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In Vitro Propagation of *Cornus capitata*: Challenges and Progress in Callus Formation and Shoot Differentiation

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Abstract

The culture of apical buds, epicotyls, and hypocotyls in MS media supplemented with different concentrations of IBA, IAA, and NAA is the main focus of this work, which examines the in vitro propagation of *Cornus capitata*. Contamination caused problems in the early trials, which prevented any growth in the first or second efforts. Nonetheless, growth was seen in the third and fourth trials, with the fourth trial showing effective callus development. Callus induction was successful, however differentiation into roots and shoots was not. Our first attempt at tissue culture for *Cornus capitata* is this study, which shows both advancements and challenges. While callus formation from hypocotyls was successful, contamination concerns and a lack of following shoot/root growth emphasize the need for additional optimization. Future research will concentrate on improving culture conditions, media composition, and hormone concentrations to promote plant regeneration from callus and improve overall in vitro cultivation results. This study contributes valuable insights into the tissue culture of *Cornus capitata* and sets the stage for future research aimed at successful plant propagation and conservation of this species.

Keywords: *Cornus capitata*, Himalayan tree, Callus, Hypocotyle, Auxillary bud Strawberry.

Introduction

Cornus capitata, the Himalayan strawberry or dogwood, belongs to the family Cornaceae (Dimari *et al.,* 2025). It is an evergreen, sub-deciduous tree that can grow up to 10 meters high. The bark is light-brown, rough, and exfoliates in broad rectangular scars (Lamichhane *et al.,* 2009). The young parts are covered with minute hairs. Leaves are oblong or elliptic-lanceolate, 4.5-9.5 cm long and 2.5-4 cm wide, acute or acuminate; entire, coriaceous above, and glabrescent beneath. Petioles are 1-2 cm long. The flowers are small, greenish-yellow, bi-sexual, and tetramerous, compactly arranged in hemispheric heads that are 1-2 cm across, subtended by 4-5 bracts. These bracts are yellowish, large, obovoid, and 1.5-3 cm long. The calyx has four reflexed teeth. The petals are four and minute, and there are four stamens. The fruit is pinkish-yellow, arranged in globose heads 2.5-3 cm across. It is succulent, with the pericarps of several drupes coalescing together, each drupe containing one seed (Tailwal and Negi, 2022). *Cornus capitata* is found in the montane Himalayas, from Himachal Pradesh to Bhutan, Nepal, and southwestern and southern China. The fruits are edible and preferred by wildlife. They are reddish, fleshy, and taste like sweet, over-ripe bananas. Some trees produce pleasant-tasting fruits, which can also be used in preserves. The spiky red fruits named the plant "Strawberry Tree." The wood is hard and close-grained but tends to warp when seasoned and is mainly used for fuel. The bark contains tannin and is an astringent (Gaur, 1999). Other different properties and their description are

highlighted in (Table 1). While *Cornus capitata* is a well-known ornamental species with ecological and horticultural value, limited research focuses on its in vitro propagation, particularly in achieving successful callus formation and subsequent shoot and root differentiation (Field *et al.,* 2001). Based on the available literature, related species confirm the necessity of optimized media and hormone concentrations to conduct tissue culture. Insufficiencies and work-related contamination of *Cornus capitata* is published rarely, and none of them are dedicated to contamination difficulties during consistent callus development. The current research is aimed at increasing its scopes and testing MS media with diverse IBA, IAA, and NAA concentrations. Except for the initial difficulties with the contamination of flasks and early death of explants, the successful callus formation execution during the further trials shows a potential for cultivation optimization. The goal of this research is to offer a preliminary understanding of tissue culture methods for *Cornus capitata* in hopes of aiding its conservation and expanding its use in horticulture.

Table 1: Various properties and uses of Cornus capitata.

