NOTES

TRIPLOIDY IN *HOPEA ODORATA*

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Hopea odorata is a potentially important tropical plantation hardwood tree of the family Dipterocarpaceae, and found to be especially suitable for degraded sites of logged over lowland rain forests (Wan Razali & Ang 1991). A riparian species, it is distributed in the Andamans, Myanmar, Thailand and Indochina, and in the upper part of Malesia (Malay Peninsula: North Perak and Terengganu northwards) (Ashton 1982). Population decline in disturbed habitats in south Perak, south Terengganu and north Kelantan due to agriculture and urbanisation highlights the need for its increased propagation and tree improvement. *Hopea odorata* reproduces through seed but cuttings have been shown to be an important alternative method of propagation (Aminah 1991).

All reliable published chromosome counts of *Hopea* are diploid or polyploid based on a single basic chromosome number x = 7. In the case of *H. odorata*, diploid (2n = 14) as well as triploid and near-triploid numbers (2n = 20, 21, 22) have been reported (see Tixier 1953, Kaur *et al.* 1978, Somego 1978, Jong 1982). This species together with two other near triploid species, *H. subalata* and *H. latifolia*, represent a group of *Hopea* based on x = 11 (2n = 22), with possible reduction to x = 10 (2n = 20) (Somego 1978). It was suggested that this genus has two cytological lines of evolution, x =7 and x = 11, the latter of which is new for the genus. This would represent a notable departure from the basic number of x = 7 that is typically found in the genus and a group of allied genera that includes the closely related *Shorea*. An alternative hypothesis is that 2n = 20 and 2n = 22 are respectively hypotriploid and hypertriploid numbers, a reflection of aneusomaty, i.e. aneuploid variation in chromosome number occurring within a single organ. Thus, uncertainty surrounds the basic chromosome number of *H. odorata*. Indeed, determination of the correct chromosome number holds the key not only to the cytotaxonomic status of the species within the genus *Hopea*, but also to its proper management and conservation, according to whether it is treated as a triploid apomict or as a sexual species, and whether intraspecific aneuploidy is involved. To establish beyond doubt the chromosome number of this species, a large cytological sample was examined.

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Fifty seeds each were collected from a *H. odorata* tree in Kuala Terengganu (northern Peninsular Malaysia) and from another tree in the grounds of the Forest Research Institute Malaysia (FRIM) (next to the clubhouse) and sown in 1:3 soil:sand mixture. Seeds produced one to four viable seedlings per seed. Where formed, multiple seedlings from each germinated seed were later separated from each other into individual polybags and labelled; these were used for determining whether variation in chromosome number occurred between seedlings of a single seed. Chromosome preparations from root tips of seedlings were made according to the methods outlined below. About three weeks after germination root tips were collected between 11.00 a.m. and 12.00 noon and pretreated with 0.002 M 8-hydroxyquinoline for four to five hours at 13 to 15 °C before fixation in 3:1 ethanol:glacial acetic acid. The root tips were hydrolysed in 5 M HCl at room temperature for 30 min and stained for two to three hours in the Feulgen reagent (prepared according to Fox 1969; see also Jong 1997). For additional softening, the stained root tips were transferred into an enzyme mixture (1:1) of 4% cellulase and 4% pectinase at 35 °C for 20 min. The meristematic tissue was squashed in a counterstain of 0.4% aceto-carmine.

The use of a fluorescent dye DAPI (4'-6-diamidino-2-phenylindole) was also tested for the first time on dipterocarp chromosomes. The schedule adopted was based on that given in Kenton (1991). Pretreated root tips, unstained, were softened in 45% acetic acid (not HCl) at 60 °C for 5 to 10 min before squashing in 45% acetic acid. After removal of coverslips using a modified freezing method as outlined in Jong (1997), the slides were air-dried for three days before staining in a freshly prepared 0.02% DAPI for 3 min in the dark, rinsed in McIlvaine's buffer pH 7.0 and mounted in an anti-fade solution, Vectashield. Slides were viewed under UV light of a fluorescent microscope.

