Figure 1. Prior arts mentioning *Narcissus tazetta.*

in the treatment of alopecia through local application. Another art taken from *Al-Jaame'-li-Mufradaat-al-Advia-wal-Aghzia*[8] refers to a formulation containing *N. tazetta* as a single ingredient used in the treatment of alopecia through local application.

The alleged invention claims the use of *N. tazetta* for the prevention and treatment of damaged skin resulting from conditions like acne vulgaris, atopic dermatitis, alopecia, vitiligo, pruritus, eczema, etc. However, *N. tazetta* has been used singly and in combination with other constituents for treating alopecia, pruritus and vitiligo through local application as is seen in the prior art.

Similar is the case of neem, which has been used in the Unani system of medicine for thousands of years. In 1994, a US Department of Agriculture granted a patent for a fungicide made from neem oil. The decision brought significant opposition from NGOs and environmental organizations all over the world and in May 2000, The European Patent Office agreed to withdraw the patent confirming that 'nothing has been invented, and that knowledge and use of neem has been widespread in India and elsewhere for many decades'.

Two other cases are worth mention – one of turmeric (*Curcuma longa*) patent granted in March 1995 which was revoked on grounds of lacking the inventiveness, and another of Basmati rice, where a US patent granted was condemned as 'biopiracy' by a worldwide coalition of 90 civil society organizations.

India and five other countries have demanded that in order to prevent biopiracy, protection of the components of biodiversity and associated traditional knowledge from theft, they must be integrated within the framework of the World Trade Organization's Trade-Related Intellectual Property Rights agreement.

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In vitro culture of *Trithuria konkanensis*, one of the smallest angiosperms

The scope for progress and achievements in plant improvement strategies in recent years is widening remarkably. Diversification in the morphology, physiology and genetic nature of the plants offers incredible opportunities for identification, isolation and characterization of new genes as well as to understand basic linkages among diverse plant groups. In this context, angiosperms are the largest plant group consisting of plants with extensive assortment and multiplicity. *Trithuria* is one of the smallest angiosperms belonging to the family Hydatellaceae and is an ideal plant for biotechnological research and innovations. Hydatellaceae is considered as an early member of the angiosperm phylogenic tree consisting mainly of dwarf aquatics of a single *Trithuria* genus. Earlier reports on this plant family have described seedling diversity[1], ovule and megagametophyte development[2], and its placement in the phylogenic tree[3]. *Trithuria* is a diminutive, moss-like semi-aquatic plant and is the closest living relative of water lilies and their allies. However, *Trithuria* differs from water lilies in that all species are extremely small and most have an annual life form and grow as semi-aquatic or aquatic. Yadav and Janarthanam[4] located this plant genus for the first time in Sindhudurg district, Maharashtra, India and named the plant as *Trithuria konkanensis*, which is an annual herb of only a 1.3 cm height (Figure 1 *a–d*). The morphological features of the plant, its habitat ecology and development have been described in the literature[4–6].

In vitro culture of plant cells, particularly plant like *Trithuria*, makes it feasible

