crease in WGA levels in the initial 4 h of germination could be due to increase in ABA levels[13,14] which enhances the half-life of the already existing lectin mRNA, as reported in rice[15], rather than enhanced transcription of the gene. It is also possible that, as reported earlier[16], WGA degradation at latter stages of germination may be exceeding its synthesis, thus causing an overall decrease in total WGA content. Further studies on changes in WGA levels in response to stress imposition in embryos at different stages of germination, which are in progress, will elucidate the molecular basis of stage × stress interaction in the germinating embryos of wheat.

Although direct evidence is lacking, our observations lend further support to its proposed role in free-radical scavenging[8] as higher WGA accumulation in germinating embryos in response to salt-, osmotic- and heat-stress may be a part of the adaptation process for protecting the cells against free radicals, since a major cause of cellular damage due to stress is the production of free radicals[10].

1. Triplett, B. A. and Quatrano, R. S., *Dev. Biol.*, 1982, **91**, 491–496.
2. Peumans, W. J., Stinissen, H. M. and Carlier, A. R., *Planta*, 1982, **156**, 41–44.
3. Singh, Prabhjeet, Bhaglal, P. and Bhullar, S. S., *Plant Physiol. Biochem.*, 1996, **34**, 547–552.
4. Mishkind, M. L., Keegstra, K. and Palevitz, B. A., *Plant Physiol.*, 1980, **66**, 950–955.
5. Etzler, E. M., *Annu. Rev. Plant Physiol.*, 1985, **36**, 209–234.
6. Cammue, B. P. A., Broekaert, W. F., Kellens, J. T. C., Raikhel, N. V. and Peumans, W. J., *Plant Physiol.*, 1989, **91**, 1432–1435.
7. Komarova, E. N., Vyskrebentseva, E. I. and Trunova, T. I., *Fiziologiya Rastenii*, 1995, **41**, 500–503.
8. Bhaglal, P., Singh, Prabhjeet, Bhullar S. S. and Kumar, Sanjay., *J. Plant Physiol.*, 1998, **153**, 163–166.
9. Peumans, W. J. and VanDamme, E. J. M., *Plant Physiol.*, 1995, **109**, 347–352.
10. Bohnert, H. J. and Jensen, R. J., *Trends Biotech.*, 1996, **14**, 89–97.
11. Lowry, O. H., Rosebrough, N. J., Forr, A. L. and Randall, R. J., *J. Biol. Chem.*, 1951, **193**, 265–267.
12. Laemmli, U. K., *Nature*, 1970, **227**, 680–685.
13. Wright, S. T. C. and Hiron, R. W. P., in *Plant Growth Substances* (ed. Carr, D. J.), Springer-Verlag, Berlin, 1972, pp. 291–298.
14. Watton, D., Harrison, M. A. and Cole, P., *Planta*, 1976, **131**, 141–144.
15. Stinissen, H. M., Peumans, W. J. and Delanghe, E., *Plant Cell Rep.*, 1984, **3**, 55–59.
16. Stinissen, H. M., Peumans, W. J. and Carlier, A. R., *Arch. Int. Physiol. Biochim.*, 1981, **89**, B132.

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Artificial seed technology: Development of a protocol in *Geodorum densiflorum* (Lam) Schltr. – An endangered orchid

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The research communication reports the production of artificial seeds through encapsulation of protocorm-like bodies (PLBs) of *Geodorum densiflorum* (Lam) Schltr. – an endangered orchid taxon of Terai Hills, North-eastern Himalaya. 30-day-old PLBs were encapsulated in sodium alginate. Germination and regeneration capacity of the encapsulated seeds were tested by germinating such seeds in modified Knudson C (KnC) medium supplemented with coconut milk 15% (v/v), peptone (2 g l^{-1}), 6-benzylaminopurine (2 mg l^{-1}), and α-napthaleneacetic acid (1 mg l^{-1}). 88% Germination was recorded. Artificial seeds stored at 4°C for 120 days showed no reduction in viability. Non-encapsulated PLBs showed no viability after 30 days at 4°C. Artificial seeds showed 28% viability when directly transferred to nonsterile soil condition after incorporating food preservative and fungicide in its encapsulating gel.

