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Systematic review on micropropagation of *Aloe barbadensis* Mill a medicinal lily of desert

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Abstract
Gwarpatha (*Aloe barbadensis* Mill.) grown in warm tropical areas and is one of the most studied perennial shrubs worldwide. It has valuable medicinal properties and commercially used in pharmaceuticals, cosmetics, food industries and for many herbal preparations. It contains antioxidant vitamins which prevent damages caused by free radical, reducing risk of chronic diseases such as cancer, heart diseases and stroke. In nature *Aloe barbadensis* is propagated through lateral shoots which shows slow growth and are low in numbers and cultivation practice is expensive. For its large scale plantation there is a need to develop suitable and alternative method like *in vitro* micropropagation to meet the demand of farmers and pharmaceutical industries. Auxins, cytokinins and auxin-cytokinin interactions are considered to be the most important for regulating growth and organized development in tissue and organ cultures, as these two classes of hormones are generally required for *in vitro* propagation of gwarpatha. The present study aimed to develop collective information on protocol for rapid and high frequency *in vitro* propagation of *Aloe barbadensis*, looking through its increasing demand for pharmaceutical industry at global level.

Keywords: *Aloe barbadensis*, explant, Gwarpatha, *In vitro* culture, lily of desert, micropropagation, root induction, shoot induction

1. Introduction
Aloe barbadensis synonymous to *Aloe vera* belongs to family *Asphadelaceae* [35] commonly known as "Gwarpatha", "Medicine Plant", "Burn Plant", "First Aid Plant", "Miracle Plant", "Lily of Desert" and "Ghritkumari" in Ayurveda [38]. It is an ancient, semi tropical desert medicinal plant indigenous to Africa, Madagascar and Arabia and introduced in India [8, 29]. Internationally, *Aloe barbadensis* is grown largely in South Texas of United States of America, India, Mexico, Central America, Australia and Africa. In India it is cultivated in Rajasthan, Gujarat, Haryana, Andhra Pradesh, Tamil Nadu and Maharashtra. In Rajasthan it is cultivated in Churu, Sikar, Jaipur, Ajmer, Bikaner, Barmer, Jaisalmer, Jodhpur, Kota, Hanumangarh and Jhunjhunu districts. In nature (*in vivo*), *Aloe vera* is propagated through lateral buds which are slow, very expensive and low income practice [26]. Sexual reproduction by seeds due to male sterility in *Aloe vera* is almost ineffective and vegetative propagation through lateral shoots only possible during growing seasons [18]. However, the number of lateral shoots/donor plant is low and so it is difficult to plan in a rational basis a production system in commercial scale for obtaining plant propagation materials. Thus large scale plantation of *Aloe vera* through *in vivo* system of plantlet multiplication is insufficient to meet the requirements of farmers and pharmaceutical industries demand [5]. Therefore, there is a need to develop suitable and alternative method for large scale propagation like *in vitro* propagation for rapid plant multiplication to meet the demand of farmers and pharmaceutical industries [2, 22]. The technique of tissue and organ culture is used for rapid multiplication of plants, for genetic improvement of crops, for obtaining disease free clones and for preserving valuable germplasm [6]. One of the major applications of plant tissue culture is micropropagation or rapid multiplication. Compared to conventional propagation, micropropagation has the advantage of allowing rapid propagation in limited time and space. To overcome slow propagation rate, micro propagation will be a very useful technique for mass multiplication of *Aloe*. Therefore, the present study aimed to develop collective information on protocol for rapid and high frequency *in vitro* propagation of *Aloe barbadensis*, looking through its increasing demand for pharmaceutical industry at global level.

2. Importance of *Aloe barbadensis*

Aloe barbadensis has valuable medicinal properties and is commercially used in pharmaceuticals, cosmetics, food industries, as nutraceuticals and for many herbal preparations. There are about more than 40 *Aloe* based formulations being marketed in the global market. Plant contains important antioxidant vitamins (A, C and E), B (thiamine), niacin, B_2 (riboflavin), B_{12} (cobalamin), choline and B_9 (folic acid) [16], which prevent damages caused by free radical, reducing risk of chronic diseases. Natural antioxidants increase the antioxidant capacity of the plasma and reduce the risk of certain diseases such as cancer, heart diseases and stroke [31]. The secondary metabolites like phenolics and flavonoids from plants have been reported to be potent free radical scavengers [25].

