disintegrate in compatible crosses, but incompatible ones induce the endothelial cells to become greatly enlarged and crush the embryo sac. In the present study by considering the behaviour of tapetum in the anther along with the behaviour of integumentary tapetum, it may be concluded that they have lost their physiological activity and fail to transfer the nutrients to the developing pollen grains and embryo sac respectively resulting in total sterility in both male and female phases.

Summary

A sweet potato plant showing sterility in male and female phases was identified in a population derived from a cross between white flowered and pink coloured clones. Studies were made on the microsporogenesis, megasporogenesis, the distribution of starch in the anther and ovules of the sterile plant and was compared with the fertile parent with pink flowers In contrast to MF, in the MS plant the tapetum is persistant and the presence of starch outside the tapetum increase as the anothers mature. The lack of nutrients to the developing pollen result in pollen sterility. Similarly, the embryo sac is also starved by the aberrant behaviour of the endothelium It is concluded that the tapetum and endothelium have lost their physiological activity and fail to transfer the nutrients to the developing pollen grains and embryo sac respectively, resulting in total sterility in both male and female phases

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Reseña de libros

MEJIA, M. *ANDROPOGON GAYANUS.* Kunth: Bibliografía Analítica. Centro Internacional de Agricultura Tropical. 1984. 176 p.

Es verdaderamente halagador encontrar un libro de esta naturaleza cuando en la bibliografía de gramíneas todo escasea.

El autor analiza concienzudamente todos los aspectos importantes de la especie tales como: una sinopsis descriptiva, la botánica, la taxonomía y distribución geográfica, las características agronómicas, adaptación, establecimiento, manejo y producción de forraje; asociación con leguminosas, suelos y fertilización, germoplasma, fitomejoramiento, plagas, enfermedades, producción de semillas, nutrición animal, valor nutritivo, consumo, composición química y producción animal; además de sus variedades seleccionadas para forraje, razón por la cual rápidamente el lector se da cuenta de qué tipo de pasto se trata en cuanto a valor nutritivo y producción animal.

Los aspectos taxonómicos y botánicos están bien investigados y lo que se relata de la especie sobre adaptación, magnífica, características que pueden dar una buena opción a muchos países latinoamericanos que presentan problemas serios en cuanto a sabanas y bosques, ya que el hombre ha efectuado un mal manejo de ellos. Las características agronómicas son muy semejantes a nuestra *Hypharrhenia rufa,* (también traída de Africa) aún en asociación con leguminosas como *stylosanthes,* puesto que esta última mantiene al ganado en la época seca. También se asemeja a *Hyparrhenia* en cuanto a su valor nutritivo moderado ya que ninguno de los dos géneros suple las necesidades de proteína cruda y minerales de crecimiento.

La digestibilidad *in vitro* y la energía digestible son bajas durante la lluvia y sequía pero la cantidad de forraje y la posibilidad de seleccionar fracciones ricas en nutrimentos podrían permitir aumentos de peso del animal; por ello los estudios muestran ganancias en peso/animal de 300-400 g/animal/día, lo que demuestra que funciona en forma adecuada. Con respecto a la producción animal es buena puesto que puede sostener 2.3 animales/ha, lográndose bajo pastoreo 457 kg/peso vivo/ha/año. En asociación con leguminosas el peso aumenta a 670 g/día con 2 animales/ha.

En Costa Rica, bajo condiciones similares de manejo, los mejores pastos sostienen 1.5 animales/ha. Con tales características, *A. guayanus* es una gramínea promisoria, que soporta suelos difíciles para el cultivo de ellas. En Costa Rica ya se ha introducido en la zona de San Carlos, pero creo que debe ser probado cuidadosamente y sólo para los suelos ácidos, pues ya tenemos experiencia con otras especies de *Andropogon* que por ser tan invasoras se convierten en malas hierbas. Considero que nuestros suelos no están tan degradados como para tener que dispersarla, ni presentan problemas tan serios como muchos sudamericanos en los cuales, como demuestra el autor, esta gramínea viene a solucionar múltiples problemas.

Otro punto por analizar es la buena viabilidad de la semilla lo que hace que la especie se disemine; en caso de que en nuestro medio no se comporte adecuadamente, se convertiría en una gramínea invasora como *Dischantelium aristatum,* que en países suramericanos funciona bastante bien y en nuestro país, en algunas zonas de Guanacaste, se ha transformado en un problema.

El libro suministra también valiosa bibliografía sobre leguminosas, resultados de proyectos y programas del CIAT, los cuales son de gran utilidad ya que ellas son las colaboradoras inmediatas de las gramíneas. Sin embargo, en el último congreso de pastos se comentó la poca importancia que ellas tenían en las asociaciones, llegándose a considerar que no pueden mantenerse en una forma adecuada, o que deberían cultivarse por separado, como lo deja entrever en sus citas bibliográficas el autor, pues en ellas se demuestra que su producción decae después de un período corto.

Considero el libro del Dr. Mariano Mejía muy valioso, una fuente de consulta que da un aporte a la agrostología latinoamericana.

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