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Comparative efficacy of different culture media on callus proliferation and regeneration of *Aloe barbadensis*

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Abstract
Aloe barbadensis is a desert medicinal herb, immensely useful for pharmaceutical and cosmetic industries. Its micropropagation through indirect organogenesis under *in vitro* condition highly influences with the composition of culture media. In the present investigation, the efficacies of different types of media *viz.* Nitsch and Nitsch, Woody plant medium (WPM), Schenk and Hildebrant, Whites medium, Knudson Solution–C and, Murashige and Skoog (MS) medium were evaluated for indirect organogenesis. For callus induction using lateral shoot explant, media were fortified with 2,4-D (2.5 mg/l) while, a combination of 2.0 mg/l Kn + 1.0 mg/l NAA were used for *de novo* shoot regeneration in callus culture. Significant differences were obtained among all media compositions for all the characters studied for callus proliferation and adventitious shoot regeneration. Woody plant medium were found best for callus proliferation (3.42 g) and number of shoot regeneration (3.40). The efficacies of MS medium were also high for callusing (3.38 g) and shoot regeneration (3.30) after Woody plant medium. The rank of the different media for their relative effectiveness for callus proliferation and organogenesis can be ordered as Woody plant medium > MS medium > Nitsch and Nitsch medium > Whites medium > Knudson Solution-C = Schenk and Hildebrandt medium. These media, Woody plant medium and MS medium interchangeably can be utilized for large scale shoot multiplication of *Aloe barbadensis.*

Keywords: Aloe, callus, culture media, micropropagation, ms medium, organogenesis, woody plant medium

Introduction
Aloe barbadensis Miller is an ancient succulent medicinal plant commonly grown in warmer regions. It is synonymously to *Aloe vera* (L.) and commonly known as Gwarpatha, First aid plant, Desert Lily and Ghritkumari in Sanskrit (Tanabe *et al.* 2006; Ahmad *et al.* 2020) [1, 2]. Due to its curative medicinal properties, Aloe gel widely used in pharmaceutical and cosmetic industries for making medicine and various herbal preparations. More than 40 well known Aloe based formulations are being marketed in India and worldwide (Jakhar *et al.* 2020) [3]. Bioactive ingredient of Aloe gel are rich sources of antioxidant vitamins (A, B, C, E) and characterized with anti-inflammatory, anti-cancer, anti-viral and anti-bacterial properties commonly used in treatment of cancer and heart diseases (Prior and Cao 2000; Jayakrishna *et al.* 2011; Ahmad, 2020) [4, 5, 2]. Gwarpatha traditionally grown by using naturally developed lateral shoots which is a tedious and expensive method of cultivation (Abrie and Staden 2001, Bhandari *et al.* 2010, Kumari and Naseem 2015) [6, 7, 8]. Alternatively, multiplication through *in vitro* culture technique can provide a better solution for large scale propagation of this perennial herb. The micropropagation highly depended on the culture condition, type of explant and genotype of plant (Murashige and Skoog 1974, Dixit *et al.* 2020) [9, 10]. Composition of media is one of the major factors affecting the regeneration process under control condition. A media is composed of major and micronutrient, carbon and nitrogen source, solidifying agent (usually agar) and vitamins. However, proportion of these component vary in different types of media such as Murashige and Skoog (MS) medium (Murashige and Skoog 1974) [9], Woody plant medium (Lloyd and McCown 1980) [11], Nitsch and Nitsch medium (Nitsch and Nitsch 1969) [12], Schenk and Hildebrandt medium (Schenk and Hildebrandt 1972) [13], Knudson Solution–C and Whites medium. The energy and nutrient requirement of plant also differ for different plant species. Therefore, an appropriate culture condition is a prerequisite for elevated propagation of plant under *in vitro* condition.

