Journal of Plantation Crops 20 (Supplement) : 191-193

SPONTANEOUS MALE STERILITY IN TWO CLONES OF *HEVEA BRASILIENSIS* (WILLD. EX ADR. DE JUSS.) MUELL. ARG.

C.K. SARASWATHY AMMA, A.O.N. PANIKKAR and JOSEPH G. MARATTUKALAM

Rubber Research Institute of India, Kottayam 686 009, Kerala

ABSTRACT

From the existing exotic and indigenous populations of *Hevea brasiliensis* (Willd. ex. Adr. de Juss.) Muell. Arg. four male-sterile clones (GT 1, Ch 2, RRII 17 , RRII 35) were identified. Among these, detailed studies on two clones(Ch 2 and RRII 35) are reported. In Ch 2 there is no abnormality in the external morphology of the flower. But in RRII 35 the male flowers fall by abscission and do not attain full maturity, and even sterile pollen grains were absent. Proliferation and persistence of tapetum were noted in Ch 2 but in the control, complete degeneration was noted. SEM studies showed that exine ornamentation is perfect and uniform in the control whereas in sterile pollen grains exine exhibited aberrant pattern.

Upto the formation of tetrads, meiosis in microspore mother cells was normal both in Ch 2 and RRII 35, as in the control clone. Subsequenly, complete degeneration of cytoplasm and nuclei was noted in male-sterile clones resulting in sterility. Male-sterile clones having good fruit-set have potential in hybrid seed production.

INTRODUCTION

The Para rubber tree, *Hevea brasiliensis* (Willd. ex Adr. de Juss.) Muell. Arg. belonging to the family Euphorbiaceae is monoecious. The male flowers are far more numerous than the female flowers which are limited and restricted to the tip of the panicles as well as its major branches. Male sterility is of utmost importance in any field crop especially for the production of hybrid seeds. Spontaneous male sterility in this crop has been reported in clone PR 104 (Ramaer 1935), GT 1 (Majumder 1964; Leconte and Nicolas 1985; Saraswathy Amma et al. 1988) and RRII 17 (Annamma et al. 1980; Sarawathy Amma et al. 1990). The details of male sterility in two clones - Ch 2 and RRII 35 - are reported for the first time in the present paper.

METERIALS AND METHODS

Flower development and morphology were studied in two male sterile clones Ch 2 of Malaysian origin and RRII 35 of Indian origin along with a male fertile control clone G1 1 (Origin: Malaysia) from the initiation of flower to maturation. Young male flowers fixed in modified Carnoy's fluid (1:1:3 Chloroform: acetic acid and ethyl alcohol) and were transferred to acetic-alcohol (1:3) after 24 hrs. Staminal columns were dissected out and stained overnight in 2 per cent acetocarmine. Preparations were made in 45 per cent acetic acid and observations were taken from temporary mounts. Pollen stainability was assessed in acetcarmine-glycerol mixture (1:1). For the studies on microsporogenesis young male flower buds were fixed in acetic alcohol (1:3), preserved in 70% alcohol after 24 hours of fixation, de-hydrated and embedded in paraffin (Johansen, 1940). Transverse serial sections were taken at 10µm thickness, stained with safranine and fast green and slides prepared following routine microtechniques. At various stages of development the radial diameter of the tapetum was recorded.

SEM studies were also carried out for which acetolysed pollen grains were placed on a piece of adhesive tape attached to an aluminium stub and the samples sputter coated with gold to a thickness of 200A. Observations were recorded with a JEOL-JSM 35 C Scanning Electron Microscope and photographs were taken at 2000 x and 6000 x. Five samples were observed from each clone.

RESULTS AND DISCUSSION

The male-sterile clones Ch 2, RRII 35 and the control G1 are all ortet selections. The morphology of flowers of the male sterile clone Ch 2 and that of the fertile clone is apparently normal. There are fully developed perianth and ten anthers in both. But in Ch 2, partial dehiscence of anther was observed. Male sterility in Ch 2 can only be detected after microscopical examination of anther. The male flowers in RRII 35 do not attain normal maturity. They are reduced in size and fall by abscission. The perianth is light yellow in colour and flowers do not open. Even though RRII 35 and Ch 2 are totally male-sterile, normal fruit set is observed indicating full female fertility.

Pollen stainability of these clones had shown that the control clone G1 1 showed 95 per cent stainable pollen. But the sterile clones were devoid of stainable pollen and furthermore, even sterile pollen grains were totally absent in RRII 35. But in Ch 2 only a few sterile pollen grains were seen.

