Indian J. Genet., **65**(1): 70-72 (2005)

Short Communication

Cytological studies in some *Citrus* species of Arunachal Pradesh

Mohesh Gogoi, B. Singh, P. Rethy and A. K. Mishra[1]

Department of Forestry, North Eastern Regional Institute of Science and Technology Nirjuli 791 109
[1]Department of Botany, Arunachal University, Rono Hills 791 112

(Received: June 2004; Revised: April 2004; Accepted: April 2005)

Arunachal Pradesh is natural home of many *Citrus* species and varieties which is indicated by its diversity of species existing in the tropical and subtropical forests and wide distribution pattern of species [1]. Phenotypic variations among the species is quite interesting as the genotype determines their characters [2] and it may further be modified due to variety of climatic and topographic conditions prevailing in the state. Now it has established that nine (n = 9) is the haploid number [3] and polyploidy is a common phenomenon for this genus [4]. On the other hand, confusion and disagreement have prevailed in the interpretation of species in *Citrus*. The earlier pomologist, Swingle [5] classified *Citrus* into 16 species, Tanaka [6] divided into 162 species and recent molecular taxonomists Deng *et al.* [7] have considered only 3 genuine botanical species namely *C. grandis*, *C. reticulata* and *C. medica*. Hence a cytological investigation was carried out to confirm the systematic position of seven important species native to the state of Arunachal Pradesh.

Somatic metaphase of root tip cell was considered for present study. Roots were collected from the germinated seedlings of identified species between 1-3pm and the mitotic study was made following by the procedure of Sharma and Mookherjea [8]. The chromosomes were studied from squash preparation of root tip cells and more than ten slides were prepared for each species. Finally photograph was taken using LEICA LEITZ research microscope with automatic microphotographic attachment at the magnification of 1000xoil. Chromosomes were measured from the microphotographs and classified as suggested by the system of Levan *et al.* [9].

Cytological studies of the seven species namely *Citrus reticulata* Blanco (var. Khasi mandarin), *C. aurantifolia* Swin. (var. Round fruited), *C. indica* Tan., *C. aurantium* Linn. (var. Chakala), *C. grandis* (L.) Osbeck (var. Pathalipahar), *C. limon* Burm f. (var. Assam lemon) and *C. medica* Linn. (var. Bira jora) have revealed diploidy, 2n = 18. It was found that seven species studied possess small, rod shaped and morphologically more or less similar chromosomes (Fig. 1). No numerical variation of chromosome was observed, but gross morphological features of the chromosome complements were variable from one species to another and also distinct for each species (Table 1). Thus, similarities of chromosome number indicate that the seven species must have originated from a common ancestral stock and inclusion of seven species under a same genus is justified. Moreover, consideration of their absolute chromosome length, TF% and karyotype formulae indicate their individuality and a strong genetic relationship. It is, therefore, recommended that the seven species must be treated as separate species.

The result has supported the view of Iwamasa and Nito [4] that diploidy has been generally widespread in the genus, *Citrus* and 9 is the basic chromosome number without any contradiction. It has been reported by Kumar and Subramaniam [10] that 18 is the diploid number in *C. reticulata* Blanco, *C. aurantifolia* Swin., *C. grandis* (L.) Osbeck, *C. aurantium* Linn., *C. limon* Burm f. and *C. medica* Linn, and the present result support the view on chromosome number of these species. The probable progenitor of mandarin orange [11], *C. indica* Tan., has also been shown to have 2n = 18 and similarity of the morphological features of the chromosome with other species has so far not been reported.

The divergent views of the classification of *Citrus* are due to the ambiguous morphological characters that have been responsible for the confusion existing in their characterization. For instance, we support the view of Herrero *et al.* [12] that the karyomorphological data can be used as supporting evidence in solving problems in taxonomy and the delineation of *Citrus* species. The present result has provided ample scope in understanding the chromosome number, morphological features of chromosome complements and taxonomic position of seven *Citrus* species of Arunachal Pradesh.

