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Characterization of Open-Pollinated Seedling Progenies of Turmeric (*Curcuma longa* L.) based on Chromosome Number, Plant Morphology, Rhizome Yield and Rhizome Quality†

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Summary Fourteen open-pollinated seedling progenies of a high yielding, released variety of turmeric, namely 'IISR Kedaram', have been characterized for chromosome number, plant morphology, fresh rhizome yield and rhizome quality, in comparison to the mother. The progenies showed significant variation from the mother variety with respect to chromosome number and most of the other characteristics studied. Chromosome number in the mother plant was $2n=63$ while in the progenies it ranged from $2n=78$ to $2n=84$, the latter being the most frequent. For the morphological characteristics namely plant height, number of tillers, leaf length, leaf breadth, petiole length and internode length of primary rhizomes, several progenies excelled the mother. Five progenies produced a significantly higher fresh rhizome yield than the mother variety. Among the quality parameters studied, curcumin and oleoresin content was found to be lower in all the progenies while oil content was found to be slightly higher in 2 progenies compared to the mother. The role of triploid segregation in generating the variation among seedling progenies and the usefulness of the variation in the improvement of turmeric is discussed.

Key words *Curcuma longa*, Curcumin, Essential oil, Oleoresin, Triploid, *Zingiberaceae*.

Turmeric (*C. longa* L., Zingiberaceae) is a popular spice crop cultivated widely in India and its underground rhizomes are used as a condiment and in dye, drugs and cosmetics, after processing and value addition. Turmeric is a certified natural food colour and has several uses in traditional Indian medicine as well as modern medicines for various human ailments (Govindarajan 1980, Purseglove *et al.* 1981, Pruthi 1998, Chattopadhyay *et al*. 2004, Ravindran *et al*. 2007). Turmeric is traditionally propagated using perennial rhizomes and cultivated annually. The chromosome number of *C. longa* $2n=63$ has been reported frequently (Chakravorti 1948, Ramachandran 1961, 1969, Nambiar 1979, Renjith *et al.* 2001, Nair 2000, Nair and Sasikumar 2009). Deviations such as $2n=32$ (Sato 1948), $2n=48$ (Das *et al.* 1999), $2n=61$ (Nair and Sasikumar 2009), $2n=62$ (Raghavan and Venkatasubban 1943, Sharma and Bhattacharya 1959), $2n=64$ (Chakravorti 1948) and $2n=84$ (Renjith *et al.* 2001, Nair and Sasikumar 2009) have also been reported. The basic chromosome number of the genus *Curcuma* is suggested as $x=21$, which in turn originated by dibasic amphidiploidy from $x=9$ and $x=12$ or by secondary polyploidy (Ramachandran 1961, 1969, Nambiar 1979).

Being propagated traditionally by underground rhizomes, crop improvement programs in turmeric were largely restricted to clonal selection and induced mutation and subsequent selection. The main emphasis was yield potential, high curing percentage and high curcumin content

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(Ravindran *et al.* 2007). Turmeric has been considered as a triploid and pollen fertility is less than 60% (Nambiar 1979, Nair *et al.* 2004). Even though it was believed earlier that *C. longa* fails to set seeds, unlike *C. aromatica* (Nambiar 1979, Nambiar *et al.* 1982, Nazeem *et al.* 1993), seed set and germination of seeds have been recorded (Lad 1993, Sasikumar *et al.* 1996, Nair *et al.* 2004). Subsequent to these reports on seed set in *C. aromatica* and *C. longa* by open-pollination as well as controlled crosses, the possibility of utilizing recombination breeding in the genetic improvement of turmeric is gaining attention (Nazeem *et al.* 1993, Sasikumar *et al.* 1996, Nair *et al.* 2004). High yielding varieties have also emerged as a result of yield and quality evaluation of open pollinated progenies of turmeric (Sasikumar *et al.* 1996). Variation in the chromosome number of seedling progenies has been reported by Nair and Sasikumar (2009). However, information on systematic evaluation of open-pollinated seedling progenies of same mother genotype is lacking.

