heat or AVS treatment. This asserts the notion that there is hardly any PLA_2 assay which could be applied universally[11]. The presented procedure has been applied successfully to some plant aqueous extracts popularly known as 'antivenom' and was found to be completely inhibiting hydrolysis. Thus it may serve as an empirical guide for screening similar plants. This is in relation to our long-term interest to address better snake-bite management in the Indian subcontinent[14,22–24].

1. Verheij, H. M., Sletboom, A. J. and De Hass, G. H., Structure and function of phospholipase A_2. *Rev. Physiol. Biochem. Pharmacol.*, 1981, **91**, 91–203.
2. Gutierrez, J. M. and Lomonate, B., Phospholipase A_2 myotoxin from Batrops snake venoms. *Toxicon*, 1995, **331**, 405–424.
3. Barlow, P. N., Liser, M. K., Sigler, P. B. and Dennis, E. A., Probing the role of substrate conformation in phospholipase A_2 action on aggregated phospholipids using contrained phosphatidylcholine analogues. *J. Biol. Chem.*, 1988, **263**, 12954–12958.
4. Arni, R. K. and Ward, R. J., Phospholipase A_2 – A structural review. *Toxicon*, 1996, **34**, 827–841.
5. Scott, D. L. and Sigler, P. B., Structure and catalytic mechanism of secretary PLA_2. *Adv. Protein Chem.*, 1994, **45**, 53–84.
6. Kini, R. M. and Evans, H. J., A model to explain the pharmacological effects of snake venom phospholipase A_2. *Toxicon*, 1989, **27**, 613–635.
7. Neumann, W. and Habermannn, E., Beitrage zur charakteriseierung der wirkstoffe des Bienengiftes. *Arch. Exp. Pathol. Pharmakol.*, 1954, **222**, 367–370.
8. Nieuwenhuizen, W., Kunze, H. and De Hass, G. H., Phospholipase A_2 from Porcine pancreas. *Methods Enzymol.*, 1974, **32**, 147–154.
9. Gatt, S. *et al.*, Assay of enzymes of lipid metabolism with colored and fluorescent derivatives of natural lipids. *Methods Enzymol.*, 1981, **72**, 351–375.
10. Raymond, A. D. and Dennis, E. A., Phospholipase A_2 from cobra venom (*Naja naja naja*). *Methods Enzymol.*, 1981, **71**, 703–710.
11. Phospholipase assays, kinetics and substrates. Section I, article nos. 1–17. *Methods Enzymol.*, 1991, **197**, 3–200.
12. Lakowicz, R., *Principles of Fluorescence Spectroscopy*, Plenum Press, New York and London, 1983, pp. 19–48.
13. Radvanyi, F., Russo-Marie, L. and Bon, C., A sensitive and continuous fluorimetric assay for phospholipase A_2 using pyrene-labelled phospholipids in the presence of serum albumin. *Anal. Biochem.*, 1989, **177**, 103–109.
14. Chakrabarty, D., Bhattacharyya, D., Sarkar, H. S. and Lahiri, S. C., Purification and characterisation of a haemorrhagin (VRH-1) from *Vipera russelli russelli* venom. *Toxicon*, 1993, **31**, 1601–1614.
15. Pittz, E. P., Lee, J. C., Bablouzian, B., Townend, R. and Timasheff, S. N., Light scattering and differential refractometry. *Methods Enzymol.*, 1973, **27**, 209–256.
16. Wollenweber, H. W., Seydel, U., Linder, B., Luderitz, O. and Rietschel, E., Th., Nature and location of amide bound (R)-3 acyloxyacyl groups in lipid A of lipo polysaccharides from various Gram-negative bacteria. *Eur. J. Biochem.*, 1984, **145**, 265–272.
17. Passen, H., Humosinski, T. F. and Timasheff, S. N., Small angle X-ray scattering. *Methods Enzymol.*, 1973, **27**, 151–209.
18. Jain, M. K., Rogers, J., Marecek, J. F., Ramirez, F. and Eibl, H., Effect of the structure of phospholipid on the kinetics of intravesicle scooting of phospholipase A_2. *Biochem. Biophys. Acta*, 1986, **860**, 462–472.
19. Warrel, D., Clinical toxicology of snake bite in Asia. In *Handbook of Clinical Toxicology of Animal's Venom and Poisons*, CRC Press, 1995, pp. 493–594.
20. Prasad, N. B., Uma, B., Bhatt, S. K. G. and Gowda, T. V., Comparative characterization of Russell's viper (*Daboia/Vipera russelli*) venoms from different regions of the Indian peninsula. *Biochem. Biophys. Acta*, 1999, **1428**, 121–136.
21. Jain, M. K., Gelb, M. H., Rogers, J. and Berg, O., Kinetic basis for interfacial catalysis by phospholipase A_2. *Methods Enzymol.*, 1995, **249**, 567–614.
22. Kole, L., Chakrabarty, D., Datta, K. and Bhattacharyya, D., Purification and characterisation of an organ specific haemorrhagic toxin from *Vipera russelli russelli* (Russell's viper) venom. *Indian J. Biochem. Biophys.*, 1999, **37**, 114–120.
23. Datta, K. and Bhattacharyya, D., *In vitro* haemorrhage like activity of Russell's viper (*Vipera russelli russelli*) venom from Eastern India with mice organ. *Curr. Sci.*, 1999, **77**, 1673–1677.
24. Chakrabarty, D., Datta, K., Gomes, A. and Bhattacharyya, D., Haemorrhagic protein of Russell's viper venom with fibrinolytic and esterolytic activities. *Toxicon*, 2000, **38**, 1475–1490.

