Improvement of Cavendish Banana Cultivars through Conventional Breeding

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Keywords: Cavendish re-synthesis, female fertility, triploid × triploid crosses

Abstract

In their article "Banana breeding: polyploidy, disease resistance and productivity", Stover and Buddenhagen (1986) reported the results of the evaluation of female fertility in Cavendish banana cultivars. They showed that the pollination of a few hundred bunches of 'Valery' (AAA) and other Cavendish clones with pollen from diploids did not yield seed. The authors concluded that "the apparent seed sterility of Cavendish cultivars (without any research to determine or overcome the blocks) precluded their use as female parents in conventional breeding programs". The scientific community accepted these observations as fact and did not carry out additional tests, because the commercial cultivars of banana for export are all triploid and parthenocarpic. The triploid condition of the Cavendish banana causes them to produce many sterile eggs, and the process of parthenocarpy allows the development of fruit without ovule fertilization. On the assumption that Cavendish cultivars have low fertility, the Banana and Plantain Breeding Program at the Honduran Foundation for Agricultural Research (FHIA), starting in 2002, pollinated 20,000 bunches, approximately 2 million fingers, of the Cavendish cultivars 'Grand Naine' and 'Williams' with pollen from 10 Cavendish cultivars for the development of Cavendish tetraploids. As a result, 200 seeds with 40 viable embryos were obtained, from which 20 tetraploid hybrids were developed. These results confirmed the assumption that Cavendish cultivars have low fertility, which allows their use in conventional breeding methods to create new progenies. The selected tetraploid progenies were crossed with improved FHIA diploids for the development of second generation triploid hybrids. As a result of this cross, two hybrids with resistance to black leaf streak and Fusarium wilt race 1, have been pre-selected. These hybrids exhibit similar performance to known Cavendish cultivars.

INTRODUCTION

From its early beginnings in the 1970s, the banana industry relied on the 'Gros Michel' (AAA) cultivar. However, because of its susceptibility to *Fusarium oxysporum* f. sp. *cubense* race 1, the causal agent of Fusarium wilt, this cultivar was replaced in the 1950s with cultivars from the Cavendish subgroup (AAA genome). In 2003, Pearce reported on the probable disappearance of the Cavendish banana within a period of 10 years. This article generated a lot of attention with the media, the banana research community, banana producers and consumers. The reason behind this gloomy forecast was the susceptibility of the Cavendish cultivars to *Fusarium oxysporum* f. sp. *cubense* tropical race 4 (Foc TR4), to *Mycosphaerella fijiensis*, the causal agent of black leaf streak (BLS), and to nematodes.

Considering the needs of the banana export industry to substitute existing Cavendish cultivars for Cavendish-like cultivars with resistance to BLS and Foc TR4, the Honduran Foundation for Agricultural Research (FHIA) initiated work to develop such new cultivars, building on the 52 years of experience of the program in conventional banana breeding. The FHIA banana breeding program, even though not located in a region where Foc TR4 is present, has developed three banana hybrids resistant to Foc TR4 (FHIA-01, FHIA-18 and FHIA-25) (Daly et al., 2007). These three hybrids all have the FHIA-improved diploid SH-3142 (resistant to BLS and Foc TR4) as their male parent.

The objective of this work was to develop Cavendish tetraploids derived from the crossing of Cavendish cultivars, to increase the likelihood of having a high concentration of Cavendish desirable traits. These tetraploids were then used as female parent in crosses with SH-3142 to produce second-generation triploid Cavendish hybrids.

FEMALE FERTILITY OF COMMERCIAL CAVENDISH CULTIVARS

The use of conventional breeding in the development of disease-resistant Cavendish hybrids has received very little attention since it was reported by Simmonds (1962), Stover and Buddenhagen (1986) and Stover and Simmonds (1987) that Cavendish cultivars are female sterile. Breeding to develop new dessert bananas has thus depended largely on 'Gros Michel' or its shorter version mutant 'Highgate'.

According to Simmonds (1962) and Stover and Simmonds (1987), the majority of banana fruits are sterile due, probably in varying degree, to specific female sterility genes, triploidy and chromosomal structural change. The relative importance of each factor depends on the nature of the clone under investigation. Seed development in many edible bananas cultivars depends not only upon maternal conditions but also upon pollen availability.

