

AN INDUCED FEMALE STERILE MUTANT IN ROSELLE (*Hibiscus sabdariffa* L.)

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ABSTRACT

A mutant phenotype in roselle (*Hibiscus sabdariffa* L.) cv. Jamaican Green (JG) was found when JG was treated with 0.6% and 0.8% ethyl methanesulfonate for six hours. Style and stigma were completely missing from the flowers produced by the mutant; however, ovules were present. Normal anthers with functional pollen that could be used for hybridization were produced by the mutant. This mutant phenotype in roselle, designated female sterile, is recessive to normal female fertile characteristics.

INTRODUCTION

It has long been known that single gene mutations of spontaneous origin contributed substantially to domestication of crop plants in ancient days. Many key characters distinguishing cultivated from wild species are controlled by one or a very few genes (Ashri, 1989). Morphological mutations in crop plants have been used extensively as markers in genetic mapping and in several instances have proven useful in agronomic improvement efforts (Percy and Turcotte, 1991).

Recently, biochemical and genetic analyses of homoeotic mutations in *Arabidopsis thaliana* (Haughn and Sommerville, 1988; Coen and Meyerowitz, 1991), *Antirrhinum majus* (Schwarz-Sommer *et al.*, 1990), *Petunia hybrida* (Angenent *et al.*, 1992), *Lycopersicon esculentum* (Pnueli *et al.*, 1991) and *Nicotiana tabacum* (Broadvest *et al.*, 1992), as well as heterochronic mutations in maize (Poethig, 1988), have been utilized for identification of genes that regulate complex growth and developmental pathways in plants.

A mutant can be assigned to a linkage group rather quickly by using appropriate genetic stocks in well researched crops such as maize (Walbot, 1991), tomato (Rick and Yoder, 1988) and cotton (Kohel *et al.*, 1970), as

compared with other less researched crops. Genetic, isoenzyme, and RFLP maps are helpful in the genetic improvement of crops. Few genetic studies have been reported in roselle. Mutations would be useful for the construction of a genetic map and for the genetic improvement of roselle. An induced mutation in roselle, designated female sterile, is reported in this communication.

MATERIAL AND METHODS

A local variety of roselle, Jamaican Green (JG), was used in the present study. A total of 1,200 seeds of JG, with a moisture content of approximately 10 to 12%, was presoaked in distilled water for four hours. Two groups of 400 presoaked seeds each were treated with 0.6% and 0.8% of freshly prepared ethyl methanesulfonate (EMS) solutions. In addition, a control group of 400 presoaked seeds was treated with buffer solution only. EMS solutions were prepared in 0.02M phosphate buffer, pH = 7.0. The duration of EMS treatment was six hours. After the EMS treatment, seeds were washed in running tap water and immediately taken to the field for planting. Seeds were planted with a plant to plant distance of 20 cm, in rows spaced 25 cm apart.

All surviving M1 plants were self-pollinated, and the selfed seed from each plant was harvested separately. For the analysis of viable morphological mutations, 30 selfed seeds from each M1 plant per EMS treatment were planted in rows with same spacing as in the M1 generation.

The morphological mutations for qualitative characters were scored visually, and plants were self-pollinated in the M2 generation.

RESULTS AND DISCUSSION

Frequencies of viable mutations, calculated using M2 plants, showed that both concentrations of EMS used in this study induced viable mutations. The spectrum of viable mutations revealed that dwarf mutants were most frequent (2.93%) in this experiment. The next most frequent were non-serrated leaf margin (0.48%) and rosette-dwarf type (0.19%) mutations in 0.6% EMS treatment; and late flowering type (0.34%) mutations in 0.8% EMS treatment. Segregation for rosette-dwarf and non-serrated leaf margin mutations in some progenies, grown from the selfed seed of apparently normal looking plants in the M2 generation, indicated that these mutations were recessive to normal plant height and serrated leaf margin, respectively.

Mutant plants with non-serrated leaf margin were unusual. They did not retain any seed bearing capsules during the entire growing season. Flowering was continuous, during growth but attempts to self-pollinate were unsuccessful. This suggested male sterility; however, visual examination of flowers in the field revealed the total absence of stigmas. Halved flowers revealed the absence of style as well as stigma, but the presence of ovules, similar to the wild-type flowers (Figure 1). Pollen from these plants was used successfully in the pollination of emasculated flowers. The number of stamens, however, does seem to be reduced in the flowers produced by mutant plants as compared with the normal plants. Some flowers produced by the mutant plants at a later stage in the growing

season do not have stamens at all. Whether this is a pleiotropic effect of female sterile mutation on the development of androecium or due to some other factor(s) needs further investigation.

Studies on the inheritance of female sterility, in addition to the linkage relationship with non-serrated leaf margin, are in progress. Genetic linkage of female sterility with non-serrated leaf margin or any other morphological marker would be useful for roguing of female sterile plants from the field if the cultivar JG is grown for seed. Female sterility in roselle was found in the cultivar JG. The Jamaican Red (JR) cultivar of roselle, however, is preferred by the local population. Therefore, female sterility from the cultivar JG needs to be transferred into JR in order to take advantage of this mutation. Flower production is a continuous process in this female sterile mutation, and only calyces are used in Jamaica for making drink. Female sterility could be used in the JR genetic background for harvesting flowers on a continuous basis. The calyces from these flowers subsequently could be used for making drink as well as jam and jelly.