Properties		Description
1. Botanical Description	Family	Cornaceae (Thakur 2014)
	Common Names	Himalayan strawberry tree, Chinese dogwood, Bentham's cornel, Evergreen dogwood (Song *et al.,* 2015,) (Abrol *et al.,* 2018)
	Plant Type	Evergreen, sub-deciduous tree or shrub (Fan and Xiang 2001)
	Height	Grows up to 10 meters (approximately 30 feet) in height
	Bark	Light-brown, rough, exfoliates in broad rectangular scars, giving the tree a distinctive appearance.
	Leaves	Oblong to elliptic-lanceolate, measuring 4.5-9.5 cm in length and 2.5-4 cm in width, with a sharp (acute) or tapering (acuminate) tip. The leaves are coriaceous (leathery), dark green on the upper surface, and glabrescent (becoming hairless) beneath. The petioles are 1-2 cm long.
	Flowers	Greenish-yellow, small, bisexual, tetramerous (four parts), and arranged in compact, hemispheric heads about 1-2 cm across. These flower heads are subtended by 4-5 large, obovate bracts, which are yellowish and 1.5-3 cm long. (Wetzstein *et al.,* 2014)
	Calyx	The calyx has four reflexed teeth.
	Petals and Stamens	The petals are small, with four in total, and the stamens are also four.
	Fruits	The fruits are pinkish-yellow, globose, and measure 2.5-3 cm in diameter. Each drupe (small, fleshy fruit) is succulent and contains one seed. The fruits form in clusters, with several drupes merging together to form a head. The ripe fruits are red and edible, with a texture and taste that some describe as similar to overripe bananas. (Kollmann 2001)
2. Habitat and Distribution	Native Range	Montane regions of the Himalayas, from Himachal Pradesh in India through Nepal, Bhutan, and southern parts of China. (Khanduri *et al.,* 2018)
	Cultivation	*Cornus capitata* is commonly found in temperate gardens and

		parks, where it is appreciated for its decorative flowers, fruits, and foliage. It grows best in well-drained soils and prefers cool, moist climates, although it can tolerate a range of soil conditions. It is also grown as an ornamental tree in many regions around the world due to its aesthetic appeal.
3. Ecological Importance	Wildlife	The fruit is an important food source for birds and other wildlife. Birds are especially attracted to the brightly colored fruits, which help in seed dispersal.
	Pollination	The flowers are visited by various pollinators, contributing to the plant's reproduction and the overall health of the ecosystem.
4. Uses	Edible Fruits	The fruits are edible and can be eaten raw. They have a sweet taste resembling overripe bananas. Some trees produce fruits that are pleasant to taste, and they can also be used to make preserves or jams.
	Wood	The wood of *Cornus capitata* is hard, dense, and close-grained. However, it tends to warp during seasoning, limiting its use to simple agricultural implements and fuel.
	Tannin Source	The bark contains tannins, which have been used as an astringent and in tanning leather.
	Ornamental Use	Due to its striking flowers, attractive foliage, and colorful fruits, *Cornus capitata* is widely used as an ornamental tree in gardens and parks.
	Medicinal Use	While not as widely known for medicinal purposes, the tannin-rich bark has astringent properties and may have been traditionally used to treat wounds or skin conditions. (Tenuta *et al.,* 2022)
5. Growth Conditions	Light Requirements	Prefers full sun to partial shade.
	Soil	Grows well in moist, well-drained soil with a slightly acidic to neutral pH
	Watering Needs	Requires regular watering, especially during dry spells. It thrives in areas with consistent moisture but should not be waterlogged.
	Temperature	While tolerant of some cold, *Cornus capitata* prefers cooler, temperate climates and can suffer from heat stress in hotter regions.
6. Propagation	Propagation	*Cornus capitata* can be propagated through seeds, cuttings, or layering. Seeds require cold stratification (exposure to cold temperatures) before germination to mimic the natural winter conditions of the plant's native habitat.
7. Cultural Significance		In the regions where *Cornus capitata* grows naturally, the tree may have some cultural importance. In traditional Himalayan communities, it has been used for its edible fruits and as a source of fuel.

Methodology

Explant Preparation

Cornus capitata (var. Bhamora) plants were selected based on phenotypic and morphological characteristics for in vitro cultivation. Explants and axillary buds of the species were used for tissue culture studies. The plant material for the present study was collected from two different natural habitats, specifically the forests of Rudraprayag and Pauri Garhwal in the Indian Himalayas. These regions provided diverse genetic material, contributing to the robustness of the study in understanding the in vitro growth potential of Cornus capitata. For explant inoculation, washing of apical buds were done with running tap water to remove debris from the surface of the buds. After that washing sample was dipped in distilled water for 10minutes. The explants were then soaked in a solution containing 4 drops of Tween 20 in 100 mL of distilled water for 5-10 minutes, ensuring thorough cleaning with constant stirring. After rinsing with distilled water, sample was treated with Bavistin, for 30-45 minutes to eliminate fungal contaminants, followed by 2-3 washes with double-distilled water. After complete sterilization, the explants were treated with mercuric chloride ($HgCl_2$) for 1-5 minutes, depending on sample size and contamination risk, and then rinsed 2-3 times with double-distilled water. Finally, they were dipped in 70% ethanol for 30-40 seconds and rinsed again with double-distilled water before being inoculated into MS media. This protocol was followed to minimize contamination and prepare the explants for successful in vitro culture (Fig. 1).