Stover and Simmonds (1987) indicated that the sterility of the clones cultivated for the international trade is a consequence of the high inherent female sterility. Within the Cavendish group, seed production was not observed in the many thousands of pollinations conducted. By contrast, 'Gros Michel' can produce one or two seeds per bunch if pollinated or if grown next to a pollen source.

Stover and Buddenhagen (1986) reported in their review the results of trials to determine the female fertility of Cavendish cultivars. In testing for seed fertility by crossing with male fertile diploids, the authors found that the Cavendish cultivars never yielded seeds. Most extensive pollination attempts were made with the Cavendish cultivar 'Valery', in which a few hundred bunches were pollinated. Several other Cavendish clones were also pollinated to a more limited extent, but also without success. The authors concluded that "it is this apparent seed sterility (without any research to determine or overcome the blocks) that has precluded the use of the "ideal triploids" as parents". The first exception to the absence of Cavendish input into conventional banana breeding was the successful yield of seed from a wild *Musa acuminata* ssp. *malaccensis* when pollinated with 'Valery' pollen in 1964. Several dwarf diploid hybrids were obtained (the dwarf character from 'Valery') which were further used in diploid breeding and are now in the pedigree of all the advanced dwarf diploids (Stover and Buddenhagen, 1986). A second exception came in the 1990s when FHIA obtained one seed from the cross of 'Williams' as female parent and 'SH-3142' as male parent; this seed developed into a tetraploid plant that was named 'FHIA-02'.

From 1960 to 2002, conventional breeding was not considered a viable method for the development of a Cavendish replacement resistant to BLS and/or Foc TR4, because of the assumption that Cavendish was female sterile. In 2009, it was reported that the sequencing of the banana genome would generate knowledge for the development of a transgenic banana with resistance to Foc TR4 (Grim, 2009), but though developing a replacement for Cavendish through transgenesis is possible, the banana industry would again be dependent on a single cultivar. However, in 1992, Phillip R. Rowe, then head of the banana breeding program at FHIA, received from Frederick Novak of the International Atomic Energy Agency (IAEA), the banana clone 'Novaria', an irradiated mutant derived from 'Grand Naine', to assess its resistance to BLS and its commercial value under growing conditions at La Lima, Honduras. After the evaluation, Rowe used 'Novaria', which was susceptible to BLS, to make crosses with improved male diploids. These crosses produced seeds with no endosperm or embryo. Rowe thought that the fertility of 'Novaria' was due to the effects of irradiation. This information provided the basis to evaluate the fertility of the Cavendish clones and a program for the development of a Cavendish replacement through conventional breeding was initiated in 2002. The first task of FHIA's breeding program was to determine rates of male and female fertility of Cavendish cultivars, to subsequently outline strategies for hybrid development.

EVALUATION OF MALE FERTILITY OF CAVENDISH CLONES

To determine the male fertility of commercial cultivars of Cavendish, the diploid wild *Musa acuminata* 'Calcutta IV' was used as female parent and Cavendish cultivars were used as male parents. 'Calcutta IV' is not parthenocarpic, and the fruit only develops if at least one ovule is fertilized. When 'Calcutta IV' is pollinated with fertile diploid pollen, it produces 1,500 to 2,000 seeds per bunch. Plants of 'Calcutta IV' that were pollinated with Cavendish produced an average of 900 seeds per bunch. This first result indicated that commercial cultivars of Cavendish are not male sterile but have intermediate male fertility.

The results of these trials provided the basis for an evaluation of the pollen fertility of Cavendish cultivars, using diploid, triploid and tetraploid females with high fertility. As a result of these crosses, diploid, tetraploid and triploid hybrids were generated, respectively. It was also shown that crosses with diploid females generated the largest number of seeds and hybrid plants, followed by crosses with tetraploid females and finally triploid females.

