Micropropagation of *Gloriosa superba* L. – an endangered species of Asia and Africa

Gloriosa superba L., (Liliaceae) is a valuable tropical medicinal plant; all of its parts find diverse usage in indigenous systems of medicine. Corms are thermogenic, abortifacient, alexteric, antipyretic and contain two important alkaloids colchicine ($C_{22}H_{25}O_6N$) and colchicoside ($C_{27}H_{33}O_{11}N$). Leaves are used to treat ulcers, piles, scrofula and to expel placenta, and seeds are used to cure cancer-related diseases[1]. The conventional method of propagation is through corm, since poor seed germination restricts their use in multiplication. Indiscriminate over-exploitation of the plant for diverse medicinal uses has endangered its survival. Tissue culture approaches are needed for the rapid propagation of the plant[2]. Here we report an efficient micropropagation protocol for *G. superba* using shoot tips and non-dormant corm apical buds.

Three-year-old plants of *G. superba* grown and maintained in our garden were used as the source of explants. Excised shoot tips and non-dormant corm buds were initially washed with a neutral detergent, Teepol at 5% for 5 min and then under running tap water for 15 min. This was followed by treatment with 0.1% mercuric chloride for 2–3 min. After washing 2–3 times with sterile distilled water, shoot tips and non-dormant corm buds were dissected out and inoculated on basal medium consisting of Murashige and Skoog[3] (MS) salts and Gamborg *et al.*[4] (B_5) vitamins, 2.5% sucrose and 0.8% agar. Basal medium was supplemented with various concentrations of 6-benzylaminopurine (BAP) and adenine sulphate (ADS) for corm shoot multiplication, BAP and 2-isopentenyladenine (2iP) for shoot tip regeneration and ADS/2iP alone for nodulation. The medium was buffered to pH 5.8 and dispensed in culture tubes and conical flasks before autoclaving at 121°C for 15 min. All cultures were maintained in a 12 h photoperiod at 24 ± 2°C and a photon flux density of 70 µmol m^{-2} s^{-1}.

Non-dormant corm buds regenerated after 35 days of culture (Figure 1 *a*) were dissected out individually for further multiplication and this process was continued repeatedly for 35 days. Finally, the cluster of shoots developed (Figure 1 *b*) was transferred to basal medium additionally supplemented with ADS in the range 2.72–8.15 µM and BAP in the range 4.44–8.88 µM.

Shoot tip explants (Figure 1 *c*) cultured in a medium supplemented with BAP in the range 0.444–4.44 µM and 2iP in the range 4.92–9.84 µM with or without addition of kinetin (Kn), 2.32 µM produced 20–35 shoots per shoot tip within 35 days of culture (Figure 1 *d*). 2iP was more effective than BAP for shoot formation. MS medium with Kn supplementation (1–4 mg/l) alone was enough to promote shooting in this plant when young sprouts were used as explants by Somani *et al.*[5]. However, the number of