In the present investigation, comparative efficacies of different culture media were tested for callus proliferation and organogenesis of *Aloe barbadensis.*

Materials and Methods

The present investigation was carried out at Tissue Culture Laboratory (TCL) of department of Plant Breeding and Genetics, S.K.N. College of Agriculture, Sri Karan Narendra Agriculture University Jobner, Rajasthan, India, during academic year 2019-2020. Standardized protocols were followed for sterilization of different equipments and preparation of culture media. All chemical used were analytical grade. Total six types of culture media namely Murashige and Skoog (MS), Woody plant medium (WPM), Nitsch and Nitsch (NN), Schenk and Hildebrant (SH), Whites medium and Knudson Solution–C (KS) were evaluated for callus proliferation and organogenesis in callus culture. For callus induction, lateral shoot explant of *Aloe barbadensis* were inoculated in media fortified with 2.5 mg/l 2, 4-D. Callus sub-cultured at same level of growth regulator for multiplication. Fresh callus further transferred for regeneration response in different media incorporated with combination of 2.0 mg/l Kn + 1.0 mg/l NAA. All the inoculated cultures were maintained at 25 ± 2^0C temperature with 14 hours light and 10 hours dark. The light intensity of 3000 lux was used during light hours.

Standard procedures had been used for data recording on various traits. For callus proliferation and regeneration response, data were collected after 45 days of inoculation on callus weight (g), number of regenerated shoot, morphogenetic response (per cent), days taken for callus initiation and regeneration, callus colour and texture. Callus growth and shoot morphology visually observed. The experiments were conducted in CRD (Completely Randomized Design) using ten replication of each treatment. Data were analyzed by using XLSTAT software for means and standard error accordingly as described by Snedecor and Cochran (1972) [14]. For comparing different treatments, test of significance were done according to Duncan's Multiple Range Test (DMRT) for different characters (Gomez and Gomez 1984) [15].

Results and Discussions

In the current investigation, six types of culture media were evaluated for callus proliferation and organogenesis using plant growth regulator 2.5 mg/l 2,4-D and 2.0 mg/l Kn + 1.0 mg/l NAA, respectively. Significant variations were present among all the culture media for callus weight, number of regenerated shoots, days taken in callus initiation and shoot regeneration.

All culture media responded for callus induction in lateral shoot explant except Knudson Solution-C and, Schenk and Hildebrant medium. Among all, significantly higher callus weight (3.42 g) in shortest days of initiation (17.90 days) was reported in Woody plant medium, followed by MS medium (3.38 g) with non-significant differences for callus weight (Table 1 and Fig. 1). Efficacy for callus proliferation and morphogenetic response of both these media was highest (80%). The proliferated calluses in both the media were characterized with texture of semi-compact and yellow to yellow brown colour. Similarly, Vasantha and Shivanna (2005) [16] in *Desmodium oojeinense*, observed profuse callus proliferation in Woody plant medium using leaf explant. The efficacies of Woody plant medium and MS medium are comparable in the present investigation. However, Roy *et al.* (2016) [17] in *Centella asiatica* and Raj *et al.* (2020) [18] in *Aegle marmelose* reported that MS media were more proficient than Woody plant medium for callus induction response. This deviation might be caused due to the differences in the genotypes of plant and other culture conditions such as growth regulators, physiological state of explant etc. Nitsch and Nitsch medium and Whites medium also responded for callus induction in lateral shoot explant but proficiency were very low.

