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Male - sterility in clone SCATC 93-114 of *Hevea brasiliensis* Muell. Arg.

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Abstract

SCATC 93-114, an introduced hybrid clone of *Hevea* at the Rubber Research Institute of India (RRII) was observed to be male-sterile. Detailed cytological and anatomical studies on microsporogenesis have been carried out. Pollen stainability and germination studies also indicated that this clone is male-sterile. Cytological studies revealed that meiotic division was normal up to the formation of tetrad stage. Soon after the formation of tetrads there was complete degeneration of cytoplasm and nucleus with-in the microspore. Observations on microsporogenesis of this clone were carried out in comparison with a male-fertile clone, RRII 105. In the sterile clone, SCATC 93-114, the microspores underwent rapid cytoplasmic degeneration and pollen were not liberated into the locule. The pollen sacs did not enlarge as in fertile clones and all the pollen grains formed were sterile. The tapetum was not utilized and the tapetal layers persisted for a long period. This study indicated that SCATC 93-114 is a cytoplasmic male sterile (CMS) clone under the climatic conditions prevailing in the traditional rubber growing region of India.

Key words : *Hevea brasiliensis*, cytoplasmic male-sterile, microsporogenesis, tapetum

Introduction

Hevea brasiliensis belonging to the Family Euphorbiaceae is commercially exploited for natural rubber. SCATC 93-114 is a Chinese hybrid clone with the parentage TR 31-45 and HK 3-11, introduced to India from China and is now under evaluation under our agro climatic conditions. The trees are tall and vigorous with straight trunk and dense dark green foliage. Usually defoliation starts in the month of January and refoliation and flowering occur in the middle of February (late flowering). Flowers are pale yellow in colour, and small in size. Average size of male flower is 5.93 x 3.06 mm and female flower is 8.02 x 3.99 mm. In male flower, the anther column contains 10 stamens arranged in two whorls as usual but shrivelled in appearance. The female flowers are normal and fertile. It is a cold tolerant clone and is suitable for the frost prone regions especially in north-eastern India (Priyadarshan and Nair, 2002). During the study for selecting male parents for hybridization programme, it was observed that this clone was male sterile. It is the first report on male sterility in this clone.

Materials and Methods

Young male flower buds were collected from the clone SCATC 93-114 and RRII 105 planted in the RRII experiment station. These were fixed in modified Carnoy's fluid 3:1:1 (ethyl alcohol: acetic acid: chloroform), for cytological studies. After 24 hrs, the materials were transferred to 70% alcohol. The anther columns were dissected out and kept overnight in 2% acetocarmine and smear preparations were made using 45% acetic acid. Pollen stainability was assessed using 1:1 acetocarmine: glycerin mixture and pollen germination was studied by hanging drop technique at room temperature. For anatomical studies on microsporogenesis, anther columns from RRII 105 and SCATC 93-114 were collected during the month of February and fixed in ethyl alcohol: formaldehyde: acetic acid 90:5:5 (FAA) mixture. Samples were processed and serial microtome sections of 8-10μm were taken and stained with Periodic acid – Schiff's reagent for observation.

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Results and Discussion

Flowers of SCATC 93-114 were apparently normal in their morphology, though smaller in size compared to RRII 105. In mature male flowers of SCATC 93-114, the anther column was fully developed but the pollen sacs contained only sterile pollen grains. Cytological studies in SCATC 93-114 revealed that meiotic division was normal showing 18 bivalents at metaphase I (Fig. 1) and equal separation of chromosomes to opposite poles at anaphase I (Fig. 2). The second meiotic division was also normal showing equal separation of chromosomes to the poles at anaphase II (Fig. 3) resulting in the formation of tetrads. Soon after the formation of tetrads, there was complete degeneration of cytoplasm and nuclei in the microspores resulting in pollen sterility (Fig. 4). Pollen squash in acetocarmine revealed complete absence of stainable pollen. Groups of sterile pollen grains (pollen conglomerates) were observed in plenty (Fig. 5). *In vitro* pollen germination revealed the absence of germinated pollen. In RRII 105, meiotic division was normal resulting in four fertile microspores from each pollen mother cell at the end of meiotic division (Fig. 6).

