Effect of NAA and picloram on the callus induction in *Aloe barbadensis*

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Abstract
Aloe barbadensis miller is the most important medicinal plant used globally in the cosmeceutical, pharmaceutical and nutraceutical industries. This present study investigates the effect of different plant growth regulators on the in vitro callus induction. The leaf explant was dissected from the 1-month plant and inoculated in the MS media containing NAA (0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4 mg/l) and picloram (0.5, 1.0, 1.5, 2.0, 2.5, 3.0 mg/l) either alone or in combination with BAP and kinetin.

Morphological parameters like color, texture and biomass of the callus were recorded after 50 days. Maximum callus induction was obtained on the MS media supplemented with 4mg/l picloram and 3mg/l NAA+0.5 mg/l kinetin. This innovated method could be utilized as a reference for the large-scale propagation of medicinal plant Aloe barbadensis.

Keywords: *Aloe barbadensis*, callus induction, plant growth regulator, medicinal plant.

Introduction
Aloe barbadensis miller is a perennial pea-green, arborescent, succulent, xerophytic plant in the Aloeaceae (Liliaceae) family. It is identical to Aloe vera (L.) and is referred to by the Sanskrit names Gwarpatha, Desert Lily and Ghritkumari[1,2]. Aloe gel is frequently utilised in the cosmetic and pharmaceutical industries to make medicine and various herbal remedies because of its healing capabilities[8]. In India and other countries, more than 40 well-known products containing Aloe are in the market[3]. Bioactive components of Aloe gel are abundant in antioxidant vitamins (A, B, C and E) and have anti-inflammatory, anti-viral, anti-inflammatory and antibacterial qualities. These substances are frequently employed in the treatment of cardiac disorders and cancer[1,4,9,12].

Traditionally, gwarpatha is cultivated by employing naturally formed lateral branches, which is a time-consuming and costly process[6,10]. As an alternative, large-scale growth of this perennial herb may be best achieved through multiplication using the *in vitro* culture method. The plant's genotype, explant type and culture conditions all had a significant impact on the micropropagation process[7,11]. One of the main elements influencing the regeneration process under-regulated settings is the media composition[5]. Major and micronutrients, carbon and nitrogen sources, vitamins and solidifying agents (often agar) make up a media. A callus is a mass of undifferentiated cells that is typically brought on by different growth hormonal concentrations in the culture media. A single differentiated cell can become a callus and many callus cells are totipotent, meaning they can regenerate the entire plant body. Plant growth regulators, such as auxins and cytokinins, can induce callus and induce organogenesis from somatic cells and tissues in the form of shoots, roots, or embryos, depending on their concentration alone or in combination. Since auxins and cytokinins are typically needed for root and shoot regeneration, they are typically regarded as the hormone classes that have the most influence on controlling plant development *in vitro*[13].

The induction of cell division and the emergence of lateral bud dormancy are two of cytokinins' main culturally beneficial characteristics. Auxins and cytokinins work together to control cell division with each influencing distinct stages of the cell cycle. While cytokinins appear to have some control over the processes leading to mitosis and cytokinesis, auxins have an impact on DNA replication[1]. Therefore, it is important to properly balance and regulate the amounts of plant growth regulators in cultures. One of the major NAA and picloram is frequently used in plant tissue culture for the micropropagation of different species.

The physiological effects of each kind of auxin vary greatly amongst plants. Its concentration, the existence or lack of additional growth regulators, the genetic composition, the physiological state of the donor plant and the type of explants all influence these effects. Different growth regulators or different combinations of growth regulators may be needed to produce the same physiological response in various tissues, even within the same plant. Consequently, the current study was conducted to assess the impact of auxin and cytokinin alone and their combinations on the *in vitro* induction of callus. In this present investigation, the effects of NAA and picloram alone and their combinations with cytokinin on the *in vitro* callus induction were analyzed.

Material and Methods
Plant material: *Aloe barbadensis* plants were collected from the RCFC herbal garden, Joginder Nagar, Himachal Pradesh. Leaves (3-4cm) were used as an explant and washed under tap water for 30 minutes followed by

treatment with antifungal and antibacterial agents for 20 minutes and rinsed with distilled water until completely washed. Washed leaves were sterilized with 70% ethanol for 30 seconds followed by different concentrations (0.01g, 0.05g, 0.1g per 100ml) mercuric chloride for 6 minutes as shown in table 1 and rinsed 3-4 with distilled water 3-4 times. The best result was shown by 0.1g/100ml of mercuric chloride.

