Degree of polyembryony among the accessions of *Commiphora wightii* collected from different natural habitats of India

Polyembryony, having more than one embryo in a single seed, was first discovered by Leeuwenhoek in 1719 in *Citrus*[1]. Later, various researchers reported and described it in detail[2–5]. The additional embryos in polyembryony are of either zygotic or non-zygotic origin and the latter is also known as adventitious polyembryony[6,7]. Adventive polyembryony is reported from a number of tropical trees species, viz. *Garcinia*[8], *Citrus*[9], *Shorea*[10], *Prunus*[11] and *Commiphora*[12]. The degree of polyembryony varies greatly among species and also within the cultivars of the same species as revealed in *Citrus*[13].

Nucellar originated polyembryony in guggul [*Commiphora wightii* (Arnott) Bhandari] was first reported by Gupta *et al.*[12]. Later, Prakash *et al.*[14] reported morphological details of guggul seeds along with seed viability, test seed weight and seed germination in relation to seed maturity.

Introduction of apomictic genes into hybrid plants has become a dream of present-day breeders all over the world. If the apomictic trait is associated with polyembryony, it would be an added advantage in increasing productivity of a genetically engineered apomictic hybrid. In the present paper we report the degree of polyembryony present within *C. wightii* germplasm.

C. wightii belonging to the family Burseraceae is a slow growing, highly branched plant that grows wild mainly in the arid rocky tracts of Rajasthan and Gujarat, India, and in Sindh and Baluchistan, Pakistan. The oleo-gum-resin produced by the plant is antiarthritic, hypocholesterolaemic and hypolipidaemic[15–17]. Overexploitation for resin extraction made the species status fall under the 'data deficient' category in the IUCN Red Data Book (1994).

The present study was carried out at the Directorate of Medicinal and Aromatic Plants Research (DMAPR), Anand, Gujarat. Thirty-two accessions collected from Gujarat and 21 accessions collected from Rajasthan were maintained in the field gene bank of DMAPR. Mature fruits from these accessions were used for the study.

The fruits are red when fully ripened and typically two-chambered drupes (occasionally three/four-chambered) with four ovules. Splitting of epicarp discloses the hard stone (putamen). The stone usually consists of a fertile one-seeded locule and a sterile locule, or both may be fertile and can easily be split into two halves (Figure 1 *a–c*). Well-developed seeds fill the entire cavity within the stony fruit wall and it is practically impossible to assess whether each half-fruit contains single or two seeds. Dissecting out the seeds often damages the thin testa and impedes germination. Hence in the present experiment, epicarp and mesocarp were removed by rubbing between the folds of cotton cloth and unfilled fruits (stones) were separated by floating on water. The stones settled at the bottom were considered filled and counted and expressed in percentage (Figure 1 *d* and *e*). Each filled stone was split into two halves and each stone half was