The present study attempts to characterize 14 open-pollinated seedling progenies of a high yielding turmeric variety 'IISR Kedaram' (Sasikumar *et al.* 2005) based on parameters namely chromosome number, plant morphology, fresh rhizome yield and rhizome quality in comparison to mother genotype.

Materials and methods

Plant materials

Rhizomes of high yielding variety 'IISR Kedaram' (Acc. 126) and its 14 open-pollinated seedling progenies, namely SLP-126/1, 126/2, 126/3, 126/4, 126/5, 126/6, 126/7, 126/8, 126/9, 126/10, 126/11, 126/12, 126/13, 126/15 harvested and stored at the breeding material repository of the Indian Institute of Spices Research, Calicut, India during February 2008, were used for the study.

Chromosome number analysis

The rhizomes were planted in seed pans filled with clean river sand and regularly watered. On initiation of sprouting and root emergence, root tips were collected for analysis. Actively growing root tips from the freshly emerging roots of 5–10 mm length were collected between 11:00 and 11:30 AM and pretreated with a 1 : 1 mixture of saturated paradichlorobenzene solution and 2 mM 8-hydroxyquinoline at 4–5°C for 4 h. After washing thoroughly in double distilled water, the root tips were fixed in a mixture of ethyl alcohol, acetic acid and chloroform (3 : 1 : 1) for 24 h.

The fixed root tips were subjected to hydrolysis with 5 M HCl at 0°C for 4 min and stained in 2% aceto orcein for 4 h, and then squashed in 45% acetic acid. Photomicrographs were taken from temporary slides using a Leica DBRB microscope fitted with MPS-28 camera. Six mitotic metaphase plates with good chromosome spread from 2–3 slides were used for counting the chromosome number in each plant.

Plant morphology and rhizome yield

For recording plant morphology and rhizome yield, a replicated trial was laid out using pot culture. Garden pots of 30 cm diameter each filled with 15 kg of 1 : 2 : 1 mixture of river sand, garden soil and farmyard manure were used. The trial was laid out in completely randomized design with three replications, composing 15 treatments including the mother genotype and 14 progenies. Fifty grams each of seed rhizomes were planted in pots in the first week of May 2008. Fertilizer applications and plant protection practices were done uniformly for all the plants in pots as per the package of practices of IISR for turmeric. Morphological observations were recorded from each plant in each replication after 6 months of planting, in the first week of November 2008.

Plant height was recorded by measuring the tallest tiller of the plant from the base up to the leaf tip. Number of tillers was counted, avoiding the poorly developed ones. Leaf length was

measured from the tip of the leaf to the point of origin of petiole. Leaf breadth was measured from the middle point of the leaf length from one side to the other. Petiole length was measured from the base of the lamina to the point at which the sheath originates. All the leaf measurements were taken from randomly selected 3 leaves from each plant avoiding those towards the base and the tip. All these measurements were expressed in cm and mean of 3 values formed the value of the treatment in respective replication.

Harvesting of rhizomes was carried out after 9 months of growth after drying of aerial stem and leaves, in the month of February 2009. Fresh weight of rhizomes was recorded after cleaning the rhizomes and expressed in gm. For recording internode length of primary rhizomes, 6 primary rhizomes were randomly selected from each treatment and 4 internodes were measured serially from each primary rhizome and expressed in mm.

Analysis of rhizome quality

For quality analysis, rhizomes samples containing 25% mother rhizomes, 60% primary rhizomes and 15% secondary rhizomes were drawn from each replication and composited together. The composited samples of each treatment were boiled in water for 1 h. After boiling, the samples were sun dried for 72 h to reach a moisture level of 10% approximately. The dried samples were powdered and used for analysis following ASTA analytical methods (ASTA, 1968a, 1968b, 1968c).

For analysis of curcumin content 3 samples weighing 0.1 g each were drawn from the total powdered sample of each treatment and analyzed separately. Curcumin was extracted from the powder in ethyl alcohol (98%) by refluxing for 3 h. The colour estimation was carried out in a Shimadzu UV 1601 spectrophotometer at 425 nm and percentage of curcumin was computed based on the concentration of pure crystalline curcumin (98%) (ASTA, 1968c).

