the core site which is enriched in chlorite, smectite, illite and kaolinite in diminishing order (Figure 3). Based upon the above observations, we propose that the sediment supply to the eastern Bay of Bengal, close to Burma and the eastern Andaman sea, was dominantly from the east (NE Himalayas, northern Burma and Andaman region) rather than from north (Ganga–Brahmaputra system). The abundance of smectite in these sediments, a characteristic product of volcanic rocks, source of which lies in proximity (the Burmese arc and Andaman Islands) further gives credence to our proposition of proximity of the source.

The silt contents at 60–65 cm and reappearance of heavy minerals at 40 cm (at 14 and 10 ky^{-1}) may be related to the oscillating sea level still stands during this time[25,31]. As the content of the clay size component is uniformly high in Holocene section, we interpret an enhanced humid phase associated with stronger monsoon and rapid sea level rise, resulting in a landward recession of sources after Younger Dryas, and corresponding reduction in the gravity-induced sedimentation to the depositional basin.

In conclusion, (i) significant variations exist in the intensity of the monsoon since Late Pleistocene and at least two dominant arid phases at Pleistocene–Holocene boundary and LGM are identified. During Holocene, the intensity of monsoon was stronger over NE India, part of Burma and Andaman Islands, and (ii) the terrigenous input to the eastern Bay of Bengal is dominantly from NE Himalayas, Andamans and Burma.

1. Sarin, N. M., Borole, D. V. and Krishnaswami, S., *Proc. Indian Acad. Sci.*, 1979, **88**, 131-154.
2. Fontugne, M. R. and Duplessy, J. C., *Palaeogeogr. Palaeoclimatol. Palaeoecol.*, 1986, **56**, 69-88.
3. Foucault, A. and Fang, N., *C. R. Acad. Sci. Paris*, 1987, Serie II, 1383-1388
4. Chauhan, O. S., Mascarenhas, A., Paropkari, A. L. and Rao, CH. M., in *Oceanography of the Indian Ocean* (ed Desai, B N), Oxford-IBH Publ. Co., Bombay, 1992, pp. 479-486.
5. Birkland, P. W., *Pedology, Weathering and Geomorphological Research*, Oxford University Press, London, 1974, pp. 255.
6. Biscaye, P. E., *Bull. Geol. Soc. Am.*, 1965, **76**, 803-832.
7. Griffin, R. E., *Clay Mineralogy*, McGraw Hill, New York, 1968, pp 565.
8. Weaver, C. E., *Clays, Muds and Shales*, Elsevier, Amsterdam, 1989, pp. 819.
9. Prell, W. L., Hutson, W. H., Williams, D. F., Be, A. W. H., Geitzenauer, K. and Molfino, B., *Quat. Res.*, 1980, **14**, 309-336.
10. Curray, J. R. and Moore, D. J , in *The Geology of the Continental Margins* (eds. Burk, C. A. and Drake, C L.), Springer-Verlag, New York, 1974, pp. 617–639.
11. Emmel, F. J. and Curray, J. R., *Geo-Mar. Let.*, 1984, **3**, 119-124.
12. Folk, R. L., *Sedimentology*, 1966, **6**, 73-93.
13. Carrol, D., *Clay minerals: A Guide to their X-ray Identification*, Geological Society of America, special paper, 1970, pp. 80.
14. Krishnaswami, S. and Sarin, N. M., *Anal. Chim. Acta*, 1976, **83**, 143-146.
15. Borole, D. V., Unpublished Ph D Thesis, 1980, pp. 155.
16. Krishnaswami, S., *Geochim. Cosmochim. Acta*, 1976, **40**, 425-434.
17. Passega, R., *J. Sediment. Petrol.*, 1964, **34**, 830-847.
18. Whitehouse, U. G., Jefferey, L. M. and Debbrecht, J. D., in *Clay and Clay Minerals*, Proceedings of Seventh National Conference, (ed. Swineford, A.), Pergamon Press, London, 1960, pp. 1–79.
19. Stanley, D. J. and Liyange, A. N., *Mar. Geol.*, 1986, **73**, 263-283.
20. Sirocko, F. Sarnthein, M., Lange, H. and Erlenkeuser, H , *Quat. Res.*, 1991, **86**, 72-93.
21. Prell, W. L. and Hutson, W. H., *Science*, 1979, **206**, 454-456.
22. Broecker W. S., Peteet, D. M. and Rind, R., *Nature*, 1985, **315**, 21-26.
23. Boyle, E. A. and Keigwin, L., *Nature*, 1987, **330**, 35-40.
24. Stoddart, D. R., in *Regional Variation in Indian Ocean Coral Reefs* (eds. Stoddart, D. R. and Younge, M.), Academic Press, New York, 1971, pp. 3–38.
25. Fairbanks, R. G., *Nature*, 1989, **342**, 637-642.
26. Banerjee, A. and Sengupta, R., in *Recent Geoscientific Studies in the Bay of Bengal and the Andaman Sea*, Geological Survey of India special publication, 1992, pp. 163–170.
27. Krishnan, M. S., *Geology of India and Burma*, Higginbothams Limited, Madras, 1968, pp. 536.
28. Haldar, D., Laskar, T., Bandyopadyay, P. C., Sarkar, N. K. and Biswas, J. K., *J. Geol. Soc. India*, 1992, **39**, 411-419.
29. Kolla, V. and Rao, N. M., *Geo-Mar. Let.*, 1990, **10**, 129-136.
30. Chowdary, G. V. V. S., Ramana, Rao, S. V. and Swamy, A. S. R., in *Recent Geoscientific Studies in the Bay of Bengal and the Andaman Sea*, Geological Survey of India special publication 1982, pp. 197–200.
31. Fairbridge, R. W., *Sci. Am.*, 1962, **202**, 70-79