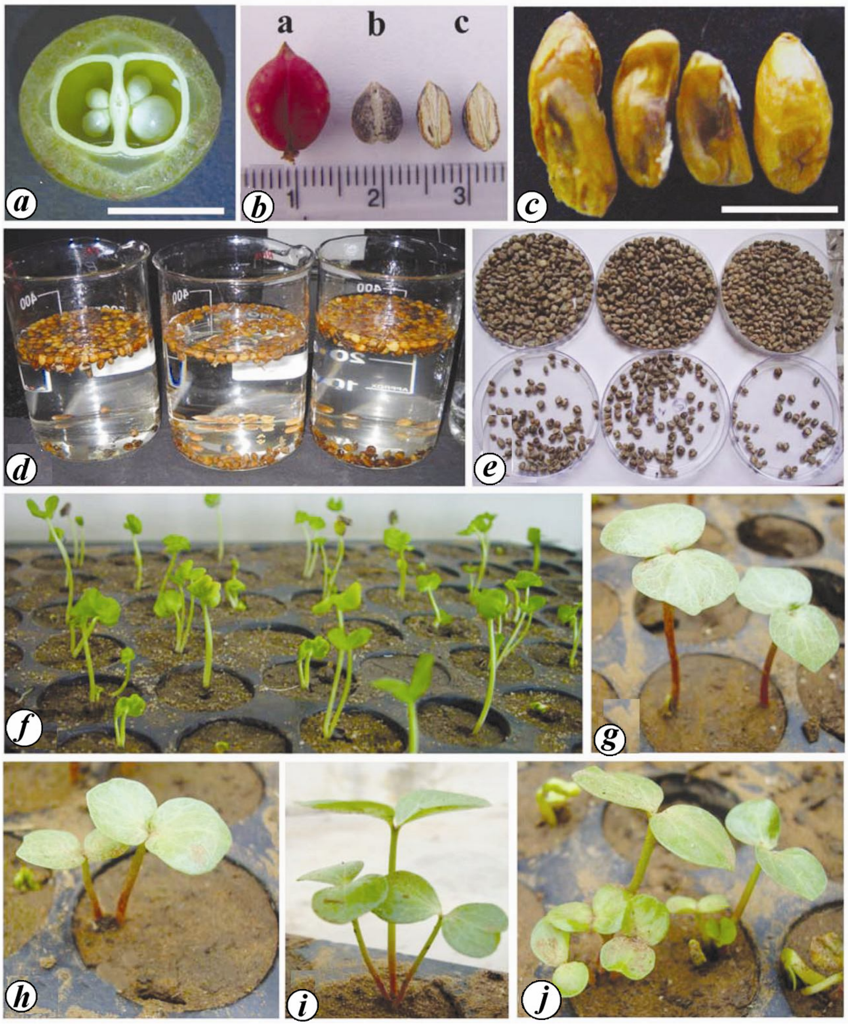

Figure 1. ***a***, Cross-section of developing fruit showing four ovules in different developmental stages; ***b***, Details of mature fruit: (a), Mature fruit; (b), Stone, i.e. fruit after removal of epicarp and mesocarp; (c), Two split halves of a stone. ***c***, Seeds dissected out from the stones; ***d***, Floating test of stones; ***e***, Stones sorted after floating test based on seed fill; ***f***, Germination tray of stones showing seedling emergence; ***g***, Single seedling emergence from both the half stone (indicating monoembryony); ***h***, Two seedlings emerging from a half stone and in the other half no seed germination; ***i***, Three seedlings emerging from a half stone and no seed germination in the other half (indicating polyembryony); ***j***, Four seedlings emerging from both the half stones (indicating polyembryony). Scale bar = 5 mm (for ***a*** and ***c***).

Table 1. Seed germination in guggul (*Commiphora wightii*) accessions collected from Gujarat