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Germination improvement in *Swertia angustifolia*: a high value medicinal plant of Himalaya

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The present communication deals with improvement in seed germination of *Swertia angustifolia* via various hormonal treatments (GA_3, IAA and KNO_3). Germination of the species under controlled conditions is found to be low (<32.0%). GA_3 is found to be the best with respect to germination (96.0%) and reducing mean germination time (7.6 days) followed by KNO_3 (81.3%; 8.4 days) and IAA (66.0%; 16.6 days). A high degree of variation with regard to the germination percentage and mean germination time in different populations and treatments is recorded. The possible reasons for such variations are discussed.

Keywords: Conservation, endangered, gibberellic acid, Himalaya, *Swertia angustifolia*.

SWERTIA angustifolia Ham. ex D. Don (family Gentianaceae), an endangered medicinal plant of west Himalaya[1], is listed among medicinal plants prioritized for conserva-

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tion[2]. It is an erect annual herb, distributed in India, Nepal, Pakistan, Bhutan, Burma, South and Southwest China. In India, the species is distributed in Jammu and Kashmir, Himachal Pradesh, Punjab, Uttaranchal, Sikkim and Assam between 600 and 2000 m asl[3] (Figure 1).

The species is useful in malarial fever and bronchial asthma and is used as a substitute of chirayita, *S. chirayita*[4,5]. The plant is also used as a blood purifier and febrifuge[6].

This species is identified for the present study because (i) it has high medicinal value and (ii) the species is often used as a substitute and/or adulterant of *S. chirayita* – a highly priced, rare medicinal plant of the Himalaya. Both these factors are responsible for its dwindling populations. Since the regeneration of this species is possible only through seeds, studies to understand germination behaviour assume great importance. The present study focuses on: (i) improvement of seed germination using different pretreatments and reducing mean germination time and (ii) analysis of the extent of variation in germination responses of seeds collected from different populations.