Essential oil and oleoresin were analyzed using pooled samples of 30 g and 10 g respectively for each treatment. Essential oil was determined by hydro-distillation using a Clevenger trap (lighter than water type). The percentage of essential oil was computed as a percentage of volume/weight (ASTA, 1968a). Oleoresin was determined gravimetrically by cold percolation of the powdered sample using acetone. The slurry was flash evaporated to a viscous mass and oleoresin was computed as percentage of weight/weight (ASTA, 1968b).

Statistical analysis

Data on morphology, yield and curcumin content were subjected to analysis of variance (ANOVA) test using MSTATC software package and means were separated using Duncan's Multiple Range Test at $p=0.05$. For essential oil and oleoresin the percentage values obtained are presented.

Results and discussion

Chromosome number of all the seedling progenies analyzed showed deviation from the chromosome number of mother genotype, $2n=63$; which is the widely accepted chromosome number of turmeric (Chakravorti 1948, Ramachandran 1961, 1969, Nambiar 1979, Renjith *et al*. 2000, Nair 2000). Among the 14 seedling progenies analyzed, 11 showed $2n=84$, 1 showed $2n=82$ and 2 showed $2n=78$ (Table 1). The progenies showed $2n=84$ were 126/1, 126/3, 126/5, 126/7, 126/8, 126/9, 126/10, 126/11, 126/12, 126/13 and 126/15. Progenies Nos. 126/4 and 126/6 showed $2n=78$ while 126/2 showed $2n=82$ (Fig. 1 A–O). Reports on chromosome number deviation from $2n=63$ are available on germplasm collections of *C. longa* (Sato 1948, Das *et al.* 1999, Raghavan and Venkatasubban 1943, Chakravorti 1948, Sharma and Bhattacharya 1959, Renjith 1999, Nair and Sasikumar 2009) as well as seedling progenies (Nair and Sasikumar 2009). However, the chromosome number variation among the different progenies of the same mother genotype is

Table 1. Chromosome number, plant morphology and yield in turmeric cultivar Kedaram (ACC. No. 126) and its 14 opens-pollinated progenies. Data on morphology and yield were derived from pot culture experiment with 3 replications.

Plant Identity	Chro. Number ($2n$)	Morphological characteristics*						Yield/Pot* (g)
		Plant height (cm)	Number of tillers	Leaf length (cm)	Leaf breadth (cm)	Petiole length (cm)	Internode length in rhizome (mm)	
Kedaram (126)	63	99.33 ABC†	8.00 AB	46.67 D	12.01 BCD	45.56 ABC	9.17 DE	776.67 CDE
126/1	84	101.67 ABC	6.00 B	57.89 A	14.11 ABC	43.33 ABCD	11.92 AB	1026.67 AB
126/2	82	96.67 ABC	6.67 B	51.22 ABCD	13.78 ABC	43.33 ABCD	10.71 BC	893.33 ABCD
126/3	84	103.67 ABC	5.33 B	55.11 AB	14.28 AB	38.67 CD	11.71 AB	713.33 DEF
126/4	78	101.33 ABC	7.33AB	53.67 ABCD	13.67 ABC	44.11 ABC	7.67 F	573.33 EF
126/5	84	97.33 ABC	6.67 B	53.67 ABCD	12.55 ABCD	41.56 BCD	11.75 AB	986.67 ABC
126/6	78	108.67 A	6.33 B	57.89 A	14.72 A	45.11 ABC	12.79 A	1046.67 A
126/7	84	89.00 C	8.00 AB	48.44 BCD	11.61 CD	36.00 D	11.83 AB	793.33 BCDE
126/8	84	93.00 BC	7.67 AB	48.78 BCD	12.72 ABCD	43.53 ABC	10.88 BC	960.00 ABC
126/9	84	107.33 AB	7.67 AB	58.78 A	13.89 ABC	42.11 BCD	10.00 CD	646.67 EF
126/10	84	103.33 ABC	6.33 B	55.89 AB	14.22 AB	46.78 AB	11.67 AB	720.00 DEF
126/11	84	109.67 A	6.67 B	54.44 ABC	13.00 ABCD	50.00 A	12.54 A	666.67 DEF
126/12	84	97.00 ABC	6.00 B	54.22 ABCD	14.22 AB	40.78 BCD	8.21 EF	486.67 F
126/13	84	91.00 C	10.33 A	47.11 CD	11.17 D	40.56 BCD	11.75 AB	593.33 EF
126/15	84	108.00 AB	5.33 B	57.55 A	15.11 A	47.33 AB	9.04 DEF	680.00 DEF