The microsporogenesis in fertile and sterile anthers were almost normal. The only difference noted was in the behaviour of tapetum. In the fertile anthers after the differentiation of microspores, the tapetal cells degenerated and in the fully mature anther there was no trace of tapetum (Fig.3). But the tapetal cells in the sterile anthers persisted and proliferated. The radial diameter of tapetum of the fertile anther and sterile anther showed wide variations. In the control the maximum width of tapetum was 8µm and that of the sterile clone Ch 2, 25µm.

Detailed observations on meiosis did not indicate any abnormalities. However, after the formation of tetrads, there was degeneration of cytoplasm and nuclei resulting in sterility. In RRII 35 due to complete degeneration not even a single sterile pollen grain could be observed.

SEM studies of the pollen grains from the control clone G1 1 showed that the development of germ pores and exine ornamentation was perfect and uniform. The pore development in the sterile pollen grain showed abnormalities and exine ornamentation exhibited more or less an aberrant pattern.

The present findings of the association of abnormal tapetal activity, with the abortion of pollen grain, had been reported in other crops also (Alam and Sandal 1967; Kaul and Singh, 1966; Kaul,1988). The tapetal cells were found to be thicker in Ch. 2. The failure of the tapetal cells to disintegrate causes, in turn, a failure in making available vital substances to microspores for their development into viable pollen grains. Premature vacuolation of the tapetal cells is a universal feature indicating male sterility (Heslop-Harrison 1972). Male sterility (Majumder,1964; Leconte and Nicolas, 1985) and manifestation of cytoplasmic male sterility (Saraswathy Amma *et al.* 1988) were reported in clone GT 1.

Investigations on the development of irregular pollen have shown typical exine development in reduced, unreduced and tetrakaryotic cells as well as in atypical and abortive spores (Rogers and Harris, 1969; Heslop-Harrison, 1968; Tara and Namboodiri, 1974). Exine formed around nonfunctional pollen grain with degenerating nuclei may exhibit normal stratification but more or less of aberrant gross pattern. In *H. brasiliensis* also exine ornamentaion was noted in the sterile pollen grains but pattern was different. The exine ornamentation was not complete as in the case of stainable pollen grains.

Male sterility provides a strong outbreeding mechanism leading to the release of locked up genetic variability. Since these two male sterile clones have normal fruit set, they can be considered for incorporation in breeding gardens for the production of hybrid seeds. Further work on the genetics of male sterility is in progress.

ACKNOWLEDGEMENTS

The authors are thankful to Dr. M.R. Sethuraj, Director of Research for encouragement. Thanks are due to the NBRI Lucknow for the SEM pictures and to Shri KP Sreeranganathan for the photographs.

REFERENCES

ALAM, S. and SANDAL, P.C. 1967. Cyto-histological studies in male sterile sudan grass. *Crop Sci.* **7** : 587-589.

ANNAMMA, Y., MARKOSE, V.C. and NAIR, V.K.B. 1980. Male sterility in *Hevea brasiliensis*. *Int. Rubb. Conf. India.* 1980 (Abt.).

HESLOP-HARRISON, J. 1968. Pollen wall development. *Science* **161** : 230-237.

HESLOP-HARRISON, J. 1972. Sexuality of Angiosperme. pp.133-271. In : *Plant Physiology - A treatise* Vol.VI (Ed.) Steward F.C. Academic Press, New York, London.

JOHANSEN, D.A.1940. *Plant microtechnique.* McGraw Hill, New York.

KAUL, C.L. and SINGH, S.P.1966. Studies on male sterile barley. 11. Pollen abortion. *Crop Sci.* **6**: 539-541.

KAUL, M.L.H.1988. *Male sterility in higher plants.* Springer Verlag, Berlin.

LECONTE, A. and NICOLAS, D. 1985. Male sterility in Hevea GT 1 clone. *Revue generale des Caoutchoucs et Plastiques.* **649** : 127-129.

MAJUMDER, S.K. 1964. Chromosome studies of some species of *Hevea*. *J.Rubb. Res.Inst. Malaya,* **18** : 269-275.

ROGERS, C.M.and HARRIS, B.D.1969. Pollen exine deposition, a clue to its control. *Amer.J.Bot.* **56**: 1209-1211.

SARASWATHY AMMA, C.K., NAMBOODIRI, A.N. and PANIKKAR, A.O.N. 1990. Meiotic abnormalities in a sterile clone of *Hevea brasiliensis* (Willd. ex Adr. de Juss.) Muell. Arg. *Cytologia.* **55** : 225-229.

SINGH, S.P. and HADLEY, H.H. 1961. Pollen abortion in cytoplasmic male sterile *Sorghum*. *Crop Sci.* **1**: 430-432.

TARA, C.P. and NAMBOODIRI, A.N. 1974. Aberrant microsporogenesis and sterility *in Impatiens sultani* (Balsaminaceae). *Amer.J. Bot.* **61** : 585-591.