Mature seeds of *S. angustifolia* were collected during October–November 2001 from five populations located at different altitudes (1200–2200 m asl) of Kumaon Himalaya, Uttaranchal (Table 1). Immediately after collection, the seeds were dried at room temperature for one week (20 ± 5°C), and stored (at room temperature) in brown-paper bags till the start of germination experiments (January 2002). The mean seed weight showed only marginal difference between the studied populations. Ten seeds in three replicates from each population were studied for seed length and width[7] using a research microscope (Hund Wetzlar, Japan). The length and width of the seed were calculated by multiplying the measurement of one occular unit to the number of occular units occupied by the length and width of the seed. Moisture content was determined using three replicates of 50 seeds collected from each population. The fresh weight was recorded and seeds were kept at 60°C for 48 h. Thereafter, the seeds were reweighed and the difference calculated as moisture content. To determine water absorption capacity, three replicates of 50 seeds were drawn from pooled seeds of selected populations. These seeds were weighed and soaked in distilled water and kept in dark. Seed weight was recorded after every 3 h and this procedure was repeated until the constant weight of seeds[8]. Viability test using 2,3,5, *Triphenyle tetrazolium* chloride solution could not be performed. The seeds were so small that the embryo could not be detached to observe the staining pattern.

Figure 1. A flowering individual of *Swertia angustifolia*.

Seeds were surface-sterilized by dipping in 0.5% aqueous solution of mercuric chloride for 2 min to remove bacterial and fungal contamination and then rinsed thoroughly (four times) with distilled water and then soaked in different concentrations of chemicals, i.e. GA_3 and IAA (100, 200, 400 µM) and KNO_3 (100, 200, 400 mM), for 15 h. Treated seeds were then rinsed thoroughly with distilled water and 50 seeds of each in three replicates were sown in plastic petri plates (95 × 17 mm) on a moistened filter paper (Whatmann No 1). Petri plates were kept in the growth chamber for incubation at fixed temperature (25 ± 2°C). One set of untreated seeds acted as control.

Seeds were considered to have germinated upon the initiation of radical. Number of seeds germinated was counted daily. Mean germination time (MGT) was calculated as: $MGT = \sum(nd)/N$; where n is the number of seeds which germinated after each incubation period in days d, and N is the total number of seeds emerged at the end of the test[9]. Analysis of variance was applied for all the experiments. Least significant difference (LSD) was estimated separately for comparison of populations and treatment means[10].

Imbibition test revealed that seeds absorb water rapidly during the first 15 h and thereafter attaining a plateau. Maximum seed length was recorded for Sa_1 and minimum for Sa_5, whereas seed width was maximum in Sa_3 and minimum in Sa_4 (Table 1). There was no influence of seed size on germination percentage. Seed moisture content varied from 22.2 (Sa_2) to 28.9% (Sa_1). Germination responses among treatments and populations varied significantly (Table 2). Untreated seeds of different populations showed variation between 18.7 and 32.0%, which was significant ($P < 0.01$). The highest mean germination in Sa_4 (32.0%) was significantly ($P < 0.05$) better than Sa_3 (18.7%) and Sa_2 (22.7%) responses.

Across the population, compared to control, GA_3 treatments improved the per cent germination significantly ($P < 0.05$). Maximum germination (96.0%) was observed in Sa_4 population with 100 µM GA_3, which was significantly ($P < 0.05$) higher than the responses in other populations (Table 2). Different populations did not show uniformity of germination in a particular concentration of GA_3. For instance, populations Sa_1 and Sa_2 responded the best with

Table 1. Location and general features of identified populations of *Swertia angustifolia*

Locality	Altitude (m asl)	Seed weight (mg/50 seeds)	Seed length (μm)	Seed width (μm)	Moisture content (%)	Habitat
Sa_1-Jalna	1920	0.86	482.99	315.64	28.9	Dry grassy slope with *Pinus roxburghii* and *Quercus leucotrichophora*
Sa_2-Majkhali	1700	0.83	477.55	292.15	22.2	Dry steep slope with *P. roxburghii*
Sa_3-Katarmal	1200	0.86	476.17	318.36	28.4	Gentle grassy slope with *P. roxburghii*
Sa_4-Killbury	2200	0.76	459.86	285.71	25.9	Grassy steep slope with *Q. leucotrichophora*
Sa_5-Dinapani	1770	0.96	404.44	314.28	24.0	Moist sloppy area with *P. roxburghii* and *Q. leucotrichophora*
LSD ($P < 0.05$)		0.30	33.63	267.32	10.96	
F		0.57^{ns}	3.69*	0.98^{ns}	0.67^{ns}	

*$P < 0.05$, ^{ns}non-significant.

