together can serve the water requirement of about 50 lakh people, i.e. 53% of the city population. Better management and judicious usage of the water resources available within the city will prevent the uneconomical and anti-environmental proposals like diversion of water from the west-flowing rivers like the Nethravati in the Western Ghats and/or from distant river systems like the Krishna.

Groundwater is now not a sustainable resource. It is susceptible to the vagaries of rainfall. It needs to be considered only as a stand-by resource to be used during scarcity or drought. The groundwater table should be allowed to revive up to the unconfined aquifer horizon. Also, there should not be exploitation of groundwater resources beyond 60% of the corresponding annual groundwater recharge.

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Brick pieces soaked in liquid culture medium – a new matrix for seed germination and plantlet development for orchid *Flickingeria nodosa* (Dalz.) Seidenf.

In vitro seed germination, growth and development of plants are largely determined by the composition of the culture medium. The main components of most plant tissue culture media are mineral salts and sugar as carbon source. Other components may include organic supplements, growth regulators and a gelling agent[1,2]. The growth of cultures and production of shoots or roots are strongly influenced by the physical consistency of the culture medium. Gelling agents are usually added to the culture medium to increase its viscosity because of which plant tissues and organs remain above the surface of the nutrient medium[3]. The most commonly used gelling agent in plant tissue culture media is agar due to its stability, high clarity, non-toxic nature and resistance to plant metabolites[4–6]. Several attempts have been made to look for an alternative gelling agent as agar is expensive and also due to the threat of exhausting the sources of agar. Some of these alternatives include starches and plant gums[7,8], alginates[3,9], gelrite[10], agarose[11], isubgol[12], starch[13–15], cotton fibre[16], glass wool[17], polystyrene foam[6], glass beads[14,18], filter paper[17] and glass marbles[19]. Furthermore, several alternative supporting matrixes have been tried for *in vitro* germination of orchid seeds and nodal culture using glass beads for *Vanilla* nodal culture[3]; polyurethane foam disc, coconut coir, betel nut coir, leaf litter for asymbiotic seed germination and plant regeneration of *Cymbidium aloifolium*[20]. But, most of these materials are less explored in application than agar and have their own limitations. Hitherto, there is no report on the use of brick pieces as supporting matrix for the germination and development of orchids. However, brick pieces and charcoal are the materials that are being used for *in vivo* cultivation and hardening of orchid plants. Keeping this in view, an economical method with brick pieces soaked in liquid culture medium has been formulated for *in vitro* seed germination and development of a medicinally important orchid, *Flickingeria nodosa*. Furthermore, not much work has been documented on the *in vitro* studies of *F. nodosa*.

F. nodosa (Dalz.) Seidenf. is a medicinal epiphytic orchid found in the Eastern Himalayas, Sri Lanka and in parts of the Western Ghats in India, namely Kodagu, Hassan, Uttara Kannada and Udumbansholai[21,22]. It has a creeping rhizome sending erect pseudobulbous greenish-yellow or yellow shoots and occurring in large colonies on tree trunks. The white flowers with pink lips produced singly or in twos last only for a day. The sidelobes of the lips are deeply spotted with violet-pink, and midlobe is white with red markings[21,22].

Bricks were collected from the field for preparation of the supporting matrix. They were broken into small pieces of approximately 1 cm and sieved to remove the mud particulates. Brick pieces were surface-sterilized by thoroughly washing several times in running tap water to remove the mud particulates. The brick pieces were then soaked in 2% (w/v) potassium permanganate solution for 30 min to kill the fungal spores that might be present in them, and rinsed several times with running tap water and twice with 70% ethanol to remove the traces of potassium permanganate. They were immersed in sterile water and the pH was adjusted between 5.6 and 5.8 as the brick pieces may have some acidic or

basic ions that may affect the pH of the medium. After adjusting the pH, the water was decanted and the brick pieces were autoclaved at 121°C with 15 lb/in^2 for 15 min. They were then dried in the hot-air oven at 180°C for 1 h.

Four different types of media, i.e. Vacin and Went basal liquid medium (VWBLM)[23], Knudson C basal liquid medium (KCBLM)[24], Burgeff's N3F basal liquid medium (BN3FBLM)[25] and Murashige and Skoog's basal liquid medium (MSBLM)[26] with 2% sucrose were prepared using the standard procedure. The pH was adjusted between 5.6 and 5.8 and 15 ml of the medium was dispensed into a culture bottle. Then the sterilized 7–8 brick pieces were added into the culture bottles containing the medium. These culture bottles were autoclaved at 121°C with 15 lb/in^2 for 15 min. The four different media with 2% sucrose and 1% agar were also prepared as control and autoclaved.