* Mean from 3 replications † Means followed by the same letter are not significantly different at $p=0.05$ of Duncan's Multiple Range Test.

reported for the first time, in the present study.

As all the seedling progenies analyzed were derived from the same mother plant, having $2n=63$, the chromosome number variation might have resulted from union of gametes produced subsequent to the triploid segregation. Polyploids with an odd number of genomes, like triploids, exhibit higher degrees of meiotic abnormalities (Gupta 1997). However, only the chromosome constitution of maternal parent is known in the present study, as the progenies were resulted from open pollination and turmeric is a cross-pollinated plant. Varying degrees of chromosome abnormalities and associations were observed during meiosis of *C. longa* by Nambiar (1979). He observed quadrivalents, trivalents, bivalents and univalents in varied frequency depending on the cultivar studied, with a tendency towards formation of bivalents in higher frequency. Thus, it is evident that the trivalents and univalents have a crucial role in generating gametes with variable chromosome number thereby progenies with higher chromosome numbers than $2n=63$. The highest frequency of progenies in the present study had $2n=84$, a tetraploid number. This indicate that gametes having $n=42$ may have some selective advantage in both the parents. Nair and Sasikumar (2009) also presented similar opinion while reporting chromosome number variation among germplasm collections and seedling progenies of turmeric. Such gametes may originate by a perfectly normal triploid segregation or by unilateral inclusion of 21 bivalents into one gamete as a result of nondisjunction. A detailed meiotic analysis will help to understand the exact process, at least in the mother genotype. Chromosome number variation among the progenies of triploids has been reported earlier in horticultural plants like gladiolus (Jones and Bamford 1942), black pepper (Nair *et al*. 1993) and citrus (Zhu *et al*. 2009).

Variation with respect to different morphological characteristics was observed among seedling progenies, compared to mother genotype. Four progenies showed significantly higher plant height, while 3 showed significantly lower plant height compared to the mother. Number of tillers was