Definite counts of 2n = 21 were obtained from 13 seedlings from Kuala Terengganu, nine multiple seedlings from FRIM and an additional eight seedlings from separate seeds, also from the same FRIM tree. Thus both accessions of *H. odorata* from this study were triploid with 2n = 3x = 21, with no variation in chromosome number detected between multiple seedlings derived from single seeds. Counts of 2n = 20 and 2n = 22 were sometimes encountered within single root tips, but the majority of counts were 2n = 21 (Figure 1). One metaphase had a hexaploid number 2n = 42, most probably the result of endomitosis. Metaphase chromosomes of *H. odorata* ranged from 1 to 2.5 μm in size; in favourable cells, the morphology of the chromosomes was discernible (Figure 2), the chromosomes mostly with median to submedian centromeres. At least one, and in some cases two, chromosomes with satellites could be seen. Blocks of centromeric heterochromatin were prominent amongst prometaphase chromosomes.

There were no diploids detected in the samples studied. The only known diploid cytotype of 2n = 14 is reported from India (Roy & Jha 1965). The unconfirmed count of 2n = 20 by Tixier (1953) remains in doubt, as with the other counts of Vietnamese dipterocarps made by the author. Thus Somego's (1978) proposition that counts of 2n = 20, 21 and 22 of Malaysian *H. odorata* represent diploid numbers is difficult to sustain. With reference to *H. subalata*, Kaur *et al.* (1978) reported a chromosome number of 2n = 21. In addition, some 21% of fruits produced multiple seedlings, and embryological evidence suggests that this species is apomictic. As sexual reproduction is usually not an option to a triploid, agamospermy provides an escape from sterility, that is, a means of "clonal" propagation through seed. Germinating seeds of *H. odorata* produce up to 90% with multiple seedlings (Kaur *et al.* 1978, 1986) and it is highly probable that these, as in *H. subalata*, are also agamospermous in origin. The occurrence of genetic variation among multiple seedlings from single seeds has been inferred from isozyme and RAPD analyses (Wickneswari *et al.* 1995). However, variation detected by these genetic methods does not necessarily

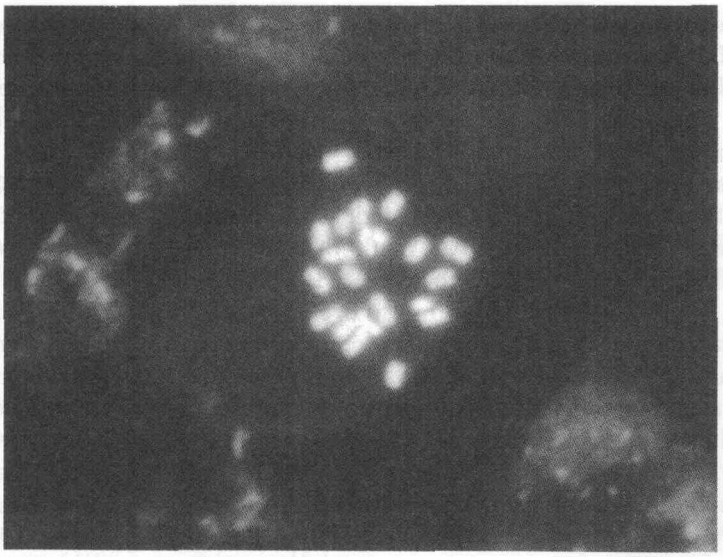

Figure 1 Metaphase chromosomes of *Hopea odorata* (2n = 3x = 21) stained with DAPI (2000 × magnification)

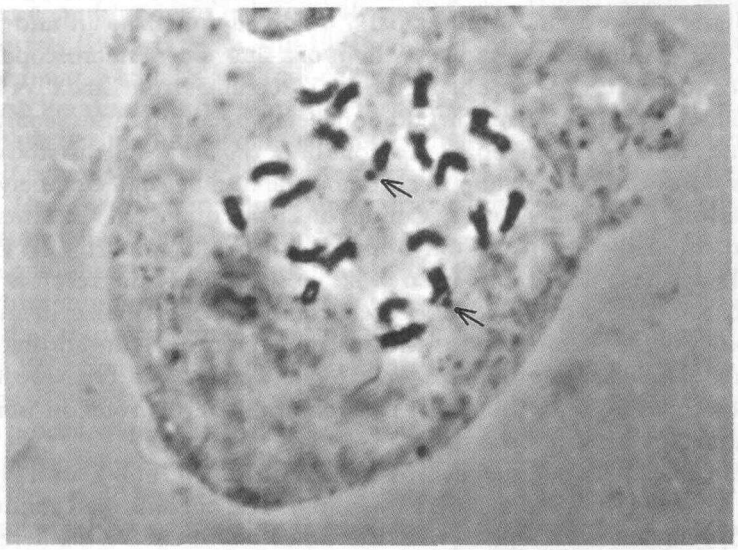

Figure 2 Chromosomes of *Hopea odorata* (arrows showing satellites) (2000 × magnification)

contradict the present cytological findings, as the multiple seedlings may have been of mixed origins, e.g. from nucellar adventitious polyembryony as well as possibly from mutant/aneuploid nucellar or embryo-sac cells. A closer investigation into the reproductive biology and embryology of *H. odorata* is clearly desirable, with particular attention paid to meiotic behaviour.