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Plant regeneration from internode segments of *Cucurbita maxima* Duch. × *Cucurbita moschata* Duch.

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High frequency shoot formation from internode explants of a hybrid (*Cucurbita maxima* × *C. moschata*) squash was achieved on Murashige and Skoog's medium. Explanted internodal sections were induced to develop multiple shoots through direct regeneration without intervening callus phase. Maximum frequency of shoot bud formation was obtained when MS medium was supplemented with 4.4 μM BA and 0.54 μM NAA. Only one subculture of shoot bud producing explants to the same nutrient combination was required for development of shoots from shoot buds. Rooting of *in vitro* regenerated shoots was obtained in half strength MS medium with 0.54 μM NAA.

DIFFERENTIATION leading to the formation of plantlet in tissue cultures derived from plants of many families has been reported. The plants of Cucurbitaceae provide a major portion of vegetables and they need to be investigated for maximum utilization. In *Cucurbita*

pepo[1], watermelon[2] and cucumber[3–5], plantlet formation from *in vitro*-grown tissues has been reported. A hybrid between *Cucurbita maxima* × *Cucurbita moschata* is an economically important crop among the vegetable species that is now becoming popular in Bangladesh. There have been no reports showing plant regeneration of this hybrid squash. The present work describes a technique that allows the production of multiple shoots from seedling-derived internode explants of *C. maxima* × *C. moschata.*

Internodal segments (4–5 mm in length) were excised from 4-week-old aseptically grown seedlings and cultured on MS medium[6] containing 0.54–4.4 μM of 6-benzyladenine (BA), 2,4-dichlorophenoxyacetic acid (2,4-D) and α-naphthaleneacetic acid (NAA). Growth regulators were used either individually or in certain combinations. The effects of casein hydrolysate (CH 100 mg l^{-1}) and coconut milk (CM 15%) on adventitious bud initiation were tested by adding them separately. Morphogenic response of the explants was recorded after four weeks of culture. For shoot differentiation, the explants that developed shoot buds were subcultured in the same nutrient medium after five weeks of incubation on primary culture medium. Proliferated calluses from two preculture media were also subcultured to see their regenerating ability. The number of adventitious shoots per explant was recorded after four weeks of subculture. *In vitro* raised shoots were rooted in half-strength MS medium supplemented with 0.54 μM NAA. The pH of the medium was adjusted to 5.8 before sterilization; media were solidified with 0.7% Difco bacto-agar. The explants were cultured singly in 150 × 25 mm culture tubes each containing 25 ml of culture medium fitted with cotton plugs and maintained at 25 ± 2° C under fluorescent light of about 50 $\mu Em^{-2} s^{-1}$ for 16 h per day.

Three kinds of response from explanted internodal tissues were noticed: formation of callus, root or shoot bud formation. In most of the treatment combinations the explants underwent callogenesis. More than 70% explants produced callus in 2,4-D containing media and maximum frequency was observed when CM was added (Table 1). The calluses in these media were translucent to opaque in appearance and milky white to yellow or yellow green in colour and appeared as loosely packed friable regions of vacuolated cells that produced more callus and sometimes roots. The surface of the callus was often shiny. NAA singly or in combination with BA also produced callus but at lower frequency. The calluses produced in NAA containing media were compact and light green in colour. Generally it was observed that 2,4-D or NAA in combination with BA yielded more callus.