Another possible use for this mutation is that it could serve as a pollinating parent in combination with a male sterile seed parent (none available at present) for production of hybrid seed as suggested in tomato (Larson and Paur, 1948) and alfalfa (Brown and Bingham, 1984).

ACKNOWLEDGMENTS

The author expresses thanks to the Board for Postgraduate Studies and Research and Publication Fund Committee, University of the West Indies, Mona Campus for financial assistance.

RESUMO

Um mutante em rosela (*Hibiscus subdariffa* L.) cv. "Jamaica Green" (JG) foi descoberto quando JG foi tratado com 0,6% e 0,08% de etil metanosulfonato por seis horas. Ausência de etilo e estigma foi constatado nas flores produzidas pelo mutante. Entretanto havia óvulos. O mesmo produziu anteras normais com pólen funcional, que poderiam ser usados para hibridização. Além disso, este fenótipo mutante em rosela, designado como fêmea estéril, é recessivo para características de fertilidade das fêmeas normais.

REFERENCES

- Angenent, G.C., Busscher, M., Franken, J., Mol, J.N.M. and van Tunen, A.J. (1992). Differential expression of two MADS box genes in wild-type and mutant petunia flowers. *Plant Cell* 4: 983-993.
- Ashri, A. (1989). Major gene mutations and domestication of plants. In: *Plant Domestication by Induced Mutation*. IAEA, Vienna, pp. 3-9.

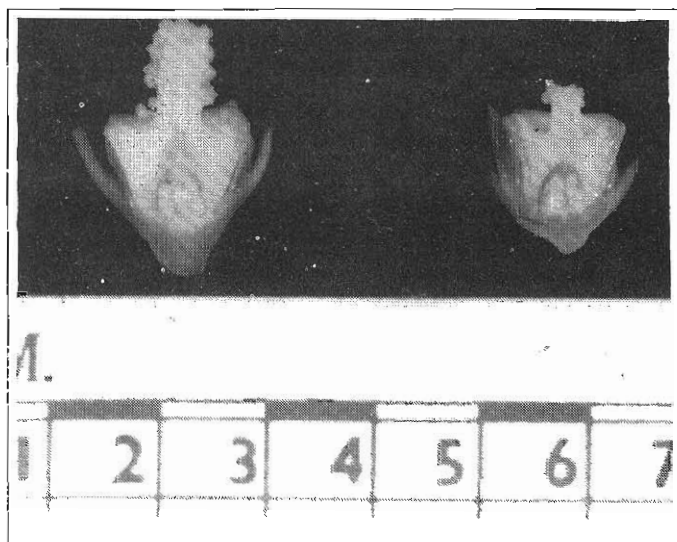


Figure 1 - Normal flower (Left) and flower of a female sterile mutant (Right). Note the presence of ovules in both flowers, and absence of style and stigma in the flower produced by the female sterile mutant.

- Broadhvest, J., Daigle, N., Martin, M., Haughn, G.W. and Bernier, F.** (1992). Appendix: a novel type of homeotic mutation affecting floral morphology. *Plant J.* 2: 991-997.
- Brown, D.E. and Bingham, E.T.** (1984). Hybrid alfalfa seed production using a female sterile pollenizer. *Crop Sci.* 24: 1207-1208.
- Coen, E.S. and Meyerowitz, E.M.** (1991). The war of the whorls: Genetic interactions controlling flower development. *Nature* 353: 31-37.
- Haughn, G.W. and Sommerville, C.R.** (1988). Genetic control of morphogenesis in *Arabidopsis*. *Dev. Genet.* 9: 73-89.
- Kohel, R.J., Richmond, T.R. and Lewis, C.F.** (1970). Texas Marker-1. Description of a genetic standard for *Gossypium hirsutum*. *Crop Sci.* 10: 670-671.
- Larson, R.E. and Paur, S.** (1948). The description and inheritance of a functionally sterile flower mutant in Tomato and its probable value in hybrid tomato seed production. *Proc. Amer. Soc. Hort. Sci.* 52: 355-364.
- Percy, R.G. and Turcotte, E.L.** (1991). Inheritance of male sterile mutant ms13 in American pima cotton. *Crop Sci.* 31: 1520-1521.
- Pnueli, L., Abu-Abeid, M., Zamir, D., Nacken, W., Schwarz-Sommer, Z. and Lifschitz, E.** (1991). The MADS box gene family in Tomato: Temporal expression during floral development, conserved secondary structures and homology with homeotic genes from *Antirrhinum* and *Arabidopsis*. *Plant J.* 1: 255-266.
- Poethig, R.S.** (1988). Heterochronic mutations affecting shoot development in maize. *Genetics* 119: 959-973.
- Rick, C.M. and Yoder, J.I.** (1988). Classical and molecular genetics of tomato: highlights and perspectives. *Annu. Rev. Genet.* 22: 281-300.
- Schwarz-Sommer, Z., Huijser, P., Nacken, W., Saedler, H. and Sommer, H.** (1990). Genetic control of flower development by homeotic genes in *Antirrhinum majus*. *Science* 250: 931-936.
- Walbot, V.** (1991). Maize mutants for the 21st century. *Plant Cell* 3: 851-856.

(Received June 15, 1993)