Table 2. Effect of different treatments on germination of *S. angustifolia*

Treatment	Population					LSD ($P < 0.05$)	F-ratio
	Sa_1	Sa_2	Sa_3	Sa_4	Sa_5		
Control	31.3	22.7	18.7	32.0	25.3	7.65	5.48**
GA_3							
100 (μM)	69.3	74.0	62.7	96.0	69.3	17.19	5.91**
200	62.0	61.3	80.7	93.3	57.3	11.94	18.96**
400	73.3	78.0	44.0	50.7	50.7	13.33	14.36***
IAA							
100 (μM)	10.7	16.0	20.0	25.0	49.3	16.30	4.76**
200	38.0	30.7	43.0	55.0	58.0	17.27	4.76**
400	66.0	22.7	26.0	30.0	35.3	18.69	9.26**
KNO_3							
100 (mM)	72.7	73.3	58.7	67.3	76.0	10.62	4.46*
200	81.3	80.0	24.7	23.3	37.3	17.49	29.56***
400	53.3	35.3	44.0	29.3	30.0	17.10	3.57*
LSD ($P < 0.05$)	14.20	13.34	11.81	15.25	20.20		
F	22.01***	33.99***	26.65***	29.10***	6.10***		

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

400 μM GA_3 treatment, Sa_3 responded best under 200 μM GA_3, and Sa_4 and Sa_5 under 100 μM GA_3 concentrations.

IAA treatments improved germination per cent significantly (Sa_1-400; Sa_3-200; Sa_4-200 and Sa_5-100 and 200 μM) compared to control. The highest germination percentage was obtained in 400 μM IAA, 66 in Sa_1 population, which was a significant ($P < 0.05$) improvement over the other populations (Table 2) under the same treatment.

Compared to control, KNO_3 treatments also proved beneficial in improving germination percentage of seeds from all populations. The highest germination percentage (81.3) was observed in 200 mM KNO_3 treatment for Sa_1 population. This increase was significant ($P < 0.05$) from the responses of Sa_3, Sa_4 and Sa_5 populations (Table 2). Among populations, Sa_1 and Sa_2 showed increase in germination with increase in KNO_3 concentration up to 200 mM. Whereas for Sa_3, Sa_4 and Sa_5 increase in KNO_3 concentration beyond 100 mM was deleterious.

Among all germination enhancing treatments, 100 μM GA_3 improved germination percentage in Sa_4 (96.0) and reduced the MGT (7.6 days) significantly compared to control (17.3 days). IAA (100 μM) also reduces the MGT (Sa_5-8.2 days), which was a significant reduction compared to control (17.9 days). Also, 200 mM KNO_3 showed maximum germination (81.3% in Sa_1 population) and reduced MGT up to 8.4 days, which was a significant reduction (control 11.4 days; Table 3).

Germination studies among different populations provide helpful clues on genetic make-up of the species and its existence in the natural population[11]. Knowledge of such variations in germination of species is essential for selection of best (elite) provenance of seeds[12]. Reports suggest that populations of a species differ in their germination responses[13]. Similar trends were observed in the present study. Causes of such variations are multiple, which range from genetic characteristics of source population[14,15] to impact of mother plant environment[16–18].