The explants underwent rhizogenesis mostly in NAA-supplemented media and maximum rooting frequency was observed when NAA was used alone (Table 1). Shoot bud differentiation occurred only in two combinations, 4.4 μM BA + 0.54 μM NAA (Figure 1 *a*) and 4.4 μM BA + 0.54 μM NAA + 15% CM. The shoot buds appeared after two weeks of culture and oozed out on the entire surface of the explants as knob-like structures, loosely arranged side by side. Maximum

Table 1. Effects of different growth regulators on morphogenetic response of internode explants (20 replicates/treatment)

Growth regulator (μM)	Other addenda[a]	Callus-inducing explants (%)	Root-inducing explants (%)	Callus- and root-inducing explants (%)	Shoot bud-inducing explants (%)	No. of shoot buds per explant
4.4 μM NAA	–	20	60	10	–	–
4.4 μM NAA	CH	20	50	5	–	–
4.4 μM NAA	CM	30	55	5	–	–
4.4 μM 2,4-D	–	75	–	10	–	–
4.4 μM 2,4-D	CH	70	10	10	–	–
4.4 μM 2,4-D	CM	85	–	–	–	–
4.4 μM BA	–	–	–	–	–	–
4.4 μM BA	CH	10	15	10	–	–
4.4 μM BA	CM	40	–	–	–	–
4.4 μM BA + 0.54 μM NAA	–	15	–	10	55	42
4.4 μM BA + 0.54 μM NAA	CH	20	30	20	–	–
4.4 μM BA + 0.54 μM NAA	CM	40	–	–	25	30
4.4 μM BA + 0.54 μM 2,4-D	–	80	–	–	–	–
4.4 μM BA + 0.54 μM 2,4-D	CH	75	10	–	–	–
4.4 BA + 0.54 μM 2,4-D	CM	85	–	–	–	–

[a]CH, 100 mg l^{-1}; CM, 15%.

Table 2. Response of shoot buds after subculturing the internode explants

Explant type	Preculture and subculture media (μM)	Average number of shoots per explant	Average number of rootable shoots per explant
Shoot bud-forming explants	4.4 μM BA + 0.54 μM NAA	31	21
	4.4 μM BA + 0.54 μM NAA + CM	17	11
Callus	4.4 μM BA + 0.54 μM 2,4-D	–	–
	4.4 μM BA + 0.54 μM 2,4-D + CM	–	–