Hopea is a genus of about 102 species of Southeast Asian dipterocarps (Ashton 1982), and only a very small sample of 10 has been cytologically examined so far. We regard the somatic number of 2n = 21 in Malaysian *H. odorata* as another example of triploidy based on x = 7 and that occasional numbers 2n = 20 and 22 as aneusomatic variants, not diploid numbers. Other triploids include *H. subalata*, *H. latifolia* and also *S. resinosa* in the closely allied genus *Shorea* (Kaur *et al.* 1986, Jong 1982). Tetraploidy (2n = 4x = 28) is also known, so far only in *H. nutans* and *S. ovalis* (Jong & Lethbridge 1967, Somego 1978). The triploid status of *H. odorata* and its strong polyembryonic tendencies are features consistent with the well-known close association among dipterocarps between polyploidy, polyembryony and apomixis (Kaur *et al.* 1978, 1986).

From our study, we concluded that there was no convincing evidence to indicate that *Hopea* contained two widely disjunct basic chromosome numbers, x = 7 and x = 11 (10), as suggested by Somego (1978).

This case study provided a useful example of the importance and relevance of cytological information to the taxonomy as well as to the proper management and conservation of an indigenous potential plantation species.

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References

AMINAH, H. 1991. A note on the effect of leaf number on rooting of *Hopea odorata* stem cuttings. *Journal of Tropical Forest Science* 3(4): 384–386.

ASHTON, P. S. 1982. Dipterocarpaceae. *Flora Malesiana Series I* 9: 237–290.

FOX, D. P. 1969. Some characteristics of cold hydrolysis technique for staining plant tissues by the Feulgen reaction. *Journal Histochemistry Cytochemistry* 17: 266–272.

JONG, K. 1982. Cytotaxonomy of Dipterocarpaceae. *Flora Malesiana Series I* 9(2): 268–273.

JONG, K. 1997. *Laboratory Manual of Plant Cytological Techniques.* Royal Botanic Garden Edinburgh. 96 pp.

JONG, K. & LETHBRIDGE, A. 1967. Cytological studies in the Dipterocarpaceae. I. Chromosome numbers of certain Malaysian genera. *Notes of Royal Botanic Garden Edinburgh* 27: 175–84

KAUR, A., HA, C. O., JONG, K., SANDS, V. E., CHAN, H. T., SOEPADMO, E. & ASHTON, P. S. 1978. Apomixis may be widespread among trees of the climax rain forest. *Nature* 271: 440–442.

KAUR, A., JONG, K., SANDS, V. E. & SOEPADMO, E. 1986. Cytoembryology of some Malaysian dipterocarps, with some evidence of apomixis. *Botanical Journal of the Linnean Society* 92: 75–88.

KENTON, A. 1991. Heterochromatin accumulation, disposition and diversity in *Gibasis karwinskyana* (Commelinaceae). *Chromosoma* 100: 604–610.

ROY, R. P. & JHA, R. P. 1965. Cytological studies in the Dipterocarpaceae. *Journal Indian Botanical Society* 44: 387–397.

SOMEGO, M. 1978. Cytogenetical study of Dipterocarpaceae. *Malayan Forester* 41: 358–365.

TIXIER, P. 1953. Données cytologiques sur quelques Guttiférales du Viet-Nam. *Revue de la Cytologie et Biologie Vegetale* 14: 1–12

WAN RAZALI, W. M. & ANG, L. H. 1991. The early growth of two indigenous commercial tree species planted on degraded sites of logged over forest. Pp. 22–29 in Appanah, S. Ng, F. S. P. & Roslan, I. (Eds.) *Malaysian Forestry and Forest Products Research.* Forest Research Institute of Malaysia.

WICKNESWARI, R., LEE, S. L. & MARIAM, D. 1995. Utility of RAPD markers in detecting genetic variation in multiple seedlings of *Hopea odorata* Roxb. (Dipterocarpaceae). *Malayan Forester* 58: 42–50.