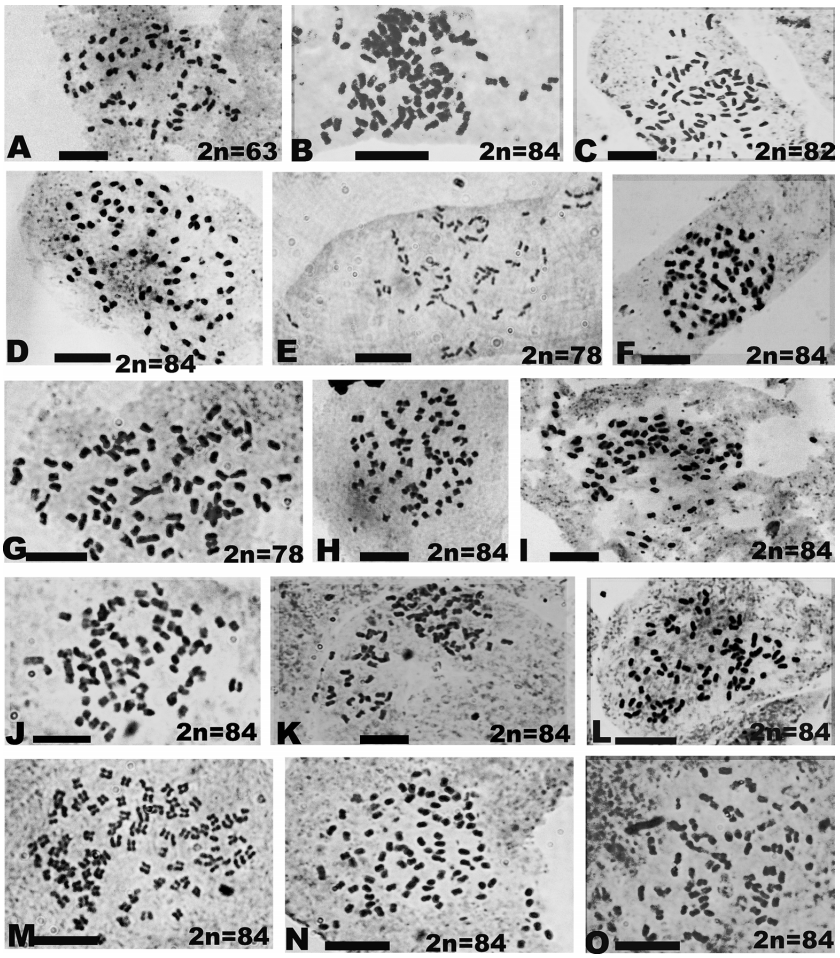

Fig. 1. Chromosome number variation among the open-pollinated seedling progenies of turmeric cultivar IISR Kedaram. A. IISR Kedaram-Acc.126 (2*n*=63), B. 126/1 (2*n*=84), C. 126/2 (2*n*=82), D. 126/3 (2*n*=84), E. 126/4 (2*n*=78), F. 126/5 (2*n*=84), G. 126/6 (2*n*=78), H. 126/7 (2*n*=84), I. 126/8 (2*n*=84), J. 126/9 (2*n*=84), K. 126/10 (2*n*=84), L. 126/11 (2*n*=84), M. 126/12 (2*n*=84), N. 126/13 (2*n*=84), O. 126/15 (2*n*=84). Bars represent 5 μm.

significantly higher in 1 progeny while lower in 9 progenies. Leaf length was higher in all progenies while leaf breadth was higher in 12 progenies and lower in 2 progenies. Higher petiole length was recorded in 3 and lower in 8. All except 3 progenies showed higher internode length of rhizomes while others showed lower values. Among the progenies, 126/6 recorded highest values for 4 morphological characteristics out of the 6 characteristics studied. The data on morphology of seedling progenies in comparison to the mother genotype is presented in Table 1. Variation among the morphological characters of open-pollinated progenies of turmeric has been reported earlier by Menon *et al*. (1992). They observed significant differences among 38 open pollinated seedling progenies of the cultivar Nandyal in respect of all plant characteristics except the number of tillers. Significant difference in leaf morphology was observed among the open-pollinated progenies of triploid citrus by Zhu *et al*. (2009)

Average yield per pot varied between the mother genotype and progenies as well as among progenies (Table 1). Five progenies out-yielded the mother while 8 progenies produced low yield. The progeny 126/6 produced the highest yield of 1046.67 g/pot followed by 126/1 which produced

1026.67 g/pot. The lowest yield was recorded in 126/12 (486.67 g/pot). Variation in yield among open-pollinated seedling progenies of turmeric has been reported earlier by Menon *et al*. (1992) and Sasikumar *et al*. (1996).

The data on quality analysis of rhizomes of seedling progenies and the mother is presented in Table 2. The major colour component of turmeric, curcumin, showed wide variation among progenies ranging from 0.90% in 126/4 to 3.24% in 126/1. But none of the progenies showed as high a value as the mother (5.67%). Oleoresin showed a range of variation from 6.43% in progeny 126/8 to 10.90% in 126/6, which are lower than the 14.24% of the mother. Oil content ranged from 3.20% in 126/8 to 6.00% in 126/13. Two progenies showed higher oil content than the mother (126/10, 126/13), while another 2 were on par with the mother (126/12, 126/15). Menon *et al*. (1992) observed a variation in curcumin content of seedling progenies of the cultivar Nandyal ranging from 2.1 to 5.1%. Sasikumar *et al*. (1996) reported high curcumin (6.21%, 6.52%), oleoresin (15.00%, 16.20%), and essential oil (6.5%, 6.2%) content in 2 seedling progenies of turmeric.