Artificial pollination technique was employed for fruit setting of *F. nodosa* (Dalz.), wherein the pollinia from the flowers were removed and placed on the sticky surface of the stigma. The mature pods were harvested after 60 days of pollination, washed thoroughly under running tap water, surface-sterilized using 2% (v/v) Savlon, 6–8 drops of Tween-20 for 15 min, rinsed with 70% ethanol for 30 s, disinfected with 0.05% (w/v) $HgCl_2$ for 6 min and rinsed in sterile water several times to remove traces of $HgCl_2$. The sterilized pods were longitudinally excised on a sterile petri plate and the seeds were scooped out. A small portion of the scooped out seeds was subjected to differential staining technique[27] using 2,3,5-triphenyl-2-H-tetrazolium chloride (TTC-$C_{19}H_{15}C_1N_4$) to confirm the viability of seeds and their frequency was calculated. In the present study, it was observed that the embryo of viable seeds turned red in colour due to conversion of TTC to triphenyl formazone (TF) and seed coat became green due to malachite green dye (Figure 1 *a*) with 35% viability, whereas the embryo in non-viable seeds did not turn red and only seed coat turned green. This is an agreement with the findings of Singh[27] and Gayatri *et al.*[28]. The other portion of the scooped out seeds was immersed in the respective liquid medium prepared separately and shaken thoroughly for uniform mixing of the seeds.

The liquid medium containing seeds (0.5 ml) was inoculated into the culture bottles containing different types of culture media such as VWBLM, KCBLM, BN3FBLM, MSBLM and brick as a supporting matrix using micropipette separately. Some of the seeds were cultured on the same medium gelled with agar as control. The cultures were incubated in the culture room as stationary cultures at a temperature of 25 ± 2°C under built-in white fluorescent light at a photon flux density of 30–50 μEm^{-2} s^{-1} under a photoperiodic regime of 16 h light and 8 h dark cycles. The medium that revealed the highest germination response was chosen for differentiation and development of protocorm-like bodies (PLBs). The chosen fresh liquid basal