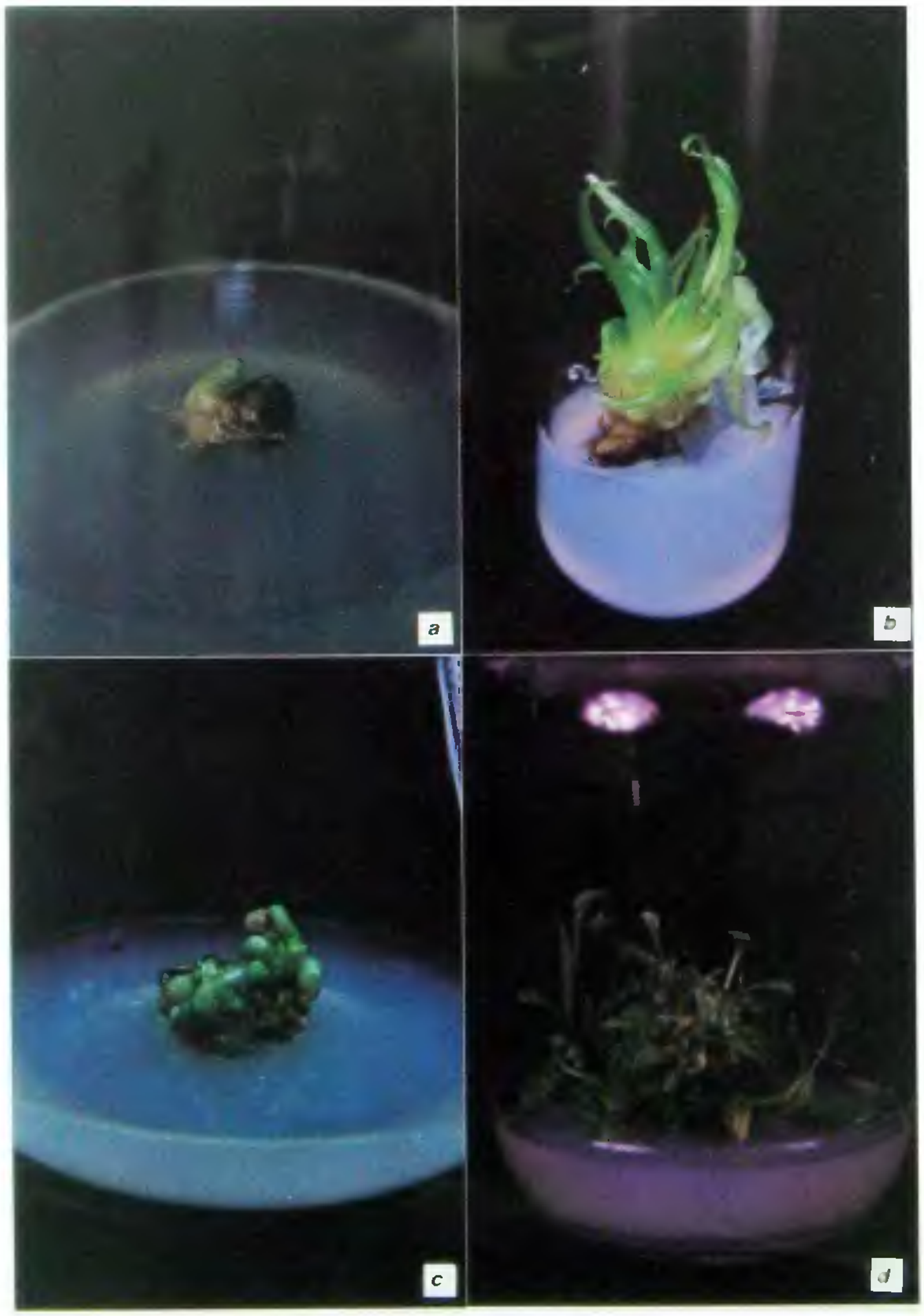

Figure 1. Micropropagation from non-dormant corms and shoot tip. *a*, Induction of shoot from non-dormant corm; *b*, Induction of multiple shoots from non-dormant corm; *c*, *d*, Initiation and further development of multiple shoots from shoot tip.

shoots produced in their system was less than six on an average and any further increase in Kn concentration did not result in increase in the number of multiple shoots.

The shoots derived from non-dormant corm buds and shoot tips were subcultured in a fresh medium containing the same hormonal combinations; such a subculture showed a different trend in multiplication within another 35 days of culture. A maximum number of shoots was obtained in a combination of ADS (5.44 µM) after two rounds of multiplication (70 days) after the initial period of 35 days. The number of new shoots formed per explant increased from the first to third subculture. However, BAP (7.77 µM) when used alone was found to induce multiple shoots that were weak and fragile (Table 1). Incorporation of ADS with BAP was stimulatory for shoot multiplication from corm bud culture. This observation is consistent with that of Chunsheng *et al.*[6] in *Leontochir ovallei*. Similarly Ayabe and Sumi[7] have also used a combination of auxin and cytokinin for the micropropagation of *Allium sativum*.

More morphologically distinct multiple shoots were developed from the shoot tip in a medium containing BAP or 2iP combined with Kn. Multiple shoots developed with a combination of 2iP (9.84 µM) and Kn (2.32 µM) grew faster, while those initiated in BAP (4.44 µM) and Kn (2.32 µM) combination grew slower (Table 2). White-greenish nodular structures developed directly from the cut ends of multiple shoots cultured on MS basal medium fortified with 2iP in the range 2.46–4.92 µM and ADS in the range 2.72–4.08 µM within two weeks of culture. After 25 days of initiation, these structures differentiated into shoot buds. After 5 weeks comprising 2 subcultures, an average of 15–20 shoots were obtained. ADS replaced with Kn produced 1–2 shoot buds and 4–7 roots preceded by whitish nodulation (Figure 2 *a*). The number of shoots initiated from shoot tip culture was greater than those initiated from corm explants. Moreover, 2iP produced large and vigorous shoots, and gave the highest relative growth rate.