Media preparation and callus formation: Prepared explants were cultured on the MS media containing all the essential micro-nutrients and macro-nutrients enriched with different concentrations of NAA (0.5,1.0,1.5,2.0,2.5 and 3.0 mg/l) alone and with the combination of kinetin and BAP and picloram (0.5, 1.0,1.5,2.0,2.5,3.0,3.5 and 4 mg/l) alone as shown in table 2 and 3. Agar (8g/l) and sucrose (30g/l) were supplemented into the MS media. Charcoal (100mg/l) was used for reducing the phenol content and to prevent the browning of callus. The pH was adjusted to 5.6-5.7 before autoclaving having a temperature of 121°C and pressure of 1.06 kg cm^{-2} for 15 min. The cultures were kept in the dark growth chambers having 25±2˚ C temperature. Callus was induced after 4-6 weeks of inoculation. After callus induction, the callus parameters like colour, texture were analysed.

Results and Discussion

Callus induction in this present investigation was influenced by plant growth hormones. Callus initiation was observed on the cut surface of the leaf within 15 days after inoculation on MS media blended with NAA, picloram, NAA+BAP, NAA+ kinetin. Maximum callus was observed at the end of 6-7 weeks. All the callus obtained was friable and light green or yellowish green in colour. Maximum callus frequency was observed on the MS media supplemented with 4mg/l picloram, NAA+BAP (3mg/l_0.5mg/l) and NAA+Kinetin (3mg/l+0.5mg/l). Days taken for callus induction depended on the concentration of plant growth regulators. Maximum concentration of auxin and in combination with cytokinin reduced the no. of days for callus induction.

3mg/l NAA, 3+0.1 mg/l NAA+BAP, 3+0.1 mg/l NAA+Kinetin and 4mg/l picloram takes 30 days for callus induction. But the best callus biomass was shown by the MS media supplemented with 4mg/l picloram and 3mg/l NAA or NAA+BAP (3+0.5 mg/l) ranging up to 0.3g-1.0g. Callus frequency was calculated from the following formula.

CIF = (number of explants with calli / number of incubated explants) × 100

The callus biomass was higher in the media fortified with 4mg/l picloran as about 0.95g and the media fortified with NAA (3mg/l) was 0.4g or the lowest biomass of callus was observed in the media fortified with the combination of NAA+BAP (0.3+0.5) or NAA+ kinetin (3+0.5) i.e. 0.1-0.2g. Data were analyzed by using XL software for mean and standard error. Analysed data is depicted in the table as Mean ± Standard Error (SE).

Table 1
Effect of different concentrations of mercuric chloride on the explant *of Aloe barbadensis.*

Treatments	Concentration of $HgCl_2$ (g/100ml)	Time (minutes)	Total flasks	Flasks free of contamination	Sterilization (%)
1.	0.01	6 minutes	30	10	33.3%
2.	0.05	6 minutes	30	15	50%
3.	0.1	6 minutes	30	28	93.3%