PRODUCTION of artificial seeds has unravelled new vistas in plant biotechnology. The artificial seed technology is an exciting and rapidly growing area of research in plant cell and tissue culture. The idea of artificial seeds was first conceived by Murashige[1] which was subsequently developed by several investigators. Initially, the development of artificial seeds had been restricted to encapsulation of somatic embryos in a protective jelly. It had been considered that the induction of somatic embryogenesis (SE) and/or pollen embryogenesis which genetically differs from zygotic embryogenesis, is the prerequisite for the preparation of artificial seeds. Their induction has been reported in a number of cereals, millets, tuberous plants, vegetables, and other commercially important plants like soybean, mustards, coffee, tobacco, and cotton. However, because of certain inherent problems, the rate of production of uniform and high quality embryos is much lower as a result of which the preparation of efficient and quality seeds has been successful in only a few crop plants like carrot[3] and alfalfa[4].

Recent advances in the area have revealed that besides somatic embryos, encapsulation of cells and somatic tissues obtained following tissue culture techniques has

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become popular as a simple way of handling cell and tissue, protecting them against strong external gradients, and as an efficient delivery system[4,9].

A number of encapsulating agents have been tried out of which agar, agarose, alginate, carragenan, gelrite, and polyacrylamide are important[2]. Recently, nitrocellulose and ethylocellulose have also been tried out for encapsulation[11]. However, the present investigation by us on *Geodorum densiflorum* (Lam) Schltr., as well as by those of a few others[7–9], suggests the that most suitable encapsulating agent for orchid protocorm-like bodies (PLBs) is sodium alginate, due to its solubility at room temperature and its ability to form completely permeable gel with calcium chloride ($CaCl_2.2H_2O$). Our findings have revealed that this method provides an efficient mechanism for handling and storage of orchid PLBs.

To date, encapsulation methodologies have met with success in only a few non-orchid angiosperms. Orchids, the most precious and costly ornamentals, are one of the few flowering plants to be propagated *in vitro*, both through seed and tissue culture. The most sensational development has been the use of *Cymbidium* apical shoot meristem as a means of clonal propagation by Morel[5,6] which revolutionized the orchid industry and triggered the global explosion of tissue culture for rapid clonal propagation of other ornamentals as well. Uptil now, synthetic seed production by encapsulating PLBs has been achieved in only a few orchids like *Dendrobium wardianum*[7], *Phaius tankervillae*[8], and *Spathoglottis plicata*[9]. The present communication of successful artificial seed production in *G. densiflorum*, an endangered terrestrial orchid, will be a further addition. Attempts have also been made for direct transfer of the artificial seeds to the field following treatments with antifungal and antibacterial agent in the encapsulating matrix.

For production of PLBs, mature undehisced capsules of *G. densiflorum* were collected and washed thoroughly with Tepol (BDH) under running tap water. They were then surface-sterilized with 3% sodium hypochlorite solution (v/v) for 15 min and were subsequently rinsed in sterilized double-distilled water. The capsules were cut longitudinally with the help of a sharp sterilized surgical blade and the seeds were inoculated in modified Knudson C (KnC) medium[10] supplemented with 2 mg l^{-1} of 6-benzylaminopurine (BAP), 1 mg l^{-1} α-napthaleneacetic acid (NAA), 2 gl^{-1} peptone and 15% coconut milk (CM). The pH of the medium was adjusted to 5.6–5.8. The cultures were maintained at 25 ± 2°C under 16 h photoperiod from cool-white-light giving 1000 lux at culture level. After 5 weeks following inoculation, green pin-head-like PLBs appeared.

For encapsulation of PLBs, 30 days following seed germination the PLBs were collected and washed in liquid KnC medium. Sodium alginate solution (4%; w/v) was prepared by mixing with liquid KnC medium. PLBs were mixed with sodium alginate solution and were subsequently singly dropped into an autoclaved-50 mM solution of calcium chloride ($CaCl_2.2H_2O$). Calcium alginate beads were formed within 15–20 min on a rotary shaker moving at 80 rpm. Beads were taken out by decanting off the $CaCl_2$ solution, washed with sterilized double-distilled water, and surface-dried with sterilized blotting paper. Freshly prepared beads were directly inoculated in KnC medium; the organic additives and the concentrations of growth regulators being the same as used during seed germination. The cultures were kept at same conditions as before.

A set of 150 artificial seeds was stored in dark at 4°C in sterile petri dishes, sealed with parafilm. They were taken out at regular intervals of 30 days, and inoculated to see their germination percentage. Another set of 150 PLBs was kept at room temperature (25 ± 2°C). Nonencapsulated PLBs were kept both at room temperature and at 4°C. Each treatment had 10 replicates and was repeated at least thrice.