Accession	Fruit (stone) settled (%)	Fruit (stone) germination (%)	Frequency** (%)			
			One seedling per half stone	Two seedlings per half stone	Three seedlings per half stone	Four seedlings per half stone
DMAPR CW-1	14.02	34.94	47.62	38.10	9.52	4.76
DMAPR CW-2	64.85	2.02	66.67	0.00	33.33	0.00
DMAPR CW-3	5.41	87.50	29.17	29.17	41.67	0.00
DMAPR CW-7	2.94	17.14	12.50	75.00	12.50	0.00
DMAPR CW-8	35	78.57	50.00	27.27	13.64	9.09
DMAPR CW-9	8.13	12.50	0.00	100.00	0.00	0.00
DMAPR CW-11	9.70	50.00	50.00	50.00	0.00	0.00
*DMAPR CW-14	25.58	41.80	82.31	16.15	1.54	0.00
DMAPR CW-15	17.28	7.14	100.00	0.00	0.00	0.00
DMAPR CW-16	30.43	14.29	44.10	44.10	11.81	00.00
*DMAPR CW-17	64.52	60.00	51.25	28.75	17.50	2.50
DMAPR CW-20	42.86	65.38	86.67	13.33	0.00	0.00
DMAPR CW-21	35.13	24.91	92.40	7.60	0.00	0.00
DMAPR CW-23	16.54	40.91	91.67	8.33	0.00	0.00
DMAPR CW-26	5.13	7.32	50.00	50.00	0.00	0.00
*DMAPR CW-29	31.67	31.58	83.35	0.00	0.00	16.65
DMAPR CW-30	18.46	50.00	50.00	16.67	16.67	16.67
DMAPR CW-31	7.95	34.69	63.33	36.67	0.00	0.00
DMAPR CW-37	57.38	37.14	91.67	8.33	0.00	0.00
DMAPR CW-39	8.21	27.27	33.33	33.33	33.33	0.00
DMAPR CW-41	7.85	26.32	33.33	66.67	0.00	0.00
DMAPR CW-43	5.37	18.42	54.17	45.83	0.00	0.0
DMAPR CW-45	4.20	18.18	50.00	50.00	0.00	0.00
DMAPR CW-33-F7	9.47	25.00	100.00	0.00	0.00	0.00
DMAPR CW-33-F10	11.27	12.50	100.00	0.00	0.00	0.00
DMAPR CW-33-F20	33.33	17.65	83.33	16.67	0.00	0.00
DMAPR CW-33-F31	22.62	26.32	62.50	37.50	0.00	0.00
DMAPR CW-33-F32	23.08	66.67	100.00	0.00	0.00	0.00
DMAPR CW-46	6.16	55.56	60.00	40.00	0.00	0.00
DMAPR CW-48	3.08	16.67	100.00	0.00	0.00	0.00
DMAPR CW-75	8.33	22.22	75.00	0.00	25	0.00
DMAPR CW-76	5.71	87.50	100.00	0.00	0.00	0.00

*Hermaphrodite accessions; ** Sample size ($N = 50$).

considered as an individual germination unit. About 100–1500 fruits (since fruit filling varied among the accessions) were collected from each individual accession for seed germination.

Germination was carried out in germination trays. Two split halves of a single stone were placed per cavity filled with soil mixture in the germination tray. Seedlings emergence from each half stone was counted. Emergence of more than two seedlings from any one of the two half stones was taken as presence of polyembryony in a single fruit. Observations were also taken on the number of seedlings developed from individual half stones and also its frequency within an accession for expressing polyembryony among the accessions. Percentage of seed germination was calculated based on germinated fruits only (Tables 1 and 2).

Stone filling varied from 2.94% to 64.85%. Maximum stone filling was seen in accession DMAPR CW2 (64.85%) and minimum in DMAPR CW7 (2.94%). Endosperm is transitory and absorbed during embryo development in guggul. Gupta *et al.*[12] reported that in *C. wightii*, nearly 50% of the developing fruits lack embryonated seed formation which may be associated with the lack of endosperm development or its early breakdown. The present study also revealed that there is genotypic variability among the germplasm for embryonated seed development.

Seed germination started from the fifth day onwards and was completed by the eighth day (Figure 1*f*). Seed germination ranged from 2.02% to 87.50%. Highest seed germination was found in DMAPR CW3 and DMAPR CW50 (87.50%) and lowest in DMAPR CW2 (2.02%) (Tables 1 and 2). However, no direct correlation was found between fruit filling and seed germination percentage. This may be due to the defective embryo/endosperm formation, even though the fruits were filled in majority of the accessions.

The number of seedlings developed from individual half stones varied from 1 to 4 (Figure 1*g–j* respectively). DMAPR CW3 showed the highest polyembryony, wherein 41.67% of the germinated stones showed multiple seedling emergence per half stone. Monoembryonic seed germination was observed in six accessions, i.e. DMAPR CW15, DMAPR CW-33-F7, DMAPR CW-33-F10, DMAPR CW-33-F32, DMAPR CW-48 and DMAPR CW-76, all collected from Gujarat.

Prakash *et al.*[14] reported up to 36.25% seed germination in *C. wightii*. However, they had taken the entire stone as a single germination unit and emergence of multiple seedlings in this case did not confirm polyembryony, since the entire

Table 2. Seed germination in guggul (*C. wightii*) accessions collected from Rajasthan

Accession	Fruit (stone) settled (%)	Fruit (stone) germination (%)	Frequency* (%)			
			One seedling per half stone	Two seedlings per half stone	Three seedlings per half stone	Four seedlings per half stone
DMAPR CW-49	7.87	41.09	66.21	27.58	6.21	0.00
DMAPR CW-50	18.61	40.71	43.75	18.75	0.00	37.50
DMAPR CW-51	11.49	37.25	55.00	36.67	8.33	0.00
DMAPR CW-52	6.46	54.00	68.75	31.25	0.00	0.00
DMAPR CW-53	7.15	23.13	50.00	47.06	2.94	0.00
DMAPR CW-54	13.60	40.98	73.93	20.62	1.72	3.72
DMAPR CW-55	12.93	31.58	50.00	50.00	0.00	0.00
DMAPR CW-56	4.10	20.83	75.00	16.67	8.33	0.00
DMAPR CW-57	17.53	19.61	80.00	10.00	10.00	0.00
DMAPR CW-58	9.44	28.00	92.86	7.14	0.00	0.00
DMAPR CW-59	7.91	65.31	83.33	16.67	0.00	0.00
DMAPR CW-60	5.86	16.00	90.00	10.00	0.00	0.00
DMAPR CW-61	16.67	13.04	62.50	37.50	0.00	0.00
DMAPR CW-62	10.24	17.65	50.00	0.00	50.00	0.00
DMAPR CW-63	6.21	34.78	79.17	16.67	4.17	0.00
DMAPR CW-64	8.87	36.36	33.33	66.67	0.00	0.00
DMAPR CW-66	7.43	38.71	40.00	60.00	0.00	0.00
DMAPR CW-67	7.61	26.67	66.00	33.33	0.00	0.00
DMAPR CW-68	5.88	63.16	68.57	31.43	0.00	0.00
DMAPR CW-70	18.52	10.00	0.00	100.00	0.00	0.00
DMAPR CW-72	3.15	25.00	50.00	50.00	0.00	0.00

*Sample size ($N = 50$).

Table 3. Extent of single, two, three and four seedlings emerging among guggul (*C. wightii*) accessions

Category	Gujarat accessions (%)	Rajasthan accessions (%)
Polyembryonic accessions, i.e. accessions showing three or four seedlings per half stone	22.64	16.98
Polyembryonic/monoembryonic accessions, i.e. accessions showing two seedlings per half stone	26.42	22.64
Monoembryonic accessions, i.e. accessions showing single seedling per half stone	11.32	0

stone has the potential of developing four seeds even in the absence of polyembryony. However, in the present study, half stone was used as an individual germination unit and hence, emergence of more than two seedlings from a half stone could occur only in the case of polyembryony. The result thus showed that among 53 accessions, 21, i.e. 39.62% of the studied accessions had confirmed polyembryony (Table 3). However, in 26 accessions half stones showed the emergence of two seedlings and it was difficult to assess whether they were developed from single ovule or from the two different ovules. The species being a facultative apomict, there is the possibility of sexual embryo and apomictic embryo co-existing, sharing the same endosperm, or both the seedlings may have originated from a single ovule or from two different ovules. Thus the mature seed may have one sexual embryo and one/more apomictic embryos or both are apomictic embryos[18]. Further study is in progress to separate the seedlings of sexual and apomictic origin using molecular markers in those accessions where two seedlings per half stone had developed, so that more polyembryonic accessions can be selected out from the germplasm.

It is also noteworthy to mention here that all the monoembryonic accessions are collected from dioecious populations and are sexual in nature[18]. Further, it was also found that all the three hermaphrodite accessions, even though there was assured pollen grain availability, invariably showed polyembryonic seed germination having more than 15% polyembryonic seed frequency.

Accession DMAPR CW3 is promising in terms of expression of polyembryonic seedling formation and can be targeted in apomictic studies. Information on successful seed germination with different degrees of polyembryony in the present study would be useful for conserving this critically endangered species.

1. Webber, J. M., *Bot. Rev*., 1940, **6**(11), 575–598.
2. Strasburger, E., *Jenaische Zf. Naturwiss*., 1878, **12**, 647–667.
3. Braun, A., *Abh. Konigl. Akad. Wiss. Berlin. Phys. Kl.*, 1859, pp. 107–263.
4. Maheshwari, P., *Palaeobotanist*, 1952, **1**, 319.
5. Tisserat, B., Esen, E. B. and Murashige, T., *Hortic. Rev*., 1979, **1**, 1–72.
6. Maheshwari, P., *An Introduction to the Embryology of Angiosperms*, McGraw Hill, New York, 1950.
7. Richards, A. J., *Plant Breeding Systems*, Chapman & Hall, London, UK, 1986, 2nd edn, p. 529.
8. Maguire, B., *Taxon*, 1976, **25**, 241–244.
9. Simmonds, N. W., In *Evolution of Crop Plants* (ed. Simmonds, N. W.), Longmans, London, 1976, pp. 104–108.

10. Kaur, A., Ha, C., Sands, V. E. and Soepadmo, E., *Bot. J. Linn. Soc.*, 1986, **92**, 78–88.
11. Martinez-Gomez, P. and Gradziel, T. M., *Sex. Plant Reprod.*, 2003, **16**, 135–139.
12. Gupta, P., Shivanna, K. R. and Mohan Ram, H. Y., *Ann. Bot.*, 1996, **78**, 67–72.
13. Frost, H. B. and Soost, R. K., In *The Citrus Industry* (eds Reuther, W., Batchelor, L. D. and Webber, H. B.), University of California, Berkeley, 1968, vol. 2, pp. 290–324.
14. Prakash, J., Kasera, P. K. and Chawan, D. D., *Curr. Sci.*, 2000, **78**(10), 1185–1187.
15. Satyavati, G. V., In *Economic and Medicinal Plant Research* (eds Wagner, H. and Farnsworth, N. R.), Academic Press, London, 1990, vol. 4, pp. 39–56.
16. Chopra, R. N., Nayar, S. L. and Chopra, I. C., *Glossary of Indian Medicinal Plants*, Council of Scientific & Industrial Research, New Delhi, 1956.
17. Chopra, R. N., Chopra, I. C., Handa, K. L. and Kapur, L. D., *Chopra's Indigenous Drugs of India*, M/S U.N. Dhur, Calcutta, 1958, 2nd edn.
18. Geetha, K. A. *et al.*, *Trees*, 2013, **27**, 567–581.

ACKNOWLEDGEMENTS. Financial support for this work was provided by the Indian Council of Agricultural Research under the National Agricultural Innovation Project in the form of fellowship provided to the first author.

Received 22 April 2014; revised accepted 26 June 2014

AARTI KAWANE[1]
K. A. GEETHA[1,*]
M. N. REDDY[2]
SATYABRATA MAITI[1]

[1]*Directorate of Medicinal and Aromatic Plants Research,*
Boriavi,
Anand 387 310, India
[2]*Veer Narmada South Gujarat University,*
Surat 395 007, India
**For correspondence.*
e-mail: geethaka99@yahoo.com

Uranium occurrence in Proterozoic Chilpi Group, near Kanhari, Kawardha district, Chhattisgarh

Proterozoic Chilpi Group of rocks were deposited in a 30 km long and 4–7 km wide N–S zone along the northwestern margin of Chhattisgarh Basin[1]. Paleo–Meso Proterozoic Chilpi Group overlies Paleoproterozoic Nandgaon Group of rocks in the east and Paleoproterozoic Malanjkhand granitoids in the west with pronounced unconformity marked by a conglomerate horizon at the base. The Group comprises sediments deposited in a cratonic rift or island arc-related basin and consists of conglomerate, coarse arenite (grit), shale and quartzite[2]. These are well exposed in Kanhari, Koyalarjhori, Mohagaon and Chilpi Ghat section. The Chilpi Group of rocks are unconformably overlain by the unmetamorphosed argillaceous, arenaceous and calcareous sediments of Mesoproterozoic Chhattisgarh Supergroup (Figure 1).

Several NNW–SSE, NW–SE and E–W faults have dissected both the basement rocks and overlying sediments of Chilpi Group in parts of Kawardha district. A NNW–SSE trending fault, SE of Kanhari village (Figures 1 and 2) has affected both Nandgaon bimodal volcanic sequence and Chilpi sediments, and shows development of slickenside, brecciation, fault gouge and intense fracturing (Figure 1). Various sedimentary structures such as bedding, colour banding, ripple marks and penecontemporaneous deformation structures are recorded in Chilpi

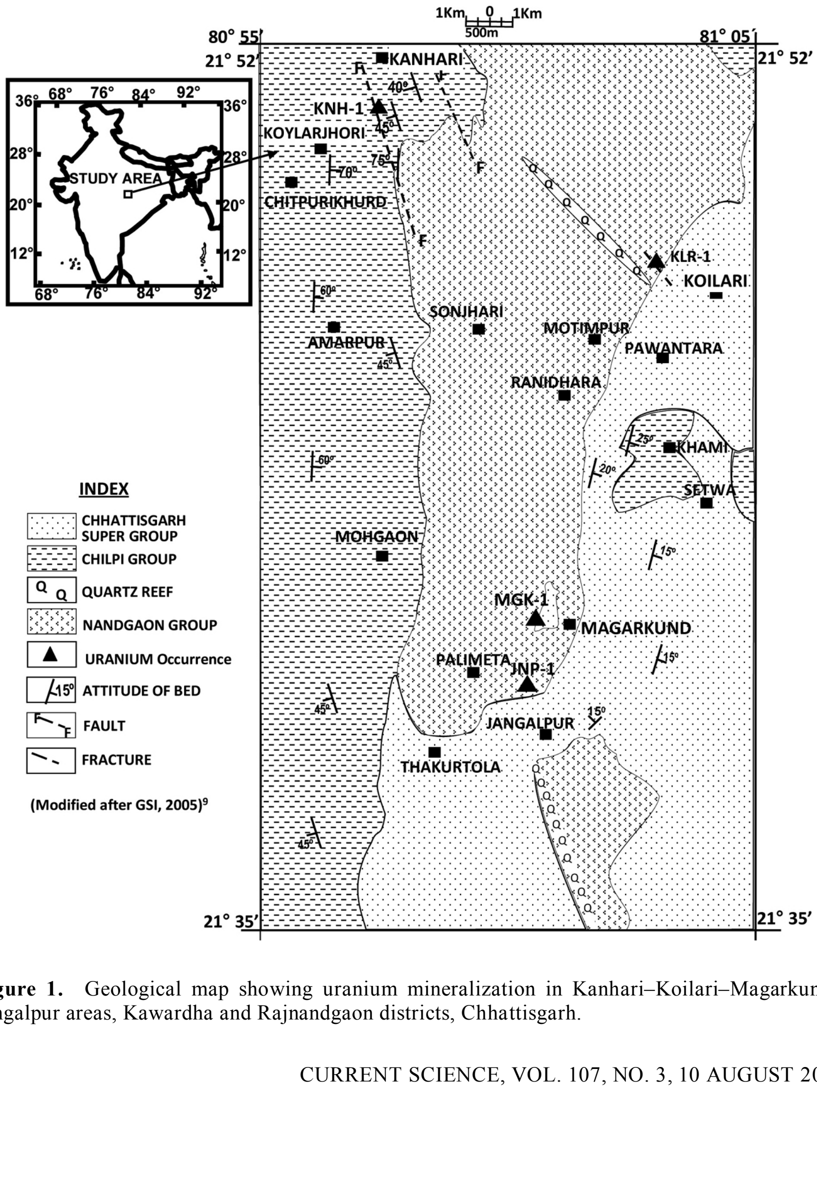

Figure 1. Geological map showing uranium mineralization in Kanhari–Koilari–Magarkund–Jangalpur areas, Kawardha and Rajnandgaon districts, Chhattisgarh.