Seed germination study of *S. angustifolia* revealed significant ($P < 0.05$) improvement in germination under GA_3 treatment compared to control. However, the extent of im-

Table 3. Effect of best responding treatments on mean germination time of *S. angustifolia*

Treatment	Population					LSD ($P < 0.05$)	F-ratio
	Sa_1	Sa_2	Sa_3	Sa_4	Sa_5		
Control	11.4	14.3	19.1	17.3	17.9	3.15	10.33*
GA_3	9.4	7.9	12.3	7.6	8.0	0.66	26.57**
IAA	16.6	11.8	21.1	12.5	8.2	2.35	47.76**
KNO_3	8.4	8.1	24.4	9.6	8.3	1.70	18.15**
LSD ($P < 0.05$)	2.03	2.26	3.27	2.60	2.28		
F	151.64**	147.86**	44.17**	46.44**	14.31**		

*$P < 0.01$, **$P < 0.001$.

provement varied among populations and concentrations. Gibberellic acid is the most commonly used hormone for promoting seed germination and is particularly considered responsible for mobilization of nutrients[9,19]. Also, gibberellic acid is used to release dormancy of seeds in many species for reducing inhibitor level or by activation of GA_3 synthesis or both. In other *Swertia* species. e.g. *S. chirayita*[20], it was found that seeds treated with GA_3 (50–400 ppm) can achieve 72–98% germination. On the contrary, treatment of 500 ppm GA_3 showed inhibitory effect on seed germination.

In the case of IAA, the maximum germination percentage (66) was achieved in Sa_1 population. IAA is known to stimulate germination in many species by playing a major role in cell division and differentiation[18,21,22].

In the present study, lower concentration of KNO_3 proved ideal. Nitrogenous compounds in various forms, particularly nitrates (e.g. KNO_3), have been used to stimulate germination[23,24]. They play a critical role in increasing the physiological efficiency[25] and influence germination through change in water relationship[26].

In general, Gentianaceae is reported to have physiological dormancy[27]. The results of *Swertia* species, with improved germination under KNO_3 and GA_3 treatments correspond well with these generalizations, as both these substances are considered best for breaking physiological dormancy[27–31].

The present study recommends GA_3 as the best treatment for improving seed germination. Highest percentage of germination in Sa_4 population indicates that it is the best source. Such populations may be marked for seed production with high conservation priority.