These trials showed that the pollen of Cavendish cultivars was fertile and that pollen meiosis occurred. As a result of this division, several types of male gametes should be produced, of which probably only the haploid gametes (n) are viable during the process of pollination and fertilization. This conclusion was reached because there were no triploid or tetraploid seedlings in the offspring of diploid females, no pentaploid or hexaploid seedlings in the offspring of triploid females, and no tetraploid or pentaploid seedlings, in the offspring of tetraploid females.

The above results have helped to formulate breeding strategies for the development of a Cavendish replacement. If the pollen from a triploid has the ability to fertilize an egg from a triploid cultivar, and as a result of this cross, a tetraploid hybrid can be obtained, then it is possible to improve triploid females. This situation is different from that understood by Simmonds (1962) and Rowe and Richardson (1975), who believed that banana breeding was essentially male breeding, because it was impossible to improve female triploids. The development of intermediate breeding materials or female tetraploids which will be crossed with improved diploids resistant to Foc TR4 can result in the development of second-generation triploid hybrids with new or similar organoleptic traits to the initial triploid Cavendish.

EVALUATION OF FEMALE FERTILITY OF CAVENDISH CLONES AND DEVELOPMENT OF FIRST AND SECOND-GENERATION CAVENDISH-TYPE HYBRIDS

The female fertility of Cavendish cultivars was confirmed at FHIA through the pollination of 20,000 bunches of Cavendish with pollen from 10 male Cavendish parents. As a result of these crosses, 200 seeds were obtained. From these seeds, 40 embryos were rescued and 20 plantlets were developed. This result shows that, although the female fertility of the Cavendish cultivars is very low, these cultivars should not be classified as sterile.

The tetraploid hybrids developed (Cavendish × pollen donor) contain the three sets of Cavendish chromosomes and one set of chromosomes from the male parent. This makes it possible to develop diploid gametes from the tetraploid female parent which would have two of the three chromosome sets of Cavendish and would be fertilized with the haploid gamete from the male parent in order to generate second-generation triploid hybrids with 66% Cavendish genes.

During 2009, tetraploid females, derived from Cavendish cultivars, were pollinated with the improved diploid SH-3142 developed by FHIA. From the field evaluation of these hybrids, we preselected hybrids with resistance to BLS and Foc race 1. These hybrids have confirmed the potential of this breeding approach to improve

Cavendish.

CONCLUSIONS AND PROSPECTS

Our work has shown that Cavendish cultivars have intermediate male fertility, and should thus not be classified as male sterile, as previously reported. The rate of female fertility of Cavendish cultivars, depending on biotic and abiotic factors, is approximately one seed for every 100 pollinated bunches. Crosses between triploid Cavendish cultivars can thus generate tetraploid Cavendish hybrids, showing that it is possible to improve Cavendish triploids through conventional breeding methods. The development of tetraploid hybrids with three complete sets of chromosomes from the Cavendish mother and one set of chromosomes from the male parent is the basis for the creation of second-generation triploid Cavendish type hybrids.

A sustainable banana industry requires the continuous development of improved Cavendish-like cultivars, to overcome new pest and disease threats and/or possible loss of resistance to existing constraints. A continuous program to develop improved males and females is needed, supported by long-term public and/or private financial support. Based on our research, the development of more than 20 additional female-fertile tetraploids derived from crosses between Cavendish and a male donor is needed to produce a minimum of 2,000 second-generation triploid hybrids, which would provide the opportunity to pre-select 200 hybrids resistant to BLS and Foc race 1. The resistance of these pre-selected hybrids should be tested under field conditions in Asia or Australia, where Foc TR4 is present. The selected male parent of the second-generation triploid hybrids needs to be resistant to BLS and Foc (race 1 and TR4).

Our results indicate that FHIA's method of banana improvement is appropriate for the development of new Cavendish hybrids through the re-synthesis "de novo" of a Cavendish with resistance to BLS and Foc TR4.

ACKNOWLEDGEMENTS

The author would like to express thanks to his wife Dra. M.B. Deléo Aguilar, as well as to Dr. A. Martínez, FHIA-Director General, Dr. D. Krigsvold, FHIA-Research Director until 2008, Dr. D. Breazeale, FHIA-Research Director 2008-2011 and Dr. V. Gonzalez, FHIA-Research Director, for their critical review of the manuscript.

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