International Journal for Multidisciplinary Research (IJFMR)
E-ISSN: 2582-2160 ● Website: www.ijfmr.com ● Email: editor@ijfmr.com

# Optimizing in Vitro Propagation of an Ornamental and Medicinal Plant *Crassula Ovata* (Mill.) Druce Using Leaf, Stem and Shoot Tip

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**Abstract**

*Crassula ovata* (Mill.) Druce, commonly known as Jade plant, is native to South Africa, globally popular due to its ornamental and medicinal value. It is characterized by its limited growth and slow propagation, contributing to high production costs. Countries like China and Turkey are known for exporting Crassula globally. Tissue culture offers an efficient method for mass-producing unique and beautiful species such as C. ovata. to fulfill its increasing demand in Pakistan. The current study investigates the response of explant types of the plant under different plant growth regulators (PGRs) and their optimum concentrations on in vitro regeneration. The present work describes optimal methodology derived from stem, shoot and leaf explants. The explants were sterilized using 70% ethanol and after that 10% sodium hypochlorite solution. leaf for 2 and 9 minutes, shoot tip for 2.5 and 11 minutes and stem for 3.5 and 12 minutes respectively. The explants were grown on MS medium consist of different combinations of PGRs. The effect of different PGRs was recorded in term of regeneration of number of leaves, root length and roots. Among PGRs, the highest root and shoot induction was observed under 0.5 & 1.5 mg/l IAA, 0.5 & 1.5 mg/l 2,4-D, 2.0 mg/l KIN and 0.5 mg/l NAA in shoot tip and stem explant, while leaf explant showed the best root induction under 2.0 mg/l among all the root induction PGR concentrations. Among all explants, shoot tip was the most responsive explant. The plantlets were successfully acclimatized to continue to grow under natural conditions. This protocol provides rapid and efficient season free in vitro propagation of the plant making the plant available at affordable cost.

**Keywords:** Explant, plant growth regulators, jade, succulent plant, regeneration, acclimitization

## INTRODUCTION

The *Crassula ovata*, sometimes called the money tree or jade plant, is a member of the Orpine or Crassulaceae family. This succulent plant is indigenous to South Africa and has tiny white or pink flowers. The selected plant is evergreen that can reach a height of three meters. It features thick, smooth branches that are somewhat rounded, along with leaves that have fleshy ends [1]. The plantlet production may be inadequate, With the growing demand for ornamental succulents [2, 3, 4], Plant tissue culture offers an

alternative method for rapidly producing a large number of plantlets in a short period [5].

The regulated, sterile cultivation of entire plants, organs, tissues and cells in a lab environment is known as tissue culture. This technique is frequently used to create exact replicas of plants [6, 7]. An ideal habitat for the culture's development and reproduction is produced by the carefully controlled settings. These requirements include supplying necessary nutrients, preserving the medium's pH, guaranteeing the right temperature, and controlled the liquid and gaseous environment [8]. Significant proliferation of plants is a common tool of plant tissue culture methodology. for the plant tissue culture technology. In recent years, Plant tissue culture techniques have become highly relevant in industry, extending beyond their role as a research tool to applications such as plant propagation, disease control, genetic improvement, and the production of secondary metabolites [7, 9, 10]. Various high-yielding tissue culture systems, including defined organ cultures, suspension cell cultures, and high-density cultures, have been developed [10, 11]. They used as a perpetual source for production of industrially important bioactive compounds such as alkaloids, flavonoids, terpenoids, organosulfur compounds, phenolics and secondary metabolites etc. [12]. The purpose of *in vitro* propagation is to provide a viable alternative method to propagate those plants that are not easily propagated vegetatively or by seeds. *In vitro* culture also helps in propagating plants to be re-established in their natural habitats, whose populations are heavily declining due to a lack of rapid multiplication propagation methods. C. *ovata* plants can be propagated using methods specific to succulent ornamental species (Crassulaceae), such as leaf or stem cuttings following vegetative propagation principles, or through *in vitro* propagation techniques [4, 13, 14, 15]. Tissue culture technology are known as an exceptional method for propagating medicinal plants, tissue culture enables the production of pathogen-free plants under controlled conditions, with precise regulation of environmental factors. Among them, micropropagation protocol is a necessary step for conservation and development [3, 16, 17].

Micropropagation has enabled the threatened, rare and endangered species to be multiplied, cultured and conserved successfully [7, 17]. The benefit of *in vitro* proliferation is that it can quickly produce large numbers of similar plantlets from seed or single explant. Thus, develop pathogen free plants rapidly [18, 19]. Initiating an aseptic culture, propagating explants under sterile conditions in vitro, and ultimately obtaining whole plants ready to acclimate to soil are all steps in the *in vitro* propagation process [20, 21]. such as macronutrients, micronutrients, vitamins, iron sources, and plant growth regulators (PGRs)—that can influence organ development and overall plant growth processes. However, PGRs usually contain certain compounds that either promote or inhibit plant growth and development. PGRs may indirectly control gene expression by utilizing enzymes from the biosynthesis pathway [15, 22]. However, depending on the dosage, type of retardant and application method, frequency of treatment, nutritional phase, environmental factors, and age of plant, may vary significantly even among members of the same genus [15, 23].