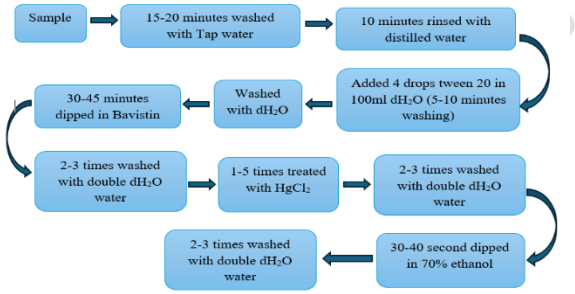

Fig.1: Flow diagram for the preparation of explants for inoculation**.**

Inoculation of Explants

This study examined the four different treatments with the use of growth hormones for the purpose of initiating callus formation and in vitro growth of *Cornus capitata*.

i. **Treatment 1:** Axillary buds of Cornus capitata were first inoculated in MS media with Indole-3-butyric acid (IBA) and Indole-3-acetic acid (IAA) and after a period of growth, callus formation was recorded.
ii. **Treatment 2:** Axillary buds were then inoculated in MS media containing IBA and IAA, and then Naphthalene acetic acid (NAA) and again growth was recorded.
iii. **Treatment 3:** Epicotyls, hypocotyls, AND axillary buds were inoculated in MS media containing NAA, IAA, and IBA, thus attempting to observe the growth effects of numerous explant varieties.
iv. **Treatment 4:** Epicotyls, hypocotyls, and axillary buds were inoculated in MS media with NAA and IBA.

These treatments aimed to study the different effects of the different combinations of growth hormones for the in vitro study of *Cornus capitata*.

Results of the Experiments

(i) **Treatment 1:** MS media inoculated *Cornus capitata* with 5 PPM, 10 PPM, 15 PPM, and 20 PPM of growth regulators in varying concentrations. In this treatment, however, the cultures were contaminated by bacteria and fungi, which caused this treatment to fail.

(ii) **Treatment 2:** Axillary buds of Cornus capitata were inoculated in MS media with 5 PPM, 10 PPM, 15 PPM, and 20 PPM IBA, IAA, and NAA combinations. Despite these combinations, there were stagnant no buds of growth, and their axillary buds showed were no in growth response to the concentrations of these IAA combinations and thus no significant response to this of the hormones.

Table 2: MS media supplemented with growth regulators at axillary buds of Cornus capitata

Growth regulator with different concentration	Number of tested axillary buds	Initiation observed
NAA 5PPM	8	1
NAA 10PPM	8	1
NAA 15PPM	8	2
NAA 20PPM	8	0
IBA 5PPM	8	2
IBA 10PPM	8	0
IBA 15PPM	8	0
IBA 20PPM	8	0
IAA 5PPM	8	1
IAA 10PPM	8	0
IAA 15PPM	8	0
IAA 20PPM	8	0

Fig. 2: Sprouting of *Cornus capitata* in NAA 10, NAA5.

(iii) **Treatment 3:** In this treatment, some *Cornus capitata* axillary bud (explant) were responsive when IBA, IAA, and NAA growth in 5 PPM, 10 PPM, 15 PPM, and 20 PPM NAA. Sprouting was in IBA combinations showed response. 2 samples of IBA 5 PPM and NAA 5 P, 1 of NAA 15 P, and 1 of IAA 5 P showed response to the 5 P concentration. This indicates some response to the growth hormone at these lower concentrations.

(iv) **Treatment 4:** In this treatment, axillary buds, epicotyls, and hypocotyls of *Cornus capitata* were inoculated in MS media supplemented with IBA and NAA at 5 PPM and 10 PPM concentrations. Sprouting was observed in several samples: NAA at 5 PPM (6 samples), IBA at 5 PPM (4 samples), and NAA at 10 PPM (5 samples). Callus formation occurred at IBA 5 PPM, but there was no differentiation into shoots or roots in the callus, indicating that further development was not successful while callus induction was achieved.