Microsporogenesis of SCATC 93-114 was studied in comparison with RRII 105. Anatomical sections of the anther column of SCATC 93-114 revealed that the pollen sacs did not dehisce and pollen grains within the anther sacs were all sterile. The tapetal layers were not utilized since their cells were deeply stained (Fig. 7). On the other hand, in RRII 105, deeply stained fertile pollen grains were present in the pollen sac and the tapetum was fully utilized for the development of the pollen grains. In the fertile clone at maturity, the anther sacs dehisced and the pollen grains dispersed (Fig. 8).

In *H. brasiliensis*, microsporogeneis is reported to be perfectly regular and conformed to the classical pattern described for angiosperms (Maheshwari, 1950; Laser and Lerston, 1972). Cuco and Bandel (1995) studied mega and microsporogenesis of 13 clones of *H. brasiliensis* and reported that the megaspore and microspore production were normal in all the clones. In the present study, development of anther was more or less similar in both fertile and sterile clones with the exception of the behavior of tetrads and tapetal cells after the beginning of meiosis. There are few clones already identified as male sterile in *Hevea brasiliensis* (Saraswathyamma, 1990).They are GT 1, Ch 2, RRII

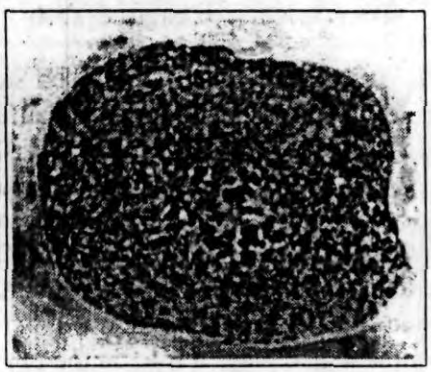

Fig. 1. Pollen mother cell (PMC) showing 18 bivalents at metaphase I (x 3000)

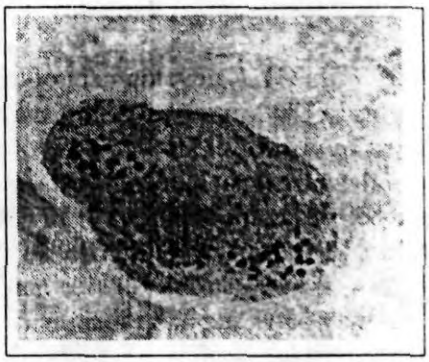

Fig. 2. PMC showing chromosomes in the poles at anaphase I (x 3000)

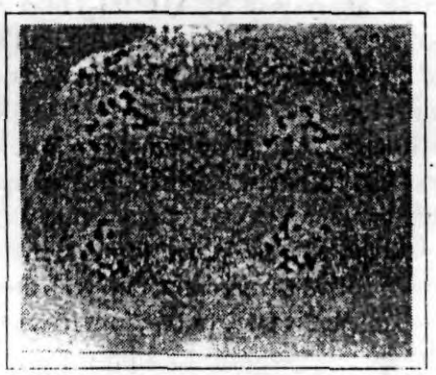

Fig. 3. PMC showing chromosomes in the poles at anaphase II (3000)

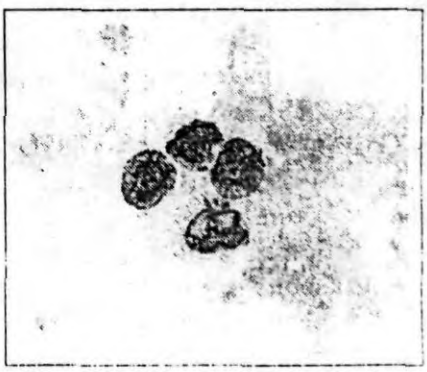

Fig. 4. Microspores of male sterile clone (x 2000)