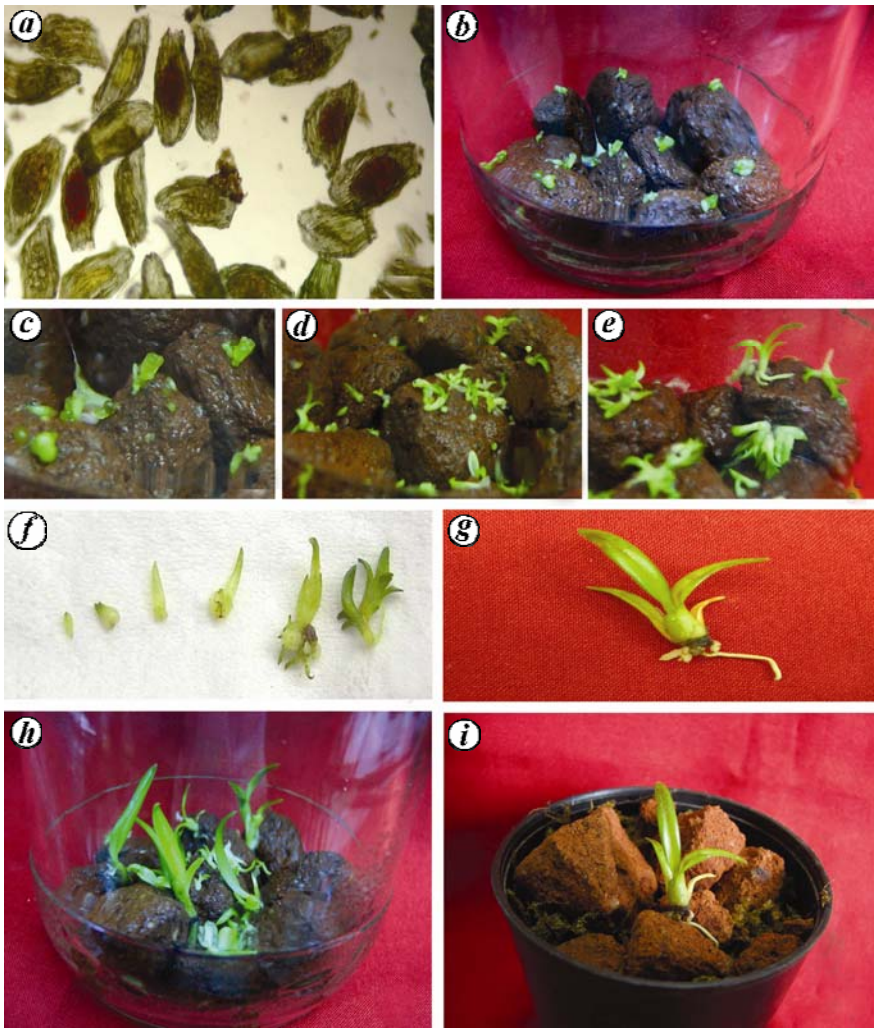

Figure 1. *In vitro* seed germination of *Flickingeria nodosa* on brick-based liquid medium. ***a***, Seeds showing viable and non-viable embryos. ***b***–***e***, Developmental stages of protocorm-like bodies (PLBs) and shoot development. ***f***–***g***, Developmental stages of the PLBs into plantlets. ***h***, Formation of complete plantlets and ***i***, Hardened plantlet.

Table 1. Effect of different nutrient media on *in vitro* seed germination of *Flickingeria nodosa*

Type of nutrient media	Culture duration (days)	Germination response $\bar{X}$* ± SE
VWBLM	35	70.2 ± 0.60
KCBLM	35	68.5 ± 0.76
BN3FBLM	28	95.4 ± 0.88
MSBLM	60	19.2 ± 1.36

*Mean of 10 replications; SE, Standard error.

Table 2. Effect of different combinations and concentrations of growth regulators on shoot and root formation from protocorm-like bodies of *F. nodosa*

Basal liquid medium	Culture duration (days)	6-Benzylamino purine (μM)	Kinetin (μm)	Naphthaleneacetic acid (μM)	No. of shoots/ explant $\bar{X}$* ± SE	No. of roots/ explant $\bar{X}$* ± SE
BN3FBLM	135	2.22	0	2.68	0	0
	135	4.44	0	2.68	2.7 ± 0.67	2.4 ± 0.49
	135	6.66	0	2.68	4.9 ± 0.93	5.8 ± 0.74
	135	8.88	0	2.68	3.4 ± 0.52	2.1 ± 0.57
	135	2.22	0	5.37	0	0
	135	4.44	0	5.37	1.9 ± 0.74	1.3 ± 0.83
	135	6.66	0	5.37	1.5 ± 0.71	2.2 ± 0.67
	135	8.88	0	5.37	1.2 ± 0.62	2.4 ± 0.57
	135	0	2.32	2.68	0	0
	135	0	4.64	2.68	1.5 ± 0.24	2.74 ± 0.28
	135	0	6.96	2.68	1.6 ± 0.20	3.2 ± 0.24
	135	0	9.28	2.68	1.9 ± 0.17	2.5 ± 0.29
	135	0	2.32	5.37	0	0
	135	0	4.64	5.37	1.3 ± 0.75	1.1 ± 0.74
	135	0	6.96	5.37	2.3 ± 0.43	1.4 ± 0.79
	135	0	9.28	5.37	2.5 ± 0.59	1.2 ± 0.52

*Mean of 10 replications; SE, Standard error.

medium was used to replace the spent medium every 2–3 weeks with the addition of different combinations and concentrations of growth regulators such as 6-benzylaminopurine (BAP) (2.22–8.88 μM), kinetin (Kn) (2.32–9.28 μM) and naphthaleneacetic acid (NAA) (2.68–5.37 μM) in the same culture bottle without disturbing or removing the culture. The cultures were monitored regularly and analysed statistically. For each treatment ten replicates were used and each experiment was repeated thrice.