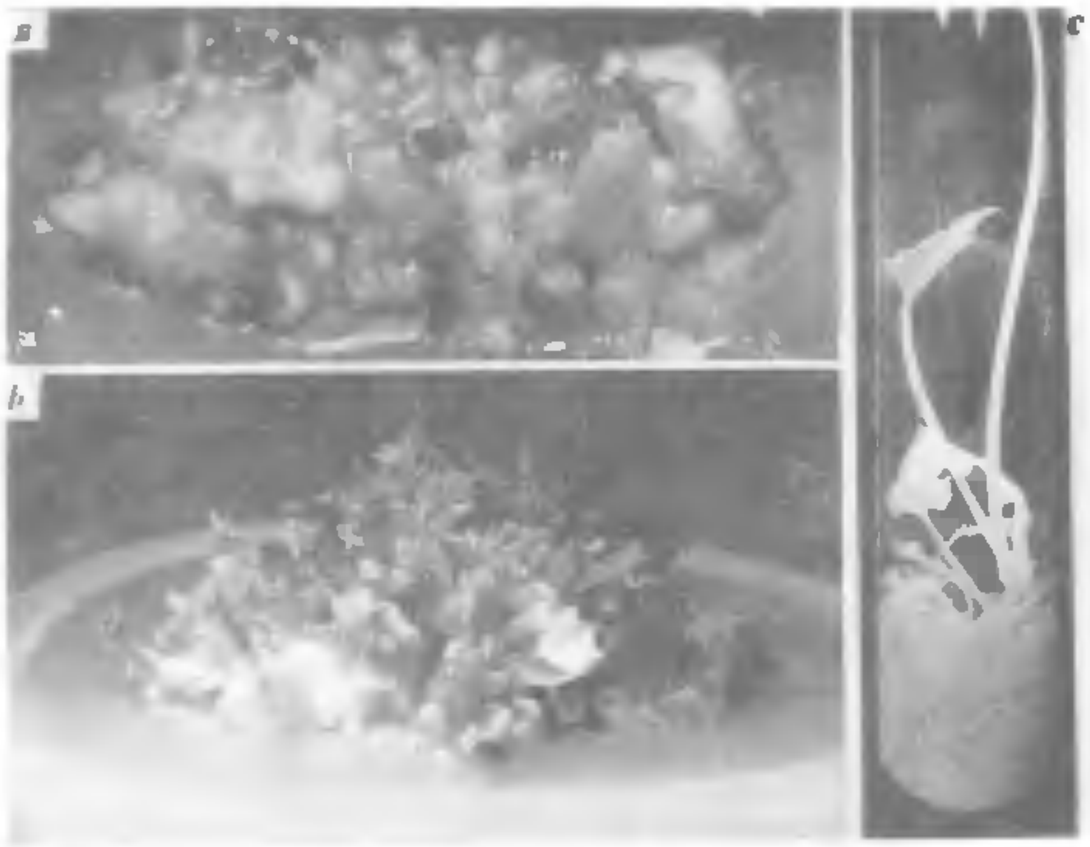

Figure 1. Organogenesis from internode explants of *C. maxima* × *C. moschata*. *a*, Development of adventitious shoot buds on internode explant in MS medium containing 4.4 μM BA + 0.54 μM NAA, after two weeks of culture. *b*, Development of adventitious shoots from shoot buds in MS medium containing 4.4 μM BA + 0.54 μM NAA, after two weeks of subculture. *c*, Development of roots from the base of excised shoot on half-strength MS medium containing 0.54 μM NAA, after four weeks of culture.

diameter of these knob-like structures was 0.5 mm. There were at least 25–50 such structures on every explant. Later, morphologically significant changes were observed after four weeks of culture. At this stage the shoot bud was recognizable as a monopolar structure developing procambial strands that established a connection with the preexisting vascular tissues dispersed within the cultured explants.

After subculturing in the same nutrient medium, most of the shoot buds developed into shoots (Table 2 and Figure 1 *b*). Presence of CM in the medium was not helpful for shoot bud differentiation. Number of shoots per explant decreased considerably with CM and the presence of CH was completely inhibitory. Similar growth regulator combinations were found to stimulate adventitious bud formation and shoot regeneration through organogenesis from cotyledonary explants of watermelon[7–9]. However, only cytokinin (kinetin or 2-isopentenyladenine) was highly effective for maximum frequency of shoot bud differentiation in cucumber[4]. In *Momordica charantia*, NAA + adenine and in *Cucumis melo*, indolebutyric acid + BA were responsive for developing shoot buds on cotyledonary explants[10]. The calluses developed from two preculture media failed to show any organogenesis even after 5 weeks of subculture.

Root initiation was observed within two weeks of culture (Figure 1 *c*). Rooting was also observed in auxin-free medium but roots produced were weak, number of roots per cutting was lower and time for root initiation was longer. Root induction with NAA has also been reported in watermelon[2]. The results of the present experiments present a reproducible and efficient plant regeneration system through direct organogenesis from internode explants of *C. maxima* × *C. moschata*. The system comprised three culture steps, i.e. direct multiple shoot induction without callus formation, shoot elongation and root induction. The method presented here will facilitate rapid clonal propagation of this economically important vegetable crop and is potentially useful in the transformation via *Agrobacterium tumefaciens*.

1. Jelaska, S., *Physiol. Plant.*, 1974, **31**, 257–261.
2. Dong, J. and Jia, J., *Plant Cell Rep.*, 1991, **9**, 559–562.
3. Chee, P. P., *HortScience*, 1990, **25**, 792–793.
4. Gambley, R. L. and Dodd, W. A., *Plant Cell Tissue Organ Culture*, 1990, **20**, 177–183.
5. Punja, Z. K., Abbas, N., Sarmento, G. G. and Tang, F. A., *Plant Cell Tissue Organ Culture*, 1990, **21**, 93–102.
6. Murashige, T. and Skoog, F., *Physiol. Plant.*, 1962, **15**, 473–497.
7. Cade, R. N., Wehner, T. C. and Blazich, F. A., *HortScience*, 1987, **22**, 154.
8. Kim, S. G., Chang, J. R., Cha, H. C. and Lee, K. W., *Plant Cell Tissue Organ Culture*, 1988, **12**, 64–67.
9. Wehner, T. C. and Locy, R. D., *HortScience*, 1981, **16**, 759–760.
10. Halder, T. and Gadgil, V. N., in Proceedings on the COSTED Symposium on Tissue Culture of Economically Important Plants (ed. Rao, A. N.), Singapore, 1981, pp. 98–103.

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