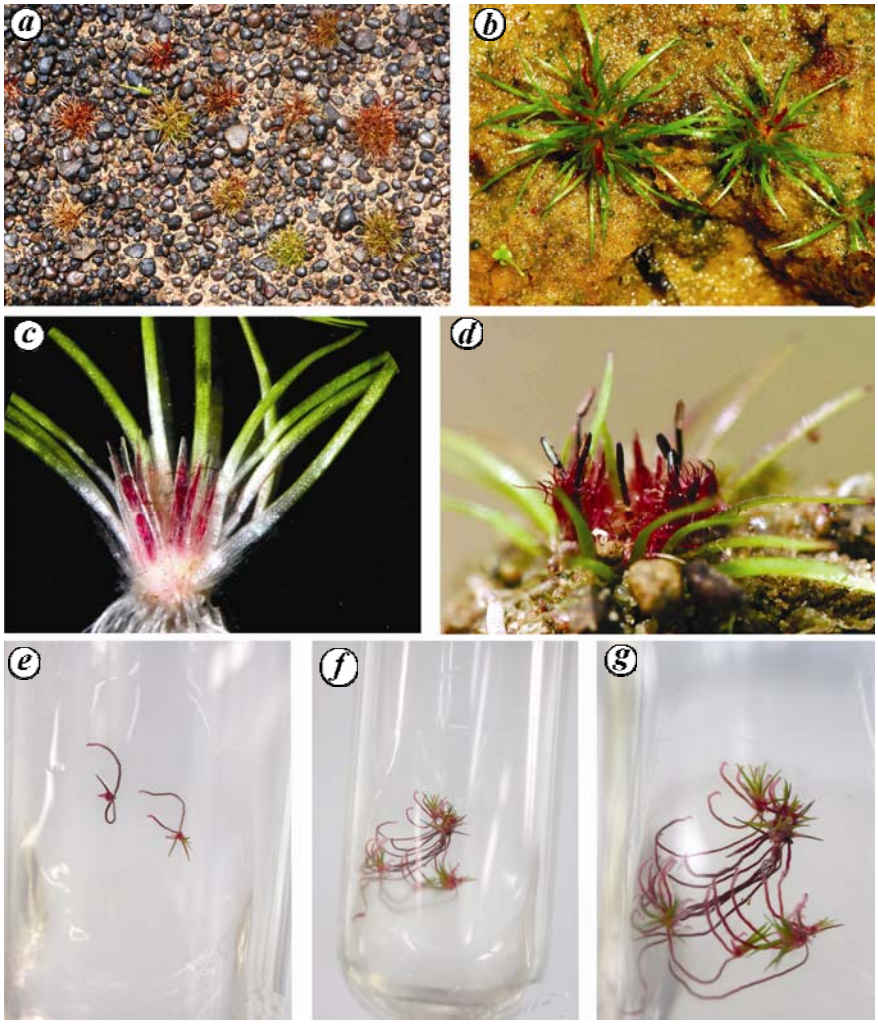

Figure 1. *In vitro* culture of *Trithuria konkanensis* S.R. Yadav and Janarthanam. ***a***, Plant in its natural habit. ***b***, Plants maintained in the garden. ***c***, Plants with young inflorescence. ***d***, Plants in flowering showing stigmatic hairs and stamens. ***e***, Young seedlings in culture medium. ***f***, Fully grown plants in culture medium. ***g***, Enlarged view of cultured plant.

to exploit the potentiality of the plant in a precise and accurate way. Besides rapid multiplication, *in vitro* culture of *Trithuria* will facilitate to understand more easily and conveniently the growth and development of this tiny plant. Against this background, attempts were made to bring this plant material under aseptic conditions and to study parameters to establish successfully *in vitro* cultures.

It was difficult to raise the cultures of this plant owing to its miniature size and fragile nature. The collection of this plant material from natural surrounding requires skilled hands and watchful eyes. The plant grows in semi-aquatic surroundings and separation of the plant from water has to be carried out gently and softly. After collection, the plants were kept in a tray with soil moistened with water and maintained in a poly house. The miniscule seeds were cautiously separated from dried plants under a microscope and transferred to an ependorf vial. It is necessary to wash the seeds repeatedly in clean water; however, escape of seeds during washing has to be avoided. The seeds were then washed in autoclaved water repeatedly, followed by treatment with $HgCl_2$ for 2–3 min. Traces of $HgCl_2$ were removed by rinsing the seeds in autoclaved water several times. Then the seeds were cultured on 1/2 MS nutrient medium slants[7]. The cultures were kept in a culture room having 16 h light and at 25°C temperature. The percentage of contaminated cultures was very high and only a few clean cultures were available for further studies. The seeds were transferred periodically to a fresh medium of the same composition. No germination was recorded for the first 45 days of culture, after which the seeds showed indication of germination. However, growth of seedlings was not rapid and it took another three months to have well-developed plants with proper roots and shoots (Figure 1 *e–g*). The established culture will be multiplied further *in vitro* to generate a large number of cultures followed by rooting of the plants and subsequently to create a large population of plants under laboratory conditions for further studies.

Genes controlling dwarf and other morphological traits of this plant will be a useful source for edible crops modifications as has been demonstrated in other plants of *Arabidopsis*, tomato, rice and maize[8,9]. The work on dwarf characteristics of *Trithuria* plant will enhance knowledge on the molecular genetics bases of the plant's architecture. Among the numerous factors controlling the increase in yield potential of crop plants, genes that manage plant height and other architecture features have been recently identified[10]. Hence, establishment of *in vitro* cultures as reported here will help in the conservation of this tiny angiosperm.

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