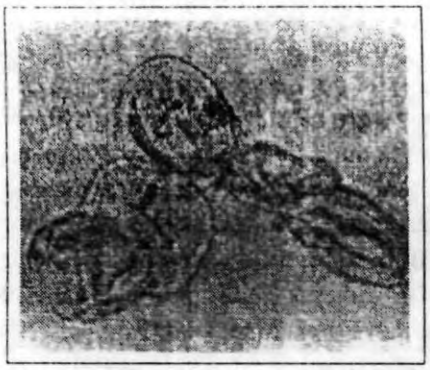

Fig. 5. Pollen conglomerate in the sterile clone SCATC 93-114 (x 500)

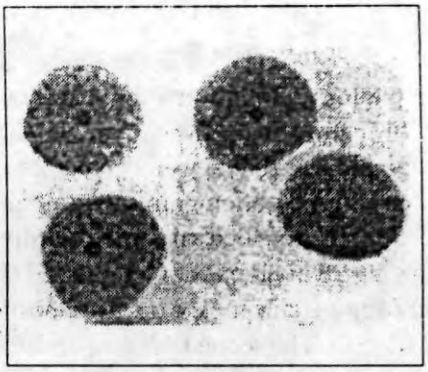

Fig. 6. Microspores of fertile clone RRII 105 (x 2000)

Fig. 7. T.S. of anther column of SCATC 93-114 showing tapetum persisting (x 500)

Fig. 8. T.S.of anther column of RRII 105 showing degenerated tapetum and fertile pollen grains (x 500)

35 and RRII 17 . In GT 1, development of male flowers was very poor. In Ch 2, morphology of the flower was similar to that of normal clone, but 30% of the anthers showed partial dehiscence. In RRII 35, the male flowers degenerated before attaining maturity. In RRII 17, both male and female flowers were sterile. In the case of SCATC 93-114, flowers attain full size and do not fall off early, but they open late and the anthers seem to be shrivelled in appearance.

Male sterility can be described as the failure or inability of the plant to produce functional male gametes. In flowering plants, three types of male sterility are observed according to their mode of inheritance. They are, nuclear or genetic male sterility (GMS), cytoplasmic male sterility (CMS) and cytoplasmic genetic male sterility (CGMS). Genetic male sterility is due to the action of nuclear genes in its inheritance, cytoplasmic male sterility is due to cytoplasmic factors and cytoplasmic genetic male sterility is due to genetic as well as cytoplasmic factors. Cytoplasmic male sterility is reported in a number of plant species such as *Datura* (Hegde and Andrade, 1982), *Cajanus cajan* (Katti *et al.*, 1994), *Glycine* (Smith *et al.*, 2002), Radish (Patil *et al.*, 1994). The causes for pollen sterility has been attributed to various factors such as malfunctioning of tapetum, untimely dissolution of callus, poor vasculature, biochemical disturbance, mutation in mitochondrial genome and the presence of viruses (Graybosch *et al.*, 1984; Grant *et al.*, 1986; Kakihara *et al.*, 1988; Kaul, 1988; Sawhney and Bhadula, 1988; Shivanna and Johri, 1989; Theis and Robbelen, 1990). The environment also plays a role on the expression of sterility/fertility, more with some cytoplasm than with others (Subudhi *et al.*, 2001).

Meiotic analysis of SCATC 93-114 revealed that meiosis proceeded normally up to the tetrad stage with no meiotic abnormalities such as formation of laggards or micronuclei noticed, but there was complete degeneration of the cytoplasm and nuclei in the microspores resulting in sterility. In the male sterile anthers, microspores lacked storage metabolites. On the contrary, in fertile anthers, accumulation of reserve metabolites in pollen grains coincided with degeneration of tapetum. The persistence of tapetum in sterile anthers implies that the tapetum fails to supply nutrients for the developing microspores and as a result the pollen grains formed are all sterile.

Male sterility in *Hevea* clones could be exploited in breeding programmes to generate hybrids, because it eliminates the expense of hand emasculation procedures. Moreover, male sterile clones having good fruit set can be used for designing seed orchards.

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