Table 3: MS media supplemented with growth regulators

Growth regulator with different concentration	Number of tested epicotyls and hypocotyls	Initiation observed
NAA 5PPM	16	6
NAA 10PPM	16	4
IBA 5PPM	16	5

Fig. 3: Sprouting of *Cornus capitata* during the fourth trial, Callus formation in *Cornus capitata* IBA

Discussion

In the present study, we observed shoot initiation in *Cornus capitata* when cultured on MS media supplemented with IBA (5 ppm), NAA (5 ppm, 15 ppm), and IAA (5 ppm) (Table 1-2 and Fig. 3-5). These results indicate that specific concentrations of growth regulators can induce shoot initiation, although callus formation and differentiation into shoots or roots remained limited. Our findings align partially with previous research by Tanaka *et al.,* (1997), who reported that leaf segments cultured on MS medium supplemented with various combinations of 2,4-D, NAA, IAA, and ABA led to callus formation. They observed that media with 2,4-D and NAA promoted callus formation, but media with IAA not only induced calli but also resulted in the formation of adventitious roots from the callus surface. Callus formation was most successful among the tested media with 2,4-D, particularly in combination with BA (benzyl adenine We inoculated epicotyls and hypocotyls into MS media having different concentrations of IBA and NAA. Callus formation was noted at IBA 5 ppm, but we did not see later differentiation into shoots or roots. This indicates that, although we succeeded in callus induction, some other aspects of the media formulation or the balance of hormones in the media might need to be fine-tuned for the callus to advance. Durkovic (2008) mentioned the high rate of shoot multiplication in another species under the influence of 0.7 mg/L BAP (6-benzylaminopurine) along with 0.05 mg/L NAA. Changes in pH and the basal medium composition (WPM) improved the shoot elongation and the overall vitality of culture for long-term maintenance. In the same way, Lattier *et al.,* (2014) reported that the greatest number of shoots was produced with WPM medium plus BAP, and after five weeks, the shoots had the highest multiplication rate and greatest elongation. From their studies, the following can be concluded: the use of other media compositions, such as WPM, and cytokinin concentrations, such as BAP, may improve rates of shoot multiplication and elongation. For instance, since our experiment has already been successful in the creation of calluses, other areas that could be investigated include the optimization of the formulation of the culture media like the use of WPM or the optimization of the cytokinin concentrations.

Future direction and Scope

Future studies related to the in vitro propagation of *Cornus capitata* should aim at several important aspects in order to increase the efficiency of

tissue culture methods. Firstly, the optimization of the tissue culture medium and the concentration of plant growth regulators would play a significant role in improving the efficiency of callus and differentiation. Researching other tissue culture mediums like WPM (Woody Plant Medium), as well as the concentration of cytokinin vs. auxins, might improve the process of shoots and roots development. Contamination issue resolution would remain a priority; implementing stronger asepsis and other innovative disinfection methods could improve efficiency. Research related to the genetic responses and physiology of *Cornus capitata* towards different plant growth regulators might play a vital role in improving plant regeneration efficiency. Moreover, the approach should involve other explants like leaf and/or cotyledon explants and other diverse light and temperature regimes that might play a pivotal role in improving our understanding related to the requirements of *Cornus capitata* at the in vitro level.

Conclusion

In this study, I attempted for the first time to culture explants of *Cornus capitata*. Apical buds were placed on MS medium supplemented with different concentrations of the growth regulators IBA, IAA, and NAA. During the initial trials, no growth was observed, most likely due to difficulties in controlling contamination. However, some growth appeared in the third and fourth trials, and in the fourth trial I was also able to obtain callus formation. Despite this progress, the callus did not differentiate into shoots or roots, so the objective was only partially achieved.

This work marked my first experience with callus tissue culture. The repeated challenges and contamination issues during the early trials were frustrating, but I was eventually able to produce some callus tissue. Although I attempted to induce further shoot development, only callus formation from the hypocotyls was observed in the fourth trial. After maintaining the callus for five days, no shoot or root development occurred, likely due to contamination. In future work, I plan to enhance the in vitro growth and differentiation of *Cornus capitata* by optimizing the culture media, implementing stricter contamination control, and exploring alternative hormone treatments.

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