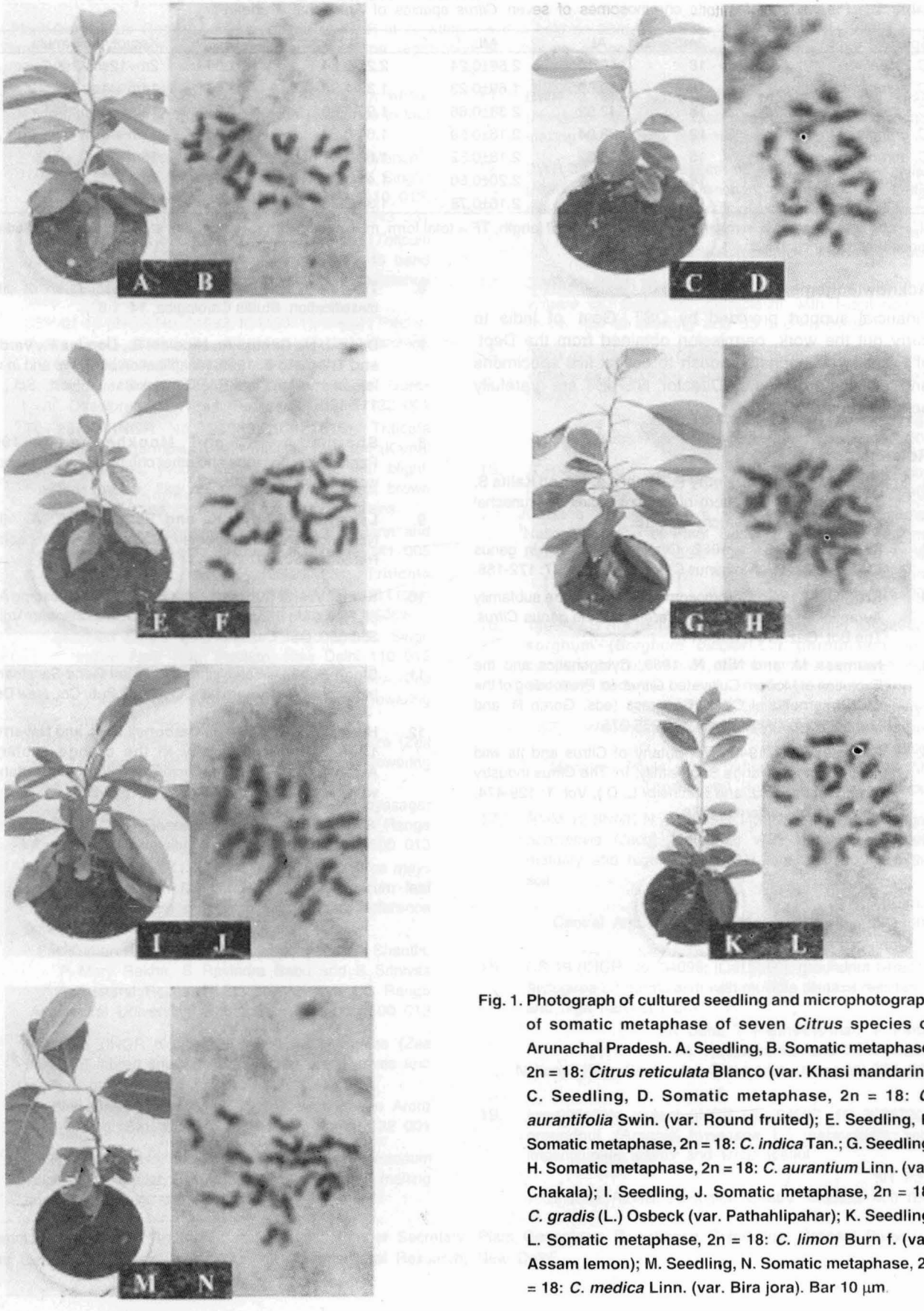

Fig. 1. Photograph of cultured seedling and microphotograph of somatic metaphase of seven *Citrus* species of Arunachal Pradesh. A. Seedling, B. Somatic metaphase, 2n = 18: *Citrus reticulata* Blanco (var. Khasi mandarin); C. Seedling, D. Somatic metaphase, 2n = 18: *C. aurantifolia* Swin. (var. Round fruited); E. Seedling, F. Somatic metaphase, 2n = 18: *C. indica* Tan.; G. Seedling, H. Somatic metaphase, 2n = 18: *C. aurantium* Linn. (var. Chakala); I. Seedling, J. Somatic metaphase, 2n = 18: *C. gradis* (L.) Osbeck (var. Pathahlipahar); K. Seedling, L. Somatic metaphase, 2n = 18: *C. limon* Burn f. (var. Assam lemon); M. Seedling, N. Somatic metaphase, 2n = 18: *C. medica* Linn. (var. Bira jora). Bar 10 μm.