Transferring the adventitious shoots developed *in vitro* to a medium additionally supplemented with gibberellic acid (GA_3) in the range 2.89–4.33 µM was necessary to ensure their normal elongation. Approximately 85% of the shoots attained lengths of 5–7 cm after

Table 1. Effect of concentration and combination of BAP and ADS on multiple shoot bud development from corm buds of *G. superba* cultured for 35 days

Hormone concentration, BAP + ADS (µM)	No. of shoots/ corm bud explant*	Hormone concentration, BAP (µM)	No. of shoots/ corm bud explant*
4.44 + 5.44	23.1 ± 0.4	4.44	21.3 ± 0.6
6.66 + 5.44	25.8 ± 0.7	6.66	21.0 ± 0.3
7.77 + 5.44	26.9 ± 0.4	7.77	23.1 ± 0.2
8.88 + 5.44	23.2 ± 0.4	8.88	17.6 ± 0.4

*Each value is the SD of three experiments with fifteen replications per experiment.

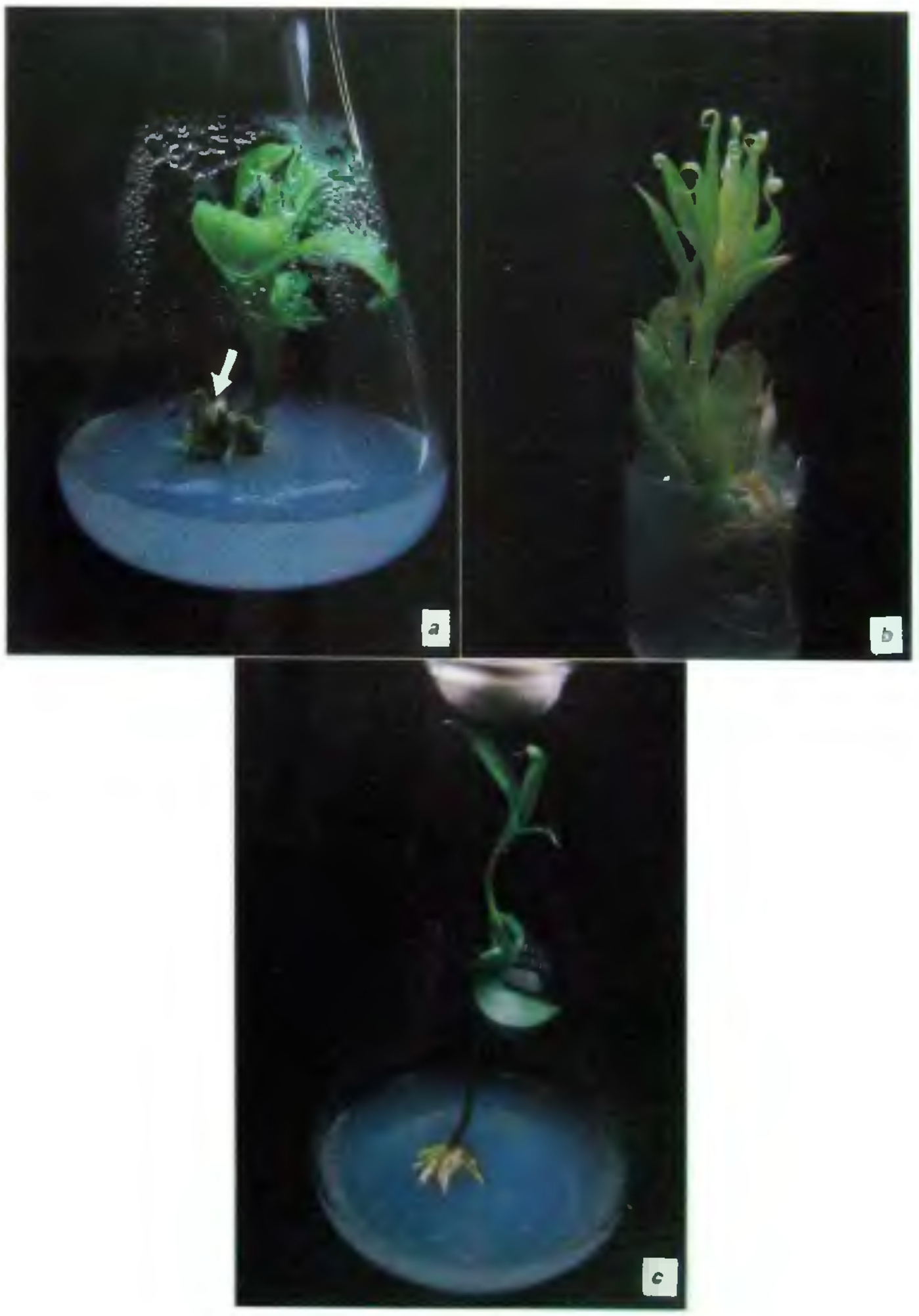

Figure 2. Micropropagation from non-dormant corms and shoot tip. *a*, Single shoot isolated from multiple shoots and used as an explant for further regeneration, the arrow mark indicates the whitish nodulation; *b*, Callus formation at the base of the shoot; *c*, Root development from isolated shoot explant grown in the rooting medium after 35 days.

Table 2. Effect of concentration and combination of 2iP, BAP and Kn on multiple shoot bud development from shoot tips of *G. superba* cultured for 35 days

Hormone concentration, 2iP + Kn (μM)	No. of shoots/ shoot tip explant*	Hormone concentration, BAP + Kn (μM)	No. of shoots/ shoot tip explant*
4.92 + 2.32	20.3 ± 0.6	1.11 + 2.32	21.1 ± 0.5
7.38 + 2.32	21.1 ± 0.8	2.22 + 2.32	22.0 ± 0.5
8.61 + 2.32	32.7 ± 0.4	3.33 + 2.32	25.3 ± 0.3
9.84 + 2.32	35.0 ± 0.1	4.44 + 2.32	34.0 ± 0.7

*Each value is the SD of three experiments with fifteen replications per experiment.