1. Samant, S. S., Dhar, U. and Palni, L. M. S., In *Medicinal Plants of Himalaya: Diversity, Distribution Potential Values*, Gyanodaya Prakashan, Nainital, 1998.
2. Sastry, A. R. K. and Chatterjee, S., Prioritization of medicinal plants of India. In *Setting Biodiversity Conservation Priorities for India* (eds Singh, S. *et al.*), World Wide Fund for Nature – India, 2000, p. 707.
3. Garg, S., In *Gentianaceae of the North West Himalaya* (a revision), International Bioscience Monograph 17, Today and Tomorrow Printers and Publishers, New Delhi, 1987.
4. Kirtikar, K. R. and Basu, B. D., *Indian Medicinal Plants, Volume III*, Bishen Singh Mahendra Pal Singh, Dehradun, 1984.
5. Ghoshal, S., Sharma, P. V. and Jaiswal, D. K., Chemical constituents of Gentianaceae. XXIII. Tetraoxygenated and pentaoxygenated xanthones and xanthone-O-glucosides of *Swertia angustifolia* Buch- Ham. *J. Pharm. Sci.*, 1978, **67**, 55.
6. Bhattacarjee, S. K., In *Handbook of Medicinal Plants*, Pointer Publishers, Jaipur, 2001.
7. Sharma, S. N., Puri, S. C., Srivastava, T. N., Handa, G. and Kaul, B. K., Enhancement of seed germination in *Nothapodytes foetida*. *J. Med. Aromat. Plant Sci.*, 2000, **22**, 206–210.
8. Li, X., Baskin, J. M. and Baskin, C. C., Physiological dormancy and germination requirements of seeds of several North American *Rhus* species (Anacardiaceae). *Seed Sci. Res.*, 1999, **9**, 237–245.
9. Hartmann, H. T. and Kester, D. E., In *Plant Propagation: Principles and Practices*, Prentice Hall, New Delhi, 1989.
10. Wilkinson, SYSTAT: A system for statistics, Systat Inc, Evaston IL, 1986.
11. Baskin, C. C. and Baskin, J. M., In *Seeds: Ecology, Biogeography, and Evolution of Dormancy and Germination*, Academic Press, San Diego, USA, 1998.
12. Jayshanker, S., Babu, L. C., Sudhakara, K. and Unnitham, V. K. G., Provenance variation in seed and germination characteristics of teak (*Tectona grandis* L.). *Seed Sci. Technol.*, 1999, **27**, 131–139.
13. Perez-Garcia, F., Iriondo, J. M. and Martinez-Laborde, J. B., Germination behaviour in seeds of *Diplotaxis erucoides* and *D. virgata*. *Weed Res.*, 1995, **35**, 495–502.
14. Witcombe, J. R. and Whittington, W. J., The effects of selection for reduced dormancy in charlock (*Sinapis arvensis* L.). *Heredity*, 1972, **29**, 37–49.
15. Babley, J. D. and Black, M., *Seeds: Physiology of Development and Germination*, Plenum Press, New York, 1994, 2nd edn.
16. Gutterman, Y., Maternal effect on seeds during development. In *Seeds: The Ecology of Regeneration in Plant Communities* (ed. Fenner, M.), Cab International, Wallingford, UK, 1992, pp. 27–59.
17. Milberg, P. and Anderson, L., Effect of emergence date on seed production and seed germinability in *Thlaspi arvense*. *Swed. J. Agric. Res.*, 1994, **24**, 143–146.
18. Anderson, L. and Milberg, P., Variation in seed dormancy among mother plants, populations and year of seed collection. *Seed Sci. Res.*, 1998, **8**, 29–38.
19. Kumar, A. and Purohit, S. S., *Plant Physiology: Fundamentals and Applications*, Agro Botanical Publishers, India, 1986.
20. Prasad, B. N., Effect of GA_3 on seed germination and seedling growth of *Swertia chirayita* and its medicinal uses in Nepal. *Vasundhara*, 1999, **4**, 93–96.
21. Chatterjee, S. K., Effect of pre sowing treatments for tung (*Aleurites fordii*). *Sci. Cult.*, 1960, **26**, 130–131.
22. Orman, A. F., Ei-Bakry, A. M. and Gawish, R. A., Effect of soaking seeds in some growth regulator solution on growth, chemical constituents and yield of akra. *Seed Sci. Technol.*, 1980, **8**, 161–168.
23. Choudhary, D. K., Kaul, B. L. and Khan, S., Breaking seed dormancy of *Podophyllum hexandrum* Royle ex. Camb. (syn. *P. emodi* Wall. ex. Honigberger). *J. Non Timber For. Prod.*, 1996, **3**, 10–12.

24. McIntyre, G. I., Cessna, A. J. and Hsiao, A. I., Seed dormancy in *Avena fatua*: Interacting effects of nitrate, water and seed coat injury. *Physiol. Plant.*, 1996, **97**, 291–302.
25. Bhargava, R. and Banerjee, V. N., Effect of N and K on root characteristics of potato. *J. Plant. Physiol.*, 1994, **37**, 130–132.
26. Anthony, M., Direct seeding technology for eucalyptus for the Rural Industries Research and Development Corporation Project, Report UWS-3A. New South Wales project, 1995.
27. Nikolaeva, M. G., Factors controlling the seed dormancy pattern. In *The Physiology and Biochemistry of Seed Dormancy and Germination* (ed. Khan, A. A.), Elsevier, 1977, pp. 51–54.
28. Toole, V. K., Factors affecting the germination of various drop seeds (*Sporobolus* spp.). *J. Agric. Res.*, 1941, **62**, 691–715.
29. Khan, A., Goss, J. A. and Smith, D. E., Effect of gibberellin on germination of lettuce seed. *Science*, 1957, **125**, 645–646.
30. Dunwell, J. M., Dormancy and germination in embryos of *Hordeum vulgare* L.: Effect of dissection, incubation temperature and hormone application. *Ann. Bot.*, 1981, **48**, 203–213.
31. Watkins, J. T. and Cantliffe, D. J., Hormonal control of pepper seed germination. *Hortic. Sci.*, 1983, **18**, 342–343.