3. Micropropagation technique

Micropropagation is one of the innovative methods of asexual propagation, which proved to be effective for *in vitro* propagation of medicinal, endangered and horticultural plants [10]. In micropropagation cultures are started with very small pieces of plants (explants) and thereafter small shoots or embryos are propagated hence the term 'micropropagation' to describe the *in vitro* methods. Through micropropagation we may produce plants free from specific viruses and diseases in large numbers. Micropropagation may facilitate the flexible adjustment of factors influencing vegetative regeneration such as nutrient, growth regulator levels, photoperiod and temperature. A number of nutrient medium are available but MS medium proved best culture media for *Aloe barbadensis* propagation. This may enable newly selected varieties to be made available quickly and widely, and numerous plants to be produced in a short while. The technique is very suitable when high volume production is essential. Slow growing plants are possible to be cloned rapidly with less energy and space requirement for watering weeding and spraying etc [13].

3.1 Explant preparation

Preparation of explant requires thoroughly washing in running tap water for 10 minutes than washed with liquid detergent for ten minutes with vigorous shaking. After washing with detergent, explants were again washed with running tap water to remove any trace of detergent for 15 minutes. Explants were surface sterilized with 0.1% $HgCl_2$ for 7-10 minutes in laminar air flow cabinet. These were thoroughly washed thrice with sterilized distilled water and inoculated on the culture media supplemented with plant growth regulators. Micro propagation using stem and lateral shoot pieces of *Aloe vera* had already been proved successful [3]. However, source of explants, size, age, genotype, media composition, culture conditions, phenolic content of explants, exogenous supply of the plant growth regulators and media discoloration greatly affect shoot regeneration from different genotypes of the same species.

3.2 Role of plant growth regulators

Auxins, ABA, cytokinins, ethylene and gibberellins are commonly recognized as the five main classes of plant hormones. Auxins, cytokinins, and auxin-cytokinin interactions are usually considered to be the most important for regulating growth and organized development in plant tissue and organ cultures, as these two classes of hormones are generally required [9]. Auxins exert a strong influence over processes such as cell growth expansion, cell wall acidification, initiation of cell division, organization of meristems giving rise to either unorganized tissue (callus) or defined organs (generally roots) and promote vascular differentiation. In organized tissue, auxins appear to be key players in maintaining apical dominance, affecting abscission, promoting root formation, tropistic curvatures, delaying leaf senescence, and fruit ripening [4, 37]. Two major properties of cytokinins that are useful in culture are stimulation of cell division (often together with auxins) and release of lateral bud dormancy. They can also induce adventitious bud formation [21]. Cell division is regulated by the joint action of auxins and cytokinins, each of which influences different phases of the cell cycle. Auxins affect DNA replication, whereas cytokinins seem to exert some control over the events leading to mitosis and cytokinesis [30, 39]. Thus auxin and cytokinin levels in cultures need to be carefully balanced and controlled.

3.3 Shoot and root induction

Due to slow multiplication rate of plantlets under natural conditions and increasing demand of *Aloe barbadensis* for its pharmaceutical uses its required demand of plantlets for large scale plantation cannot be fulfilled, hence there is a need for mass propagation of this plant through *in vitro* methods [22]. Surafel *et al.* (2018) [36] reported MS media supplemented with 0.6 mg/l BAP and 0.2 mg/l of IBA, 1.0 mg/l BAP and devoid of hormone were optimum for *Aloe vera* establishment, multiplication and rooting respectively. The higher mean number of shoots and multiplication factor were observed on MS media with 0.2 mg/l IBA and 1.0 mg/l BAP respectively [15, 17]. Kumari *et al*. (2015) [23] studied to assess the effect of different hormones like auxin and cytokinin on regeneration potential of rhizomatous stem and leaf segment used as explants of gwarpatha. Best shoot proliferation (6-7 shoots/ explants) was obtained on MS medium containing 2.5 mg/l BAP. Optimum growth of callus was achieved from rhizomatous stem and leaf segment explants on medium containing NAA+BAP+ IBA (2.5+2.0+0.5 mg/l). The best rooting of micro shoots were obtained on shoot regenerating medium containing both BAP and NAA, 2.5mg/l each [32].