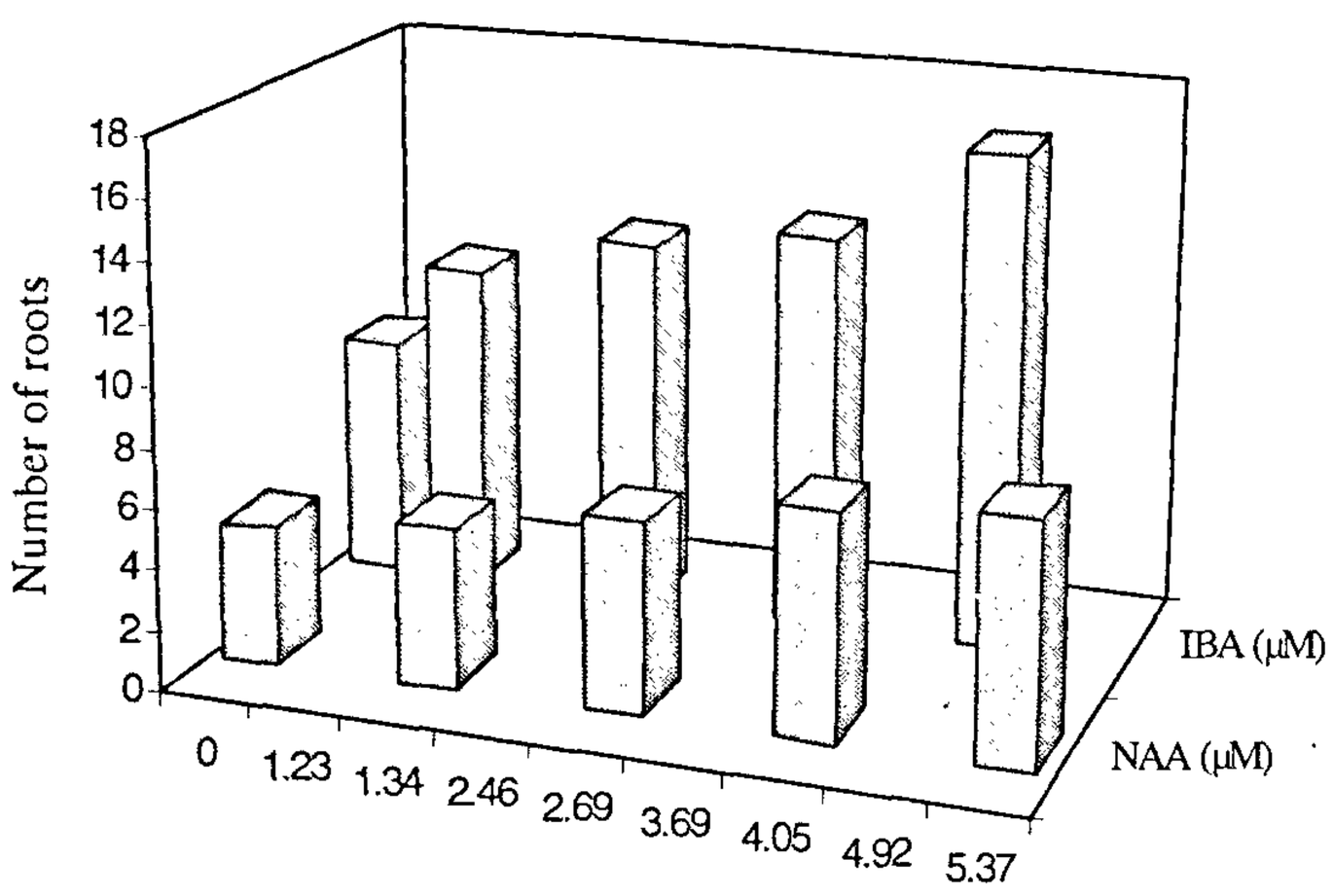

Figure 3. Effect of growth regulators on development of *in vitro* roots from multiple shoots. The number of roots differentiated from each shoot bud were scored after 5 weeks of culture on MS media containing IBA and NAA in various concentrations.

15 days of treatment with GA_3 which is known to have stimulatory effect on stem elongation in plants that do not elongate normally[8].

Roots were formed in media containing any of the auxins except indole-3-acetic acid (IAA); the latter when present in high concentration induced callus formation at the base of the shoots (Figure 2 *b*). Indole-3-butyric acid (IBA) in the range 0.492–4.92 and α-naphthaleneacetic acid (NAA) in the range 0.537–5.37 μM were found to be the best for root formation. A root system was formed within 4–5 weeks of culture. 93.3% of the explants in the rooting medium with IBA in the range 2.46–4.92 μM, and 84% explants in the same medium with IBA in the range 0.492–2.46 μM developed vigorous root systems (Figure 2 *c*). The mean number of roots produced in 93.3% of explants in a medium with 2.46–4.92 μM IBA was 16.5 while in 84% of explants in medium with 0.492–2.46 μM IBA it was 12.4. NAA in the range 2.69–5.37 μM and 0.537–2.69 μM, respectively produced roots in 82% and 70.6% of the explants (Figure 3). The mean number of roots induced in 70.6% of explants in a medium with 2.69–5.37 μM NAA was 6.3 while in 82% of explants in a medium with 0.537–2.69 μM NAA, it was 7.9.

In conclusion it may be stated that the procedures presented in this study yield efficient shoot and root regeneration from shoot tips and non-dormant corm buds. These results will encourage large scale micropropagation of this important and over-exploited medicinal plant.