Table 1. Parameters of mitotic chromosomes of seven *Citrus* species of Arunachal Pradesh

Species	2n	AL	ML	RL	TF%	Karyotype formula
C. reticulata	18	46.10	2.56±0.24	2.27-2.84	31.54	2m+12sm+2st+2stsc
C. aurantifolia	18	30.50	1.69±0.23	1.38-1.98	42.03	14m + 4sm
C. indica	18	42.92	2.38±0.66	1.95-3.68	40.21	14m + 4sm
C. grandis	18	38.94	2.16±0.59	1.64-3.22	39.85	12m + 6sm
C. aurantium	18	39.32	2.18±0.52	1.63-3.07	45.47	18m
C. limon	18	39.64	2.20±0.50	1.41-3.04	45.01	18m
C. medica	18	38.88	2.16±0.78	1.36-3.79	37.75	14m + 2st+ 2stsc

AL = absolute length, ML = mean length, RL = range of length, TF = total form, m = median, sm = submedian; st = subtelocentric and sc = secondary constriction

Acknowledgements

Financial support provided by DST, Govt. of India to carry out the work, permission obtained from the Dept. of Forests, Arunachal Pradesh to collect live specimens and facilities provided by Director, NERIST are gratefully acknowledged.

References

1. **Gogoi M., Singh B., Rethy P., Mishra A. K. and Kalita S.** 2004. Distribution pattern of *Citrus* species in Arunachal Pradesh. J. Hill Research, **7**: 13-16.
2. **Raghuvanshi S. S.** 1962. Cytological studies in genus *Citrus* IV. Evolution in genus Citrus. Cytologia, **27**: 172-188.
3. **Krug C. A.** 1943. Chromosome numbers in the subfamily Aurantioideae, with special reference to the genus *Citrus*. The Bot. Gaz., **104**: 602-611.
4. **Iwamasa M. and Nito N.** 1988. Cytogenetics and the Evolution of Modern Cultivated *Citrus*. *In*: Proceeding of the Sixth International Citrus Congress (eds. Goren R. and Mendel K.) Tel Aviv, Israel, pp. 265-275.
5. **Swingle W. T.** 1943. The botany of Citrus and its wild relatives of the orange Sub-Family. *In*: The Citrus Industry (eds. Webber H. J. and Batchelor L. D.), Vol. **1**: 129-474, University of California, USA.
6. **Tanaka T.** 1977. Fundamental discussion of citrus classification. Studia Citrologica, **14**: 1-6.
7. **Deng J. N., Gentile A., Nicolosi E., Domina F., Vardi A. and Tribulato E.** 1995. Identification of *in vivo* and *in vitro* lemon mutants by RAPD markers. J. Hort. Sci., **70**: 117-125.
8. **Sharma A. K. and Mookherjea A.** 1955. Paradichlorobenzene and other chemicals in chromosome work. Stain Tech., **30**: 1-7.
9. **Levan A., Fredga K. and Sandberg A. A.** 1964. Nomenclature for centromeric position on chromosomes. Hereditas, **52**: 201-220.
10. **Kumar V. and Subramaniam B.** 1986. Chromosome Atlas of Flowering Plants of the Indian Subcontinent, Vol. **1**: 356-360. BSI, Calcutta.
11. **Singh B.** 1981. Establishment of First Gene Sanctuary in India for Citrus in Garo hills. Concept. Pub. Co., New Delhi.
12. **Herrero R., Asins M. J., Carbonell E. A. and Navarro L.** 1996. Genetic diversity in the orange subfamily Aurantioideae. I. Intraspecies and intragenus genetic variability. Theor. Appl. Genet., **92**: 599-609.