The first response of asymbiotic seed germination was noted by the change in colour and shape of the viable seeds. These seeds were found swollen within 15 days of culture and attained a globular shape after 28 days of culture. These globule-shaped structures soon developed into PLBs (Figure 1 *b–e*) with varied percentage of germination response (Table 1). However, the best germination response of 95.4 ± 0.88 was found on BN3FBLM (Table 1). This is in agreement with the findings of Pathak *et al.*[29] and Gayatri *et al.*[28], who have reported similar results of germination response in *Goodyera biflora* and *Pecteilis gigantea* respectively. Further, the present study reports the asymbiotic seed germination of *F. nodosa* on basal medium devoid of growth regulators, which is not in accordance with the findings of Deb and Pongener[20], who have reported the necessity of synergistic effect of NAA and BA for asymbiotic seed germination of *C. aloifolium*.

The developing PLBs were provided with VWBLM, KCBLM, BN3FBLM, MSBLM supplemented with different combinations and concentrations of growth regulators in the same bottles for formation of shoots and roots. The first leaf development from PLBs was observed after 48 days of culture that multiplied subsequently to form complete plantlets after 105 days of culture (Figure 1 *f* and *g*). Rapid shoot (4.9 ± 0.93 shoots/explant) and root (5.8 ± 0.74 roots/explant) formation was observed on BN3FBLM fortified with 6.66 μM BAP and 2.68 μM NAA (Table 2). This observation corroborates the findings of Vij and Pathak[30]. However, the frequency of shoot and root formation was found to be very low for all the other concentrations and combinations of growth regulators tried. Further, in the present study, NAA was used as the sole auxin to produce roots. Similar results have been reported in several native Hong Kong orchids by Yam and Weatherhead[31].

The initiation of PLBs and the subsequent shoot development were much faster on brick pieces soaked with liquid basal medium than on agar-gelled medium. This is in agreement with the findings of Moraes-Cerdeira *et al.*[16], who have reported similar results using cotton fibres. Furthermore, in epiphytic orchids the roots will be of aerial type with the velamen tissue, because of which aeration and the space required for root development and anchorage will be better and uptake of the nutrients by the plant will be more. The brick pieces soaked in liquid culture medium have an additional advantage over agar-gelled medium as the medium can be replaced without disturbing the aseptic plantlets, as in the case of glass beads-based liquid medium[6]. Yet another advantage of using bricks as a supporting matrix is that the cost of the medium is reduced by 70–82%, in agreement with the findings of Prakash[14] and Kavyashree *et al.*[32], and also makes the hardening and acclimatization process easier.

The complete plantlets thus obtained were subjected to hardening wherein the aseptic plants adhering to the brick pieces were taken out of the culture bottles and rinsed several times with sterile water to remove traces of nutrient media to avoid microbial contamination that affects the further growth of the plantlets. The washed bricks along with the plantlets were then placed in pots containing brick, charcoal and moss, and were maintained in the hardening chamber under controlled conditions. The hardened and acclimatized complete plantlets (Figure 1 *i*) were successfully established in the greenhouse with 85% survival frequency.

The use of brick pieces soaked in liquid culture medium is an economical,

novel and reproducible protocol for germination and development of orchids. This protocol is a viable technique for large-scale multiplication of orchids.

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Impact of chilling injury on common fruit plants in the Doon Valley

The response of plants to the severities of the environment have occupied the attention of man long before the beginnings of the science of biology[1]. Biological stress may be defined as any environmental factor capable of inducing a potentially injurious strain in living organisms, which may be either a physical or chemical factor, however, any of these factors occur in severe form which lead to permanent damage to plant or organism. Plants are poikilotherms – they tend to modify their body temperature according to prevailing temperatures at their site. Tropical and subtropical plants exhibit a distinct physiological damage when they are exposed at low temperatures below 10°C (ref. 2). This is referred to as 'chilling injury'. It is observed at temperatures above 0°C and is quite distinct from freezing injury. This is mainly associated with physical disorders caused by freezing of water. Although chilling injury can be induced in complete darkness, it is more marked under weak light[3]. Chilling injury in light is regarded as a kind of photoinhibition[4]. Chilling injury can occur under certain distinct characteristics of lower threshold temperature below which it is induced in the plant[5,6]. It causes damage to the chloroplast and affect photosynthetic activities which are irreversible in nature, and leads to reduction in the rate of photosynthesis. Thereafter, visible symptoms develop in the plants after several days of hidden chilling injury.

Chilling injury may lead to indirect damage to plants. These may include starvation, in which the respiration rate may exceed the rate of photosynthesis, which gets reduced due to damage to