

ramet with the mother ramet is helpful during disturbance (e.g. herbivory) or patchy resource supply, and connected ramets may 'help each other out' and are not completely removed if all photosynthesizing aerial shoots are removed from any ramet. However, clonal growth is not a complete substitute for sexual reproduction. Rather, it is an additional means to overcome adversities<sup>17</sup>. Location of individuals on rock faces indicate that the species utilizes seeds for spreading new individuals to new niches on rock faces, where they establish and later adopt space-holder strategy<sup>17</sup> for survival.

Presently efforts are on to collect seeds of the species to raise seedlings to be transplanted to the similar microhabitats in protected areas of alpine zones of Garhwal Himalaya.

1. Nayar, M. P., *Bull. Bot. Surv. India*, 1977, **19**, 145–154.
2. Rao, R. R., *Biodiversity in India (Floristic Aspect)*, Bishen Singh Mahendra Pal Singh, Dehradun, 1994.
3. Balakrishnan, N. P., *Flora of India Introductory Volume* (eds Hajra, P. K. et al.), BSI, Kolkata, 1996, pp. 197–204.
4. Khoshoo, T. N., *Curr. Sci.*, 1995, **69**, 14–17.
5. Khoshoo, T. N., *Plant Diversity in the Himalaya: Conservation and Utilization*, GBPI-HED, Almora, 1992.

6. Singh, D. K. and Hajra, P. K., *Changing Perspectives of Biodiversity Status in the Himalaya* (eds Gujral, G. S. and Sharma, V.), British High Commission, New Delhi, 1996, pp. 23–38.
7. Mani, M. S., *Ecology and Phytogeography of High Altitude Plants of the Northwest Himalaya*, Oxford and IBH, New Delhi, 1978.
8. Rawat, D. S., Bhandari, B. S. and Gaur, R. D., *Garhwal Himalaya: Nature, Culture and Society* (eds Kandari, O. P. and Gusain, O. P.), Trans Media, Srinagar-Garhwal, 2001, pp. 69–92.
9. Majumdar, N. C., *Blumea*, 1980, **26**, 445–448.
10. Sharma, B. D. and Balakrishnan, N. P. (eds), *Flora of India* (Vol. 2), BSI, Kolkata, 1993.
11. Naithani, B. D., *Flora of Chamoli* (Vol. 1), BSI, Howrah, 1984.
12. Gaur, R. D., Rawat, D. S. and Dangwal, L. R., *J. Econ. Taxon. Bot.*, 1995, **19**, 9–26.
13. Nayar, M. P. and Sastry, A. R. K., *Red Data Book of Indian Plants*, Vol. I, BSI, Howrah, 1987, pp. 109–110.
14. Rawat, D. S. and Gaur, R. D., *Biodiversity Conservation* (ed. Trivedi, P. C.), 2007, in press.
15. Dhar, U., *Curr. Sci.*, 2002, **82**, 141–148.
16. Billings, W. D., *Arct. Alp. Res.*, 1974, **6**, 129–142.
17. Korner, C., *Alpine Plant Life*, Springer-Verlag, Berlin, 1999.

ACKNOWLEDGEMENTS. We are grateful to the Officer-in-Charge, G.B. Pant University

of Agriculture and Technology, HREC, Srinagar-Garhwal for providing laboratory facilities, and authorities of FRI, Dehradun for herbarium consultation. We also thank Dr A. K. Naithani, H.N.B. Garhwal University, Srinagar-Garhwal for his help during the preparation of the manuscript.

Received 19 September 2006; accepted 9 February 2007

D. S. RAWAT<sup>1,3,\*</sup>  
CHARAN SINGH RANA<sup>2</sup>

<sup>1</sup>G.B. Pant University of Agriculture and Technology,

Horticulture Research and Extension Centre,

Srinagar-Garhwal 246 174, India

<sup>2</sup>Herbarium and Plant Systematics Laboratory,

Department of Botany,

H.N.B. Garhwal University,

Srinagar-Garhwal 246 174, India

<sup>3</sup>Present address:

Department of Biological Science,

College of Basic Sciences and

Humanities,

G.B. Pant University of Agriculture

and Technology,

Pantnagar 263 145, India

\*For correspondence.