The phytochemical and antimicrobial potential of mentioned plant has been evaluated on several bacterial isolate and some studies has determined its potential in the pharmaceutical industry [1, 24]. *Crassula ovata* has dynamic chemical components such as carbohydrates, alkaloids, sterols, steroids and saponins which play a complex role in inhibiting microbial activity [1]. Ethnobotanically, many societies have established the tradition of using the latex extracted from leaves of Jade plant to cure warts. In Africa, Jade leaves are cooked in milk as a medication for diarrhoea and treating epilepsy. Herbalist recommends a tea of selected plant to treat diabetes [25]. *Crassula ovata* is a significant ornamental and commercial plant. It has recently become more well-liked as a bonsai, pot, and landscape plant [26]. According to Chinese custom, it is frequently positioned next to a cash register at multiple businesses in an effort to draw prosperity [27].

The Jade plant has attracted more common names including t Dollar Plant, Money Tree, and Penny Plant, Tree of Happiness in the Far East, United States and Germany [26].

Due to its slow growth, the current study focuses on the propagation of *Crassula ovata*. With some restrictions, this plant can be grown via both soft cuttings and seeds. The main purposes of the research were to investigate the response of altered explants types and concentrations of Phyto-hormones on micropropagation to upturn the manufacture of *C. ovata* plant for the first time in Pakistan. No study regarding direct regeneration via micropropagation from different explants i.e., shoot tip, stem and leaf, has been found in *Crassula ovata*. Additionally, to limit the importation of costly *C. ovata* plants and to offer an efficient micropropagation technique to encourage the proliferation of these plants for decorative, restoration, horticultural, and conservation uses.

## MATERIAL AND METHODS

### Plant procurement

The plant *Crassula ovata* (Mill.) Druce was obtained from Botanic Garden GCU, Lahore (Fig. 1A).

### Preparation of Medium

Murashige and Skoog (MS) medium [28] was used as a nutrient medium supplemented with various combinations (0.5-1.5 mg/l) of plant growth regulators; auxins like Indole-3 acetic acid (IAA), Naphthalene acetic acid (NAA) and 2, 4-dichloro phenoxy acetic acid (2, 4-D) and cytokinins such as 6-benzyl amino purine (BAP), Thidiazuron (TDZ) and Kinetin (KIN). The medium's pH was adjusted to 5.8 before autoclaving [29].

### Explant preparation

### Isolation and washing of explant

The explant preparation step started when branchlets from the parent plant with healthy shoot tips, stem and leaves were carefully removed. The first step was to thoroughly wash the branch-lets under running water for 30 minutes. The separated plant material was then rinsed for one to two minutes in 250 milliliters of distilled water with one or two drops of detergent. After that, it was treated with a few drops of tween20 (Polyoxyethylene sorbitan monolaurate) for 1 minute using a magnetic stirrer. Finally, it was washed three times in a row with sterile distilled water (Fig. 1B).

### Cutting of explant

Prior to surface sterilization, the plant material was separated cut in an aseptic environment using a laminar air flow hood. Each branchlet was carefully divided into three sections—leaf, stem, and shoot tip with two leaves—using an autoclaved scalpel, surgical blade, and forceps, all under sterile conditions in autoclaved petri dishes, without damaging the base. Explants included tips of two-leafed shoots, stem and leaves (Fig. 1C). For surface sterilization, various explants were put into autoclaved beakers one at a time (Fig. 1D).

### Surface sterilization of explant

Different explants were sterilization across a range of time periods using various sterilants in variable concentrations.

Leaves were disinfected with 70% ethanol for 2 minutes, after that 10% sodium hypochlorite for 9 minutes and then rinsed with sterile distilled water (SDW) thrice times

The tips of the shoots were dipped for two and a half minutes in a 70% ethanol solution, then disinfected with 10% sodium hypochlorite for 11 minutes and rinsed with SDW.

Stems were treated with 70% ethanol solution for 3.5 minutes, after that 10% sodium hypochlorite for 12 minutes then wash away by using disinfected purified water thrice.

All of the explants, including the leaves, shoot tips, and stems, were properly cleaned by SDW after being dipped in a 70% ethanol solution for under 30 seconds.

**Explant culture**

Following surface sterilization, stem, shoot tip and leaves explants were kept on sterile filter sheets to dry (Fig. 1E). With aid of autoclaved forceps, explants were cultured in a medium containing PGRs (Fig. 1F). Forceps were heat sterilized before to inoculating each explant every time. Test tubes were labeled with the PGR combination and the inoculation date after culture, they were firmly covered with rubber bands and a wrapping sheet.

**Incubation of cultures and regeneration**

Test tubes were incubated at 25 ± 2 °C under a 16 h dark/18 h light cycle, with 65–75% relative humidity and a light intensity of 25 µmol $m^{-2}$ $s^{-1}$ [30] (Fig. 1G). When explant shooting (baby leaves) and roots were started a few weeks later, the regeneration of plantlets was seen (Fig. 1H).