Table 2. Major chemical constituents of the processed rhizomes of turmeric cultivar IISR-Kedaram and its 14 open-pollinated progenies

Plant Identity	Chemical constituents of processed rhizomes		
	Curcumin (%)*	Oleoresin (%)	Oil (%)
Kedaram (126)	5.67 A†	14.24	5.20
126/1	3.24 B	10.17	4.00
126/2	3.20 B	9.39	4.80
126/3	1.26 I	6.48	3.60
126/4	0.90 K	7.91	4.00
126/5	3.20 B	8.84	3.73
126/6	1.15 J	10.90	4.80
126/7	2.12 D	9.01	4.40
126/8	1.66 G	6.43	3.20
126/9	1.46 H	10.52	3.47
126/10	1.81 F	9.63	5.60
126/11	1.75 F	7.19	4.00
126/12	1.99 E	7.48	5.20
126/13	2.28 C	10.54	6.00
126/15	1.52 H	9.71	5.20

* Mean of 3 replications † Means followed by the same letter are not significantly different at $p=0.05$ of Duncan's Multiple Range Test.

Observations on variation of characteristics among progenies indicate that the progenies with the same chromosome number also differ among themselves in other characteristics. This may be due to the fact that even though the number is same, chromosome constitution need not be balanced, as a result of a random process during triploid segregation. Alternatively, it is also possible that this variation might have originated through gene recombination resulting from segregation and subsequent recombination during open pollination. In the latter case, it is necessary to believe that the additional chromosome set has lesser influence on morphology. This may be due to the fact that the optimum ploidy level in turmeric has already been achieved in cultivated turmeric with $2n=3x=63$. The statements of earlier researchers that the basic chromosome number of the genus *Curcuma*, $x=21$, originated from dibasic amphidiploidy from $x=9$ and $x=12$ or secondary polyploidy (Ramachandran 1961, 1969, Nambiar 1979) also indirectly supports this view.

The wide variation observed in yield and quality characteristics among open-pollinated seedling progenies opens up the scope of creating genetic variability for selection of suitable genotypes for commercial use. The present study provides representative information of the variability available on different characteristics among open-pollinated progenies of a popular variety and extensive field trials are required for a clear understanding of the actual variability, particularly in the case of yield and quality characteristics as these are considerably influenced by soil and climatic factors. Generating more seedling progenies of turmeric cultivars may widen the scope of selecting better genotypes for commercial purposes.