Biswas *et al.* (2013) [7] observed that rooting percentage was improved in presence of low concentrations of IBA and NAA and do not support 100 per cent rooting in gwarpatha in hormone free medium. No adventitious roots were initiated in auxin free media. Old leaves and shoots greater than 10 cm in size did not induce adventitious roots under any conditions. NAA (0.5 mg/l) was most effective for bringing about improvements in induction rate (90 per cent), 5.2 numbers of adventitious roots per explant during six weeks of culture. Khanam and Sharma (2014) [19] observed maximum root induction in MS medium supplemented with 2.0 mg/l IBA and 1.0 mg/l NAA in combination.

For shoot multiplication, BAP was found more effective for shoot bud development than Kn and adenines in *Aloe barabadensis* [34]. There was no sign of meristematic shoot bud proliferation when explants were cultured in media without cytokinin. The optimum ranges of cytokinins for shooting were found 0.5-4.0 mg/l depending upon the type of explant, genotype and exposure of light and considering other factors. Further, increase in the concentration of either BAP or Kn alone or in combination had no effect on the rate of shoot bud development or multiplication. However, auxins (IBA and NAA) were found the best media for root formation with 0.5-1.0 mg/l concentration [14]. In some cases IBA was found best for induction of roots followed by IAA [11, 20].

3.4 Effect of explants

Explants are small pieces of plant parts or tissues that are aseptically cut and used to initiate a culture in a nutrient medium. The type of explants, size, position, age, physiological state and the manner in which it is cultured can affect the initiation of the cultures and further morphogenetic response [27]. Often there is an optimum size of explant suited to initiate cultures. Very small shoot tips or fragments do not survive well while it is difficult to decontaminate larger explants. Larger the sizes of the meristematic tissues are advantageous. Selecting juvenile parts of the plants yield better than older ones. The size of explant is also likely to influence the uptake of mineral salts irrespective of whether it is grown on liquid or solid medium [12]. Types of explants are one of the important factors in optimizing the tissue culture protocol like leaf, petiole, cotyledonary leaf, hypocotyl, epicotyl, embryo, internode, inflorescence axis and root explant significantly effect on tissue culture process of plants [24, 33]. This may be due to the different level of endogenous plant hormones present in the plants parts. Lateral shoot explant with sheath found better than without sheath for shoot multiplication [1]. Kiran *et al.* (2017) [20] reported leaf tip, spine did not show any response whereas, apical shoot multiplied. Among all explants, the lateral shoots and apical bud explants gave the best results and were used for further experiments.

4. Acclimatization of *in vitro* produced plantlets

The plantlets obtained through micropropagation should have roots that are capable of supporting further growth and development. They are usually transplanted into hardening media and kept in partial shade at a high ambient humidity for several days. The term media is sometimes used to describe the mixture of materials such as peat, perlite, vermiculite, rockwool, sand and soil used for transferring the plantlets from *in vitro* conditions. Compost which is commonly used for rooting conventional cuttings is suitable for transferring these plantlets, but there may be marked differences in root growth and plantlet survival with different media. Regenerated plantlets of *Aloe barbadensis* of 6-8 weeks old with well developed roots when removed from culture vessels, washed thoroughly with the tap water to remove the Agar media, the roots were treated with 0.2 per cent bavistin for 30 to 45 seconds and transferred to sterilized cocopeat in poly house and after 1 week, plants were transferred to sterilized earthen pots containing a mixture of sand, FYM, soil at a ratio of 1:2:1 and finally the potted plants were kept in the net house for acclimatization before transfer to the open field, watered at two days interval, more than 85 per cent of the potted plants survived after one month of transfer and could be successfully transferred to the field [20]. Plastic pots or cups containing garden soil or sterile sand and farmyard manure can be used for hardening prior to their final transfer to the field showed good percentage of survival (95 per cent) in both polyhouse and shade house [5, 14, 24, 28]. Filter cake and sand in 3:1 ratio gave 92.59 percent survival in rooted plantlets after fifth week [36]. Application of these protocol helps to propagate thousands of *Aloe barbadensis* plants from few initial mother plants in a year period.

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