Fungicide bavastin was incorporated into the nutrient gel at a constant concentration of 4 mg l^{-1}, as higher concentrations of fungicide were found to inhibit the growth of PLBs (data not shown). Sodium bicarbonate was used as food preservative, and was incorporated in the same way. The concentrations of sodium bicarbonate used were 5, 10, 15, 20, 25, 30 and 40 mg l^{-1}. The encapsulated PLBs were washed with sterile double-distilled water, and finally placed in autoclaved soil in petri dishes. The petri dishes were covered with lid to maintain the required humidity. Tap water was sprayed at regular intervals to keep the soil moist.

Freshly encapsulated PLBs (without storage; control) (Figure 1 *b*) when directly inoculated on KnC medium supplemented with CM (15% v/v), peptone (2 g l^{-1}), BAP (2 mg l^{-1}) and NAA (1 mg l^{-1}), showed induction of growth after second week. Subsequently, they emerged out by rupturing the alginate matrix and established contact with the media. These were then subcultured on the same media, and within 6–8 weeks well-developed plantlets were obtained (Figure 1 *c*). As the PLBs (Figure 1 *a*) were randomly selected for encapsulation, the time requirement for the encapsulated PLBs to come out through the matrix was different. 88% germination was noted at this stage, which is quite high (Table 1). Encapsulated PLBs stored at 4°C for 120 days showed 86% viability, but the same when stored at room temperature showed only 44% viability (Table 1). The germination percentage decreased gradually with increase in storage time. The nonencapsulated PLBs on the other hand showed no viability or regeneration after a storage of only 30 days at 4°C. The germination percentage of artificial seeds stored at room temperature was always much lower in comparison to those stored at 4°C (Table 1).