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References

American Spice Trade Association (ASTA). 1968a. Official analytical methods 2nd ed., New York, pp. 8–9.
American Spice Trade Association (ASTA). 1968b. Official analytical methods 2nd ed., New York, p. 21.
American Spice Trade Association (ASTA). 1968c. Official analytical methods 2nd ed., New York, p. 38.
Chakravorti, A. K. 1948. Multiplication of chromosome number in relation to speciation of Zingiberaceae. Sci. Cul. **14**: 137–140.
Chattopadhyay, I., Biswas, K., Bandopadhyay, U. and Banerji, R. K. 2004. Turmeric and Curcumin: Biological actions and medicinal applications. Curr. Sci. **87**: 44–53.
Das, A. B., Rai, S. and Das, P. 1999. Karyotype analysis and cytophotometric estimation of nuclear DNA content in some members of the Zingiberaceae. Cytobios **97**: 23–33.
Govindarajan, V. S. 1980. Turmeric-chemistry, technology and quality. CRC Crit. Rev. Food Sci. Nutr. **12**: 199–301.
Gupta, P. K. 1997. Cytogenetics. Rastogi Publishers, Meerut, pp. 137–140.
Jones, R. E. and Bamford, R. 1942. Chromosome number in the progeny of triploid gladiolus with special reference to the contribution of the triploid. Am. J. Bot. **29**: 807–813.
Lad, S. K. 1993. A case of seed-setting in cultivated turmeric types (*Curcuma longa* Linn.). Journal of Soils and Crops **3**: 78–79.
Menon, R., Valsala, P. A. and Nair, G. S. 1992. Evaluation of open pollinated progenies of turmeric. S. Indian Hort. **40**: 90–92.
Nair, R. R. 2000. Cytogenetics and reproductive biology of major spices. In: Zachariah, T. J. and Krishnamurthy, K. S. (eds.) Ann. Rep. 2000–Indian Inst. of Spices Res. Calicut. pp. 38–39.
— and Sasikumar, B. 2009. Chromosome number variation among germplasm collections and seedling progenies in turmeric, *Curcuma lon*ga L. Cytologia **74**: 153–157.
—, —, and Ravindran, P. N. 1993. Polyploidy in a cultivar of black pepper (*Piper nigrum* L.) and its open pollinated progenies. Cytologia **58**: 27–31.
—, Dhamayanti, K. P. M., Sasikumar, B., Padmini, K. and Saji, K. V. 2004. Cytogenetics and reproductive biology of major spices—Final Report. In: Rema, J. (ed.): Annual Report 2003–2004–Indian Institute of Spices Research. Calicut. pp. 38–41.
Nambiar, M. C. 1979. Morphological and cytological investigations in the genus *Curcuma* L. Ph. D Thesis, University of Bombay. p. 95.
—, Pillai, P. K. T and Sarma, Y. N. 1982. Seedling propagation in turmeric (*Curcuma aromatica* Salisb.)
Nazeem, P. A., Menon, R. and Valsala, P. A. 1993. Blossom biological and hybridization studies in turmeric (*Curcuma* spp.). Indian Cocoa Arecanut and Spices Journal **16**: 106–109.
Pruthi, J. S. 1998. Spices and condiments, National Book Trust, New Delhi. pp. 244–250.
Purseglove, J. W., Brown, E. G., Green, C. L. and Robin, S. R. J. 1981. Turmeric. In: Spices, Vol. 2, Longman, New York. pp. 532–580.
Raghavan, T. S. and Venkatasubban, K. R. 1943. Cytological studies in family Zingiberaceae with special reference to chromosome number and cytotaxonomy. Proc. Indian Acad B Biol Sci **17**: 118–132.
Ramachandran, K. 1961. Chromosome numbers in genus *Curcuma* Linn. Curr. Sci. **30**: 194–196.
—, 1969. Chromosome number in zingiberaceae. Cytologia **34**: 213–221.
Ravindran, P. N., Babu, K. N. and Shiva, K. N. 2007. Botany and crop improvement of turmeric. In: Ravindran, P. N., Babu, K. N. and Sivaraman K. (eds.) Turmeric: the genus *Curcuma*, CRC Press, Boca Raton. pp. 16–70.
—, Nazeem, P. A. and Nybe, E. V. 2001. Response of turmeric (*Curcuma domestica* Val.) to *in vivo* and *in vitro* pollination. Journal of Spices and Aromatic Crops **10**: 135–139.
Sasikumar, B., George, J. K., Zachariah, T. J., Ratnambal, M. J., Babu, K. N. and Ravindran, P. N. 1996. IISR Prabha and IISR Prathibha-two new high yielding and high quality turmeric (*Curcuma longa* L.) varieties. Journal of Spices and Aromatic Crops. **5**: 41–48.
—, —, Saji, K. V. and Zachariah, T. J. 2005. Two new high yielding, high curcumin, turmeric (*Curama longa* L.) varieties–'IIRS-Kedaram' and 'IIRS Alleppey Supreme'. Journal of Spices and Aromatic Crops **14**: 71–74.
Sato, D. 1948. The karyotype and phylogeny of Zingiberaceae. Jpn. J. Genet. **23**: 44.
Sharma, A. K. and Bhattacharya, N. K. 1959. Cytology of several members of Zingiberaceae. Cellule **59**: 297–346.
Zhu, S., Song, J., Hu, Z., Tan, B., Xie, Z., Yi, H. and Deng, X. 2009. Ploidy variation and genetic composition of open-pollinated triploid citrus progenies. Bot. Stud. **50**: 319–324.