Callus derived using lateral shoot explant further sub-cultured on different media fortified with combination of 2.0 mg/l Kn + 1.0 mg/l NAA for organogenesis. Only Murashige and Skoog, Nitsch and Nitch, and Woody plant medium showed regeneration responses in callus culture (Table 2 and Fig. 2). Highest number of shoots (3.40) per callus culture was induced in Woody plant medium, followed by MS medium (3.30) with non-significant difference among them. Both these media effectively induced shoot per callus culture; however, days taken for shoot regeneration was significantly lower in MS medium (21.30 days) than Woody plant medium. Adventitious shoot regenerated in MS medium and Woody plant medium were profuse, healthy and grow rapidly on further sub-culture on shooting media. These results of the present study were in accordance with the findings of Kumar (2018) [19], who also observed highest *de novo* shoot regeneration in Woody plant medium in Pomegranate cv. Sindhuri. The effect of Woody plant medium and MS medium found comparable, however, some published work reported that MS medium acceded then Woody plant medium for profuse adventitious shoot regeneration in callus (Thakur and Kanwar 2017, Verma 2019) [20, 21]. Moreover, Nitsch and Nitsch medium showed scantily regeneration of shoot with very short length and poor growth. White's media showed embryogenic elongations but no shoot was developed. The induced shoots transferred in shooting media for further growth and multiplication.

Table 1: Effect of different culture media on callus proliferation

S. N.	Media	Days taken in callus initiation	Morphogenetic response (%)	Callus weight (g)	Callus colour	Callus texture
1	Murashige and Skoog	17.90±0.48^{d}	80	3.38±0.06^{a}	Yellow	Semi compact
2	Nitsch and Nitsch	26.90±0.28^{b}	20	0.46±0.06^{b}	Brown	Friable
3	Woody Plant Medium	19.60±0.37^{c}	80	3.42±0.08^{a}	Yellow brown	Semi compact
4	Schenk and Hildebrant	-	-	-	-	-
5	White's medium	29.60±0.37^{a}	10	0.25±0.03^{c}	Pale yellow	Friable
6	Knudson Solution–C	-	-	-	-	-

Values followed by same letters in each column are not significantly different (p< 0.05) using DMRT

(-) = No response

Table 2: Effect of different culture media on regeneration in callus culture

S. N.	Media	Days taken in shoot initiation	Morphogenetic response (%)	Number of shoots	Callus growth	Shoot morphology
1	Murashige and Skoog	21.30±0.37[c]	70	3.30±0.15[a]	+	Medium long, medium narrow, green color, good growth
2	Nitsch and Nitsch	31.20±0.49[a]	20	0.70±0.21[b]	++	Very short, narrow, light green color, poor growth
3	Woody Plant Medium	24.40±0.31[b]	60	3.40±0.16[a]	+	Medium long, medium broad, light green color, good growth
4	Schenk and Hildebrant	-	-	-	-	-
5	White's medium	-	-	-	-	-
6	Knudson Solution–C	-	-	-	-	-

Values followed by same letters in each column are not significantly different ($p< 0.05$) using DMRT
(+) = Low growth, (++) = Medium growth, (+++) = High growth
(-) = No response

(a) MS Medium

(b) Nitsch & Nitsch medium

(c) Woody Plant medium

(d) White's medium

Fig 1: Callus proliferation using 2,4-D@2.5 mg/l in (a) MS Medium, (b) Nitsch and Nitsch medium, (c) Woody Plant medium and (d) White's medium

(a) MS Medium

(b) Nitsch & Nitsch medium

(c) Woody Plant medium (d) White's medium

Fig 2: Regeneration of shoots in callus using 2.0 mg/l Kn+1.0 mg/l NAA in (a) MS Medium, (b) Nitsch and Nitsch medium, (c) Woody Plant medium and (d) White's medium

Conclusion

The proportion of media composition greatly influences the process of callus induction and *de novo* shoot regeneration of *Aloe barbadensis.* The Woody plant medium was found best for both the steps of indirect organogenesis. The efficacy of MS medium was also high and comparable to woody plant medium. Both these media interchangeably can be utilized for callus proliferation and adventitious shoot regeneration for large scale propagation of *Aloe barbadensis*. The rank of the different media for effective callus proliferation and regeneration can be ordered as Woody plant medium > MS medium > Nitsch and Nitsch medium > Whites medium > Knudson Solution-C = Schenk and Hildebrandt medium.

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