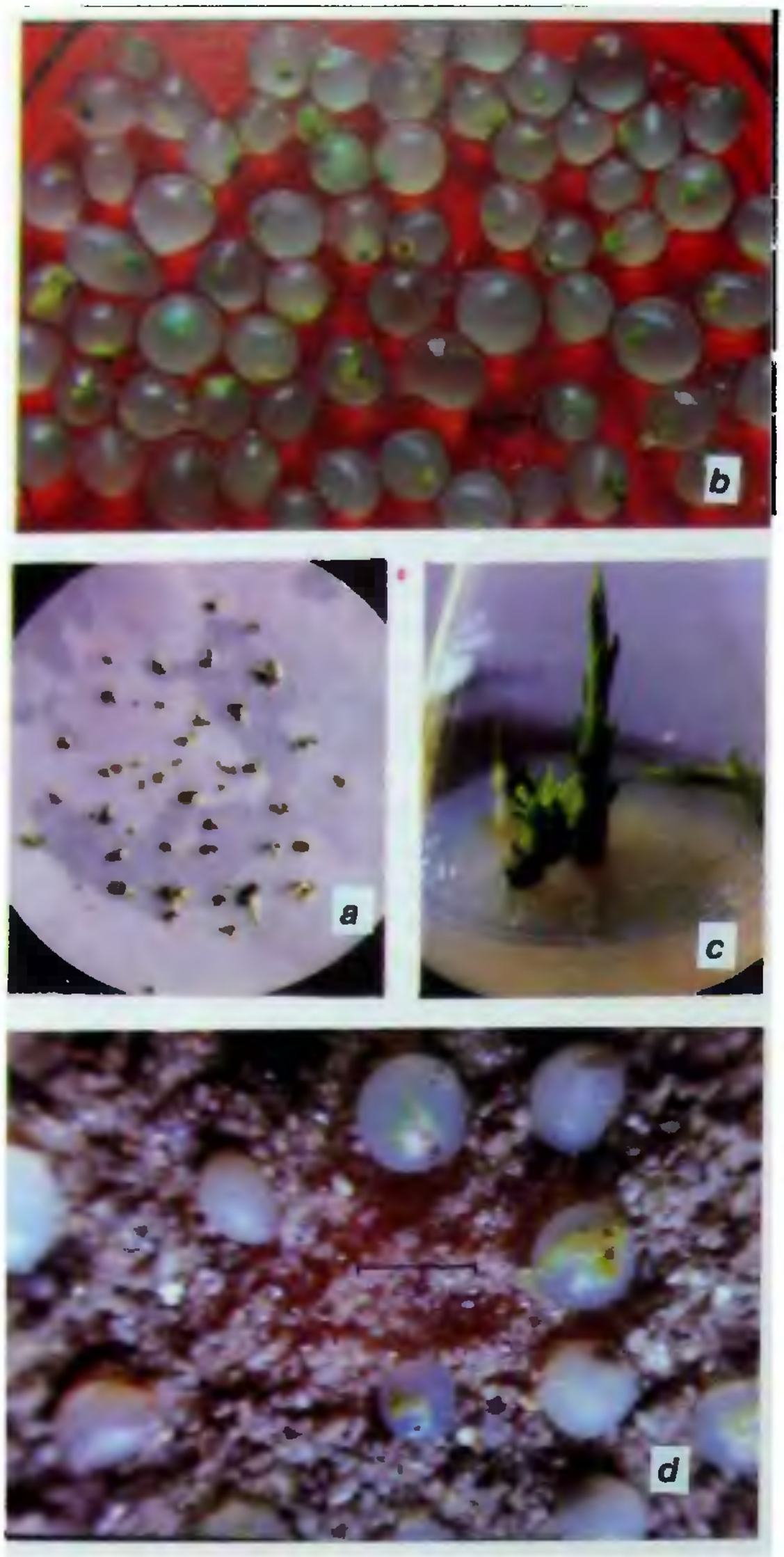

Figure 1. Protocorm-like bodies (PLBs), encapsulated PLBs and their regeneration under *in vitro* and natural condition. The bar represents 10 mm for all photographs. *a*, PLBs selected for encapsulation. *b*, Encapsulated PLBs. *c*, *In vitro* regeneration of PLBs. *d*, Regeneration and emergence of artificial seeds under *in vivo* conditions.

Table 1. Viability frequencies of the encapsulated PLBs of *Geodorum densiflorum* at different temperatures for different periods. Values are mean of 25 encapsulated PLBs in 10 replicates

Types of artificial seeds	Storage temperature (°C)	Storage time (days)	Regeneration percentage ± SE
Encapsulated	No storage	No storage	88.0 ± 1.05
Encapsulated	4	30	87.0** ± 0.83
Encapsulated	RT	30	72.0*** ± 0.78
Encapsulated	4	60	86.6* ± 1.09
Encapsulated	RT	60	64.5** ± 1.28
Encapsulated	4	90	87.0 ± 1.09
Encapsulated	RT	90	58.5*** ± 0.90
Encapsulated	4	120	86.6* ± 1.15
Encapsulated	RT	120	44.0*** ± 1.96
Encapsulated	4	150	78.0** ± 0.36
Encapsulated	RT	150	36.0** ± 1.66
Encapsulated	4	180	71.6*** ± 0.79
Encapsulated	RT	180	20.4** ± 1.04
Nonencapsulated	4	30	00.0
Nonencapsulated	RT	30	00.0

Germination percentage of artificial seeds followed by asterisks in each treatment within the same column is significantly different from control (artificial seeds without storage), using Student's *t* test at *5% level; **1% level and ***0.1% level.

The encapsulated PLBs containing fungicide and different concentrations of food preservative did not show any contamination up to 8 weeks in soil. With varying concentrations of food preservative used, optimum frequency of germination (28%) was at 20 mg l^{-1} concentration, which was followed by emergence of plantlets through the matrix (Figure 1 *d*). Although in all concentrations of sodium bicarbonate, some degree of germination was noted, the continued growth of PLBs at lower concentrations ceased due to desiccation (Table 2).

Besides rapid and mass propagation of plants, the artificial seed technology has added new dimensions not only to handling and transplantations but also for conservation of endangered and desirable genotypes. Many facets of artificial seed production by encapsulating the PLBs have been intensively investigated in the terrestrial and fast disappearing orchid, namely, *G. densiflorum*. With the ultimate objective of conservation of orchid taxon; PLB production, encapsulation, *in vitro* and *in vivo* germination of the artificial seeds (Tables 1 and 2) had been achieved.

The primary goal of artificial seed system was to recover whole plantlets from artificial seeds under *in vitro* as well as under field conditions. On immediate transplantation of artificial seeds to the same medium, a high percentage of emergence of well-developed plantlets had been noted within 6–8 weeks following subculturing. High viability percentage of stored artificial seeds at 4°C in comparison to room temperature indicated the efficiency of low temperature for storage of artificial seeds. The retention of high viability percentage up to 120 days may be due to the availability of nutrients within the gel matrix. It may be noted that while in SE-derived artificial seeds of *Santalum album*[12], only 17% germination could be obtained under nonsterile soil condition, in the present investigation an encouragingly high percentage of germination (28%) under natural condition was achieved in *G. densiflorum*, following

Table 2. Effect of different concentrations of sodium bicarbonate on *in vivo* germination of *G. densiflorum*. Each set consists of 25 encapsulated PLBs and had 10 replicates

