CHAPTER-7
CONCLUDING REMARKS
The two plant species *Coccinia grandis* and *Flacourtia jangomas* that constitute the subject matter of the present thesis have no apparent similarity among themselves and have been placed by the taxonomist in widely separated families, however, both of them are dioecious.

Chromosome analysis of *C. grandis* has been made from time to time by several workers. This plant exhibits heterogametic XX-XY type sex determination. The X chromosome in this plant is normally indistinguishable from the rest of the autosomes. The Y chromosome is quite long and acts as a marker and it also forms a heteromorphic pair with the X chromosome during meiosis. Apprehensions have been made regarding female suppresser and male promoter segments of Y chromosomes but no specific locus has so far been identified. The use of the fluorescent dye Hoechst 33258 coupled with controlled acetic carmine staining of meiotic preparations have revealed a linear differentiation of the largely heterochromatic Y chromosome into several segments. Depending on the intensity of fluorescence, very bright segments have been designated as $H_{T_1}$ regions, less bright ones are designated as $H_{T_2}$ regions and some smaller blocks exhibit dull ground-level fluorescence ($E_u$ regions), which is predominant in most of the other chromosomes of the complement.

The X chromosome(s) in the mitotic cells of either the male or the female plants of *C. grandis* could be characterised by some degree of heteropycnosity suggesting the presence of heterochromatin with repetitive sequences. It can also be distinguished during meiosis of male plant (in diakinesis and metaphase I) when it participates as a partner of the heteromorphic pair. A linear differentiation of the X chromosome also by way of differential staining with Hoechst 33258 and acetic carmine was noted. It may be suggested that the loci controlling femaleness and/or other characters are present within the unique sequences located in the euchromatic blocks having a dull 'ground level' fluorescence.
Moreover, while studying the mitotic chromosome altogether 6 chromosomes, 2 pairs of autosomes and the pair of sex chromosomes, have been found to be associated with the nucleolus indicating their role as nucleolar organiser.

Hitherto, there was no report regarding the chromosome constitution of *F. jangomas* and during the present investigation it has been worked out for the first time, though the chromosome number of another species, *F. indica* was already known. In this investigation, the chromosome number of *F. jangomas* has been found to be 2n = 18. Only one pair shows secondary constrictions. The chromosome number of *F. indica* is 2n = 22. The basic numbers of these two species belonging to the same genus are different i.e., n = 9 and n = 11.

No distinct heteromorphic pair was noticed in *F. jangomas* during meiosis. However, there might indeed be a couple of 'look-alike' chromosomes with ample homology which enable them to pair with each other during meiosis, but sufficient difference may be present at the genic level which are responsible for differential sex-expression.

One intriguing cytological feature of *F. jangomas* is the drum-stick like body attached to one member of a comparatively longer bivalent of diakinesis - metaphase 1 stage. But this can not conclusively be designated as the heteromorphic pair like that of *C. grandis*.

In course of the present work, micropropagation of this two distinct plants, *C. grandis* and *F. jangomas*, has been achieved successfully. Though in both the cases regenerated plantlets could be obtained from dedifferentiated callus tissue, for the purpose of micropropagation, direct regeneration from shoot tip and nodal section was employed instead of indirectly through callus tissue in order to get rid of somaclonal variation because chromosomal analysis of callus tissue reveals an array of aneuploids and polyploids therein.
It is interesting to note that in spite of a radical distinctiveness of the two materials with respect to their phylogeny as well as their morphology, they show some striking similarity in response to tissue culture. They prefer same basal media as well as more or less similar PGR combination. It has been found that responsiveness to callus induction as well as regeneration vary with the type of the selected explant. In both the materials, maximum callus induction was noted with leaf explant. For regeneration of plantlets, shoot apex-derived calli were most effective in *C. grandis* whereas those derived from nodal sections were more suitable for inducing plantlet regeneration in *F. jangomas*. In case of *F. jangomas*, probably due to its woody nature, root induction was found to be relatively difficult. This was overcome by manipulating the phytohormone concentration, particularly that of auxin (NAA).

In both the materials, the survival frequencies of directly regenerated rooted plantlets were not satisfactory. To have an expected survival value, further research in the line of hardening is called for. Both the materials are dioecious and with respect to callus induction, regeneration and micropropagation, no appreciable difference between the male and female individuals of any of the two species was noted.

Both *C. grandis* and *F. jangomas* constitute important raw materials for folk medicine and existence of the latter is becoming threatened in nature. The present work, involving success in micropropagation, thus promises to be an aid not only for the preservation of the germplasm, but also in pharmacological investigations to develop improved varieties having higher potentiality of the active principles.
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