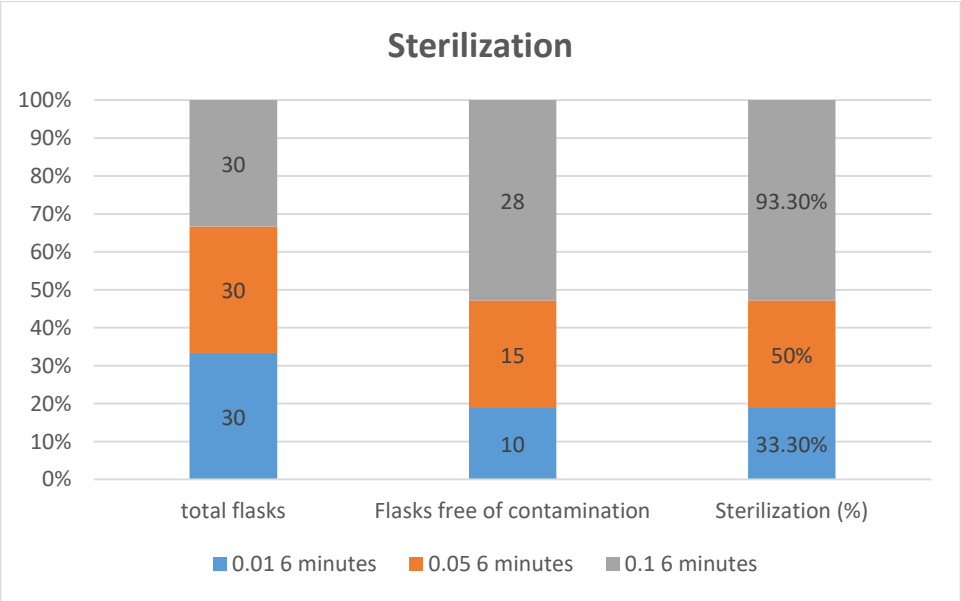

Table 2
Effect of NAA and combination with BAP and kinetin on callus induction.

PGR	Concentration (mg/L)	Days taken for callus initiation	Callus frequency (%)	Callus weight	Callus color	Callus texture
NAA	0.5	-	-	-	-	-
	1.0	-	-	-	-	-
	1.5	-	-	-	-	-
	2.0	-	-	-	-	-
	3.0	40	50	0.36±0.04	Light green	Friable and compact
NAA+BAP	3.0+0.5	35	67	0.14±0.006	Light green	Friable and compact
	3.0+1.0	30	40	0.01±0.03	Light green	Friable and compact
NAA+ Kinetin	3.0+0.5	35	60	0.8±0.01	Light green	Friable and compact
	3.0+1.0	30	30	0.04±0.01	Light green	Friable and compact

Table 3
Effect of different concentrations of picloram on callus induction.

PGR	Concentration (mg/L)	Days taken of callus initiation	Callus frequency	Callus weight	Callus color	Callus texture
Picloram	0.5	-	-	-	-	-
	1.0	-	-	-	-	-
	1.5	-	-	-	-	-
	2.0	-	-	-		
	3.5	40	80	0.45±0.02	Yellowish green	Friable and compact
	4.0	30	100	1.15±0.07	Yellowish green	Friable and compact

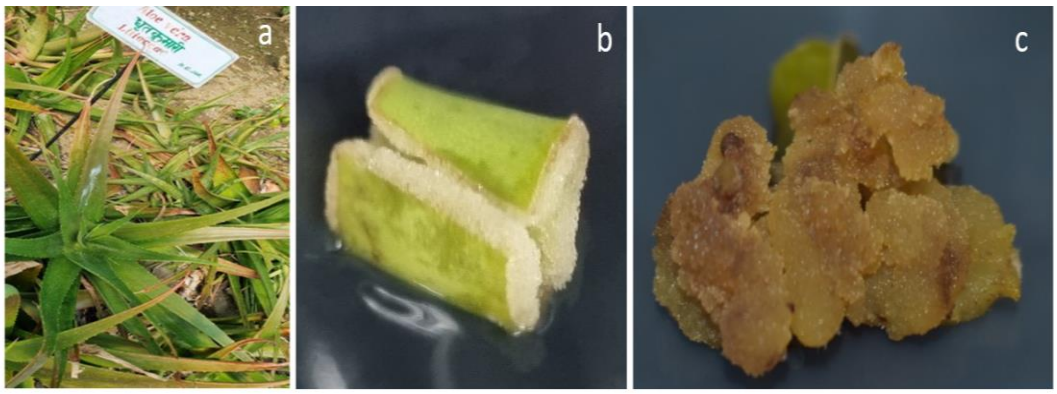

Figure 1: (a) Explant of the *Aloe barbadensis miller* (b) callus initiation in the leaf explant (c) callus induction in the *Aloe barbadensis miller*

Conclusion

Usually, auxin, cytokinin and their combinations are responsible for plants' *in vitro* growth and development. Mainly auxins are responsible for rooting whereas cytokinins are helpful in shooting and their combination in equal ratio is helpful in callus induction. In this present investigation, different plant growth regulators with their different concentrations, alone or with combinations were used for callus induction of *Aloe barbadensis.* Leaf was used as an explant inoculated in the MS media blended with picloram, NAA, NAA+BAP, NAA+ kinetin with different concentrations.

Different hormones show different response on the basis of morphological parameters of callus. In this present study, picloram gives the best result as it gives the maximum

biomass of callus (1.5g) at 4mg/l concentration which is green and friable as compared to other hormones. Therefore, picloram was found to be more efficient than NAA and their combinations with BAP and Kinetin. This study helps for further *in vitro* callus induction of Aloe vera.

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