Concentration of sodium bicarbonate (mg l^{-1})	Concentration of bavastin (mg l^{-1})	Regeneration percentage ± SE
0	4	6.00 ± 0.30
5	4	12.00 ± 1.08
10	4	20.4** ± 1.69
15	4	24.2** ± 1.28
20	4	28.40** ± 1.04
25	4	22.6* ± 0.60
30	4	20.20** ± 0.74
40	4	18.6*** ± 0.78

Germination percentage followed by asterisks in each treatment within the same column is significantly different from control (artificial seeds without $NaHCO_3$), using Student's *t* test at *5% level; **1% level and ***0.1% level.

supplementation of the encapsulating matrix with suitable food preservative and fungicide. It was interesting to note that besides preventing desiccation, the food preservative sodium bicarbonate alone was effective in checking contamination. However, in the long run, presence of a fungicide appears to be crucial to resist contamination. Development of the protocol for artificial seed production in *G. densiflorum* suggests that the lengthy and empirical process of hardening could be avoided for transplantation of *in vitro*-grown plantlets from laboratory condition to natural conditions (Table 2). Moreover, the development of the protocol for the endangered orchid *G. densiflorum* may be an useful addition to the *in vivo* germination and regeneration of plantlets for storage and transplantation of precious and costly hybrid orchids as well as for conservation of endangered germplasms. The judicious and intelligent coupling of artificial seed technology with that of microcomputer in achieving automated encapsulation and regeneration of plantlets would tremendously increase the efficiency of encapsulation and production of homogeneous and high quality artificial seeds, and will thus revolutionize the current concept of commercial micropropagation method by the beginning of twenty-first century.

1. Murashige, T., in *Frontiers of Plant Tissue Culture* (ed. Thorpe, T. A.), 1978, p. 15.
2. Kitto, S. L. and Janick, J., *J. Am. Orchid Soc. Hortic. Sci.*, 1985, 110, 277–288.
3. Fujii, J, A. Slade and Redenbough, K., *In vitro Cell Dev. Biol.*, 1989, 25, 1179.
4. Datta, S. K. and Potrykus, I., *Theor. Appl. Genet.*, 1989, 77, 820–824.
5. Morel, G. M., *Am. Orchid Soc. Bull.*, 1960, 29, 495–497.
6. Morel, G. M., *Am. Orchid Soc. Bull.*, 1964, 31, 473–477.
7. Sharma, A., Tandon, P. and Kumar, A., *Indian J. Exp. Biol.*, 1992, 30, 744–748.
8. Malemngaba, H., Roy, B. K., Bhattacharya, S. and Deka, P. C., *Indian J. Exp. Biol.*, 1996, 34, 801–805.
9. Singh, F., *Lindleyana*, 1991, 6, 61–64.
10. Knudson, L., *Am. Orchid Soc. Bull.*, 1946, 15, 214–217.
11. Wadhwa, M. K., Verma, K. L. and Singh, R., *Seed Sci. Technol.*, 1989, 17, 99–105.
12. Fernendez, P. C., Bapat, V. A. and Rao, P. C. *Indian J. Exp. Biol.*, 1992, 30, 839–841.

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On the use of symbolic computations in geosciences

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Demonstration of Computer Algebra Systems (CAS) in general, with a special reference to earth sciences, is given. Mathematical manipulations for solving complicated geophysical problems are carried out on a computer by a symbolic computational system, Maple V. Such intelligent systems can be utilized to solve large size scientific and engineering problems, which will result in enormous savings of time and manual resources.

MATHEMATICAL modelling of physical systems is a powerful tool to solve scientific and engineering problems. Although, analytical solutions to any problem are much more accurate, but scientists frequently have to resort to numerical techniques to solve for realistic models since analytical solutions for such models require gigantic human efforts and are time consuming. Even for numerical methods, algebraic formulas have to be obtained before any numerical technique is used. Symbolic manipulations of such complicated mathematical expressions are considered to be a daunting task by many practising scientists. With the advent of symbolic computation packages, also known as Computer Algebra Systems (CAS), the burden on practising engineers and scientists have become easier in handling large algebraic formulations.

Following is an account of a couple of problems in geophysics, which are quite time consuming if solved manually. For this purpose, we have used a popular symbolic computation program, **Maple V**, developed at the University of Waterloo, Canada. This is one of the first attempts at demonstrating the ability of such algebraic manipulation programs as an aid to solve complex real-world problems. The resulting enormous time saving can be utilized in comprehension of the results.

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