1. Evans, D. A., Tanis, S. P. and Hart, D. J., *J. Am. Chem. Soc.*, 1981, **103**, 5813–5821.
2. Finnie, J. F. and van Staden, J., *Plant Cell Tissue Org. Cult.*, 1989, **19**, 151–158.
3. Murashige, T. and Skoog, F., *Physiol. Plant.*, 1962, **15**, 473–497.
4. Gamborg, O. L., Miller, R. A. and Ojima, K., *Exp. Cell Res.*, 1968, **50**, 150–158.
5. Somani, V. J., John, C. K. and Thengane, R. J., *Indian J. Exp. Biol.*, 1989, **27**, 578–579.
6. Chunsheng, L., Ruan, Y. and Bridgen, M., *Plant Cell Tissue Org. Cult.*, 1995, **42**, 219–221.
7. Ayabe, M. and Sumi, S., *Plant Cell Rep.*, 1998, **7**, 773–779.
8. Phinney, B. O., in *The Biosynthesis and Metabolism of Plant Hormones* (eds Coczier, A. and Hilman, S. R.), Cambridge University Press, 1984, pp. 17–41.

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Hydrocarbon source rock potentiality of black shale from Siang district, Arunachal Pradesh

The narrow belt of Gondwana sediments from Bhutan to Siang district of Arunachal Pradesh occurs as thrust sheets in the frontal zone of Eastern Himalayas. The Siang Gondwanas are bounded to the north by the NW–SE and ENE–WSW trending Siang Group metamorphics and is overriding the autochthonous upper Tertiary rocks along the Main Boundary Fault (MBF). The laterally persistent Siang Gondwanas of Garu–Gensi and Garu–Igo road-section may be subdivided into diamictites, black shale and oscillatory streaky facies in ascending order[1]. Acharyya *et al.*[2] termed them as Rangit Pebble Slate for the basal diamictites and the overlying Rilu member. The next succeeding Garu Formation