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Direct and high frequency somatic embryogenesis and plant regeneration from hypocotyls of chickpea (*Cicer arietinum* L.), a grain legume

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A protocol for plant regeneration via somatic embryogenesis was developed in two high-yielding chickpea cultivars. Somatic embryos were induced directly from hypocotyl explants on Murashige and Skoog's (MS) medium fortified with different concentrations of 2,4-dichlorophenoxyacetic acid, 2,4,5-trichlorophenoxyacetic acid, naphthaleneacetic acid, picloram and dicamba either singly or in combination with 0.5–2.0 mg l^{-1} N^6-benzylaminopurine or 0.5–2.0 mg l^{-1} kinetin. Type of auxin, its concentration and genotype influenced the frequency of somatic embryogenesis. Picloram was better for somatic embryo induction compared to other auxins. The well-formed, cotyledonary-shaped embryos germinated into plantlets with 49.6% frequency on MS medium supplemented with 0.5 mg/l abscisic acid and 2.0 mg l^{-1} benzylaminopurine. The frequency of embryogenesis and subsequent plantlet regeneration was higher in ICCV-10 than in Annigeri. Regenerated plants were transferred to soil and grown to maturity with 80% survival and the tissue culture-raised plants produced viable seeds. This protocol to produce embryos with a high frequency and their subsequent conversion to whole plants offers potential for use in gene transfer and development of transgenics in this important grain legume and also for the production of synthetic seeds.

Keywords: *Cicer arietinum*, grain legume, hypocotyls, somatic embryogenesis.

LEGUMES are a group of economically important plants valued for food, fodder, wood, ornamentals, raw materials for industry and also for their role in biological nitrogen fixation[1]. Grain legumes are a major source of proteins for more than two billion people worldwide. Chickpea (*Cicer arietinum* L.) is an important grain legume of the Indian subcontinent, West Asia, northeast Africa, southern Europe, South and Central America and Australia. India accounts for more than 67.3% of the world's production[2]. Chickpea occupies the first place in production as well as in area under cultivation among the grain legumes in India and the third place in the world[2]. This food legume forms an important constituent of human diet in India with 20–60% proteins, 2.2% fat and 61.2% carbohydrate[3] and thus provides excellent quality of dietary proteins at affordable price to poor and average income families. However, production of this crop has remained consistently low because of its susceptibility to several fungi such as *Fusarium*, *Aschochyta blight* and also insect pest (*Heliothis*).

Few reports exist on somatic embryogenesis from leaf explants[4–6], immature cotyledons[7], immature leaflets[8] and seed explants[9]. However, the response of somatic embryogenesis remained inconsistent and often not reproducible. Some of the serious limitations also include low frequency, genotype specificity and occurrence of callus phase prior to embryogenesis. Despite the use of different methods, including desiccation and plant growth regulator treatments[10], the frequency of conversion of embryos into plantlets was not improved much. Moreover, the entire cycle of plantlet regeneration from globular to heart and torpedo-shaped embryos and then their conversion to whole plants in most of the reports is prolonged. To the best of our knowledge there are no reports on the direct somatic embryogenesis and subsequent plant regeneration in chickpea cultivars ICCV-10 and Annigeri using hypocotyl explants without the intervention of callus. The present study describes a reproducible protocol for plant regeneration via somatic embryogenesis, through hypocotyl explants in two important cultivars of chickpea.

Seeds of chickpea cultivars ICCV-10 and Annigeri were obtained from the Agricultural Research Station, Gulbarga. Seeds were surface sterilized with 0.1% (w/v) mercuric

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