e-mail: dr\_dsrawat@yahoo.com

## Occurrence of zygotic twin seedlings in mandarin orange plants of the northeastern Himalayan region

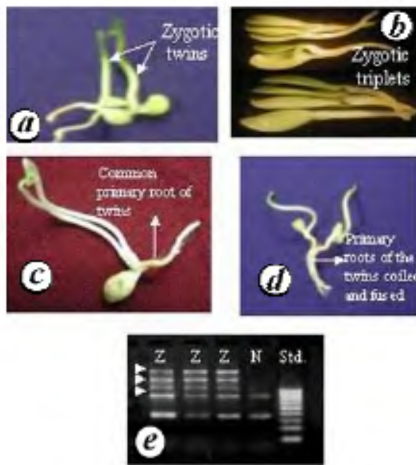
Poly-embryony commonly occurs in citrus<sup>1</sup>. Existence of multiple nucellar embryos in the seeds of a particular citrus population was reported earlier<sup>2</sup>. However, similar studies on multiple zygotic embryos in a seed developed through the cleavage of the original zygote or zygotic embryo<sup>3</sup> are rare. In a natural cross-pollinated population of citrus, embryos of zygotic origin produce heterogeneous segregated population and those of nucellar origin produce progenies identical to the mother plant. It is normally expected that from a polyembryonic citrus seed the zygotic embryo will produce a single seedling and multiple nucellar embryos will produce more than one nucellar seedling. So roguing out the off-types (zygotic) seed-

lings from the raised population may create true to the mother-type nucellar progeny<sup>4</sup>. This expectation may fail if the particular plant population has the inherent nature of producing zygotic twins or triplets. There is no precise scientific method for identification of nucellar and zygotic seedlings from a raised seedling population. DNA polymorphism analysis of the seedlings by RAPD and other markers could confirm the differences between them<sup>5</sup>.

Seeds from twenty-eight mandarin orange (*Citrus reticulata* Blanco) plants from ten different locations of Darjeeling Hills in West Bengal, Upper and Lower Assam and West Khasi Hills in Meghalaya, northeastern Himalayan region, India

were collected. Sterilized seeds were incubated in moist germinating plate and allowed for germination for 5–7 days. The germinating nucellar and zygotic embryos were identified following the procedure standardized by Tisserat<sup>6</sup> and their numbers were counted. The seedlings were grown in sterile soil:sand:organic matter mixture (2:1:1) for further observation. Seedlings of different origin were tested for their RAPD profiling with three primers selected from a set of 15, on the basis of reproducibility of bands and their efficiency of differentiation<sup>7</sup>.

Occurrence of more than one embryo within a mature seed was a common phenomenon in all the 28 selected plants.



**Figure 1.** *a*, Zygotic twin seedlings developing from a seed. *b*, Zygotic triplets, division and sharing of two original cotyledons of seeds by the triplets. *c*, Siamese twin seedlings having common radicle or primary root. *d*, Coiling and fusion of two primary roots of closely developed twins. *e*, RAPD profile of three zygotic triplet seedling and one nucellar seedling obtained from a single seed of a mandarin orange plant collected from Mirik, Darjeeling District, using the primer OPAL4 (ACAACGGTCC). N and Z represent nucellar and zygotic seedlings. (►) Position of extra amplified fragments found in zygotic triplet seedlings.

In addition to the presence of normal zygotic and nucellar embryos, in 24 plants twin and triplet zygotic embryos were also observed in the seeds. In these plants up to 30% of the seedlings were recorded either as zygotic twins or triplets embryos. These embryos were developed by fission of the original zygotic embryo as

the original two cotyledons of a zygotic embryo of the seed were also divided and shared among the twins or triplets during germination<sup>3</sup> (Figure 1 *a* and *b*). During the development of the twin embryos into seedlings, some extraordinary morphological features were observed. Some identical twin seedlings showed a common primary root (Figure 1 *c*). The twin embryos in this case germinated with common hypocotyls and primary root, but the two epicotyls were distinctly separated from the cotyledonary node. This type of development of embryos into plants was comparable with the development of Siamese twins. In other cases, during germination the two hypocotyls and sometimes also the epicotyls of the identical twins were fused. Sometimes the radicles of the twins rolled over each other from the point of transition node to ultimately fuse as the primary root common for both twin seedlings. This was interpreted as anastomosis-like development of the identical twins (Figure 1 *d*).

RAPD amplifications of genomic DNA from zygotic seedlings (twins or triplets) usually had one or more extra bands than those of the nucellar seedlings. As shown in Figure 1 *e*, all the triplet zygotic seedlings developed from a seed of a plant collected from Mirik, Darjeeling District had three extra DNA amplified fragments (above 1000 bp). This was absent in the single nucellar seedling developed from the same seed. The nature of producing a large number of zygotic twins of mandarin orange plants in this region might be one of the major causes of the wide ge-

netic variation existing within this natural population<sup>8</sup>.

1. Parlevliet, J. E. and Cameron, J. W., *Proc. Am. Soc. Hortic. Sci.*, 1959, **74**, 252–260.
2. Das, A., Mandal, B., Paul, A. K. and Chaudhuri, S., *J. Interacad.*, 2003, **7**, 343–346.
3. Frost, B. H. and Soost, K. R., *The Citrus Industry* (eds Reuther, W., Batchelor, L. D. and Webber, H. J.), University of California, Division of Agricultural Science, 1968, vol. 2, pp. 292–320.
4. Khan, J. A. and Roose, M. L., *J. Am. Soc. Hortic. Sci.*, 1988, **113**, 105–110.
5. Bastianel, M., Schwarz, S. F., Coletta-Filho, H. D., Lin, L. L., Machado, M. and Koller, O. C., *Genet. Mol. Biol.*, 1998, **21**, 123–127.
6. Tisserat, B., *Plant Cell Culture, A Practical Approach* (ed. Dixon, R. A.), IRL Press, Oxford, 1985, pp. 79–104.
7. Das, A., Mandal, B., Sarkar, J. and Chaudhuri, S., *J. Hortic. Sci. Biotechnol.*, 2004, **79**, 850–854.
8. Das, A., Mandal, B., Sarkar, J. and Chaudhuri, S., *PGR Newsl.*, 2005, **143**, 35–39.

Received 3 May 2006; revised accepted 20 February 2007

A. DAS\*  
BIDISHA MANDAL  
J. SARKAR  
S. CHAUDHURI

Department of Plant Physiology,  
Faculty of Agriculture,  
Bidhan Chandra Krishi Viswavidyalaya,  
Nadia 741 252, India

\*For correspondence.  
e-mail: anianiruddha@vsnl.net

## Bioaccumulation of heavy metals in some commercial fishes and crabs of the Gulf of Cambay, India

Accumulation of heavy metals in marine ecosystems is of global importance. Metals generally enter the aquatic environment through atmospheric deposition, erosion of geological matrix or due to anthropogenic activities caused by industrial effluents, domestic sewage and mining wastes<sup>1,2</sup>. The metal contaminants in aquatic systems usually remain either in soluble or suspension form and finally tend to settle down to the bottom or are taken up by the organisms. The progressive and irreversible accumulation of these metals

in various organs of marine creatures ultimately leads to metal-related diseases in the long run because of their toxicity, thereby endangering the aquatic biota and other organisms<sup>3–6</sup>. Fishes being one of the main aquatic organisms in the food chain, may often accumulate large amounts of certain metals<sup>7,8</sup>. Essentially, fishes assimilate these heavy metals through ingestion of suspended particulates, food materials and/or by constant ion-exchange process of dissolved metals across lipophilic membranes like the gills/adsorption

of dissolved metals on tissue and membrane surfaces.

The Gulf of Cambay, India also known as the Estuarine Delta due to the convergence of many popular rivers of diverse nature like Sabarmati, Mahi, Narmada, Dhandhar, Tapti and Vishwamitri, comprises of a special ecosystem consisting of mud flats, dunes and scattered sandy beaches<sup>9</sup>. It receives discharges (domestic and industrial effluents) in significant quantities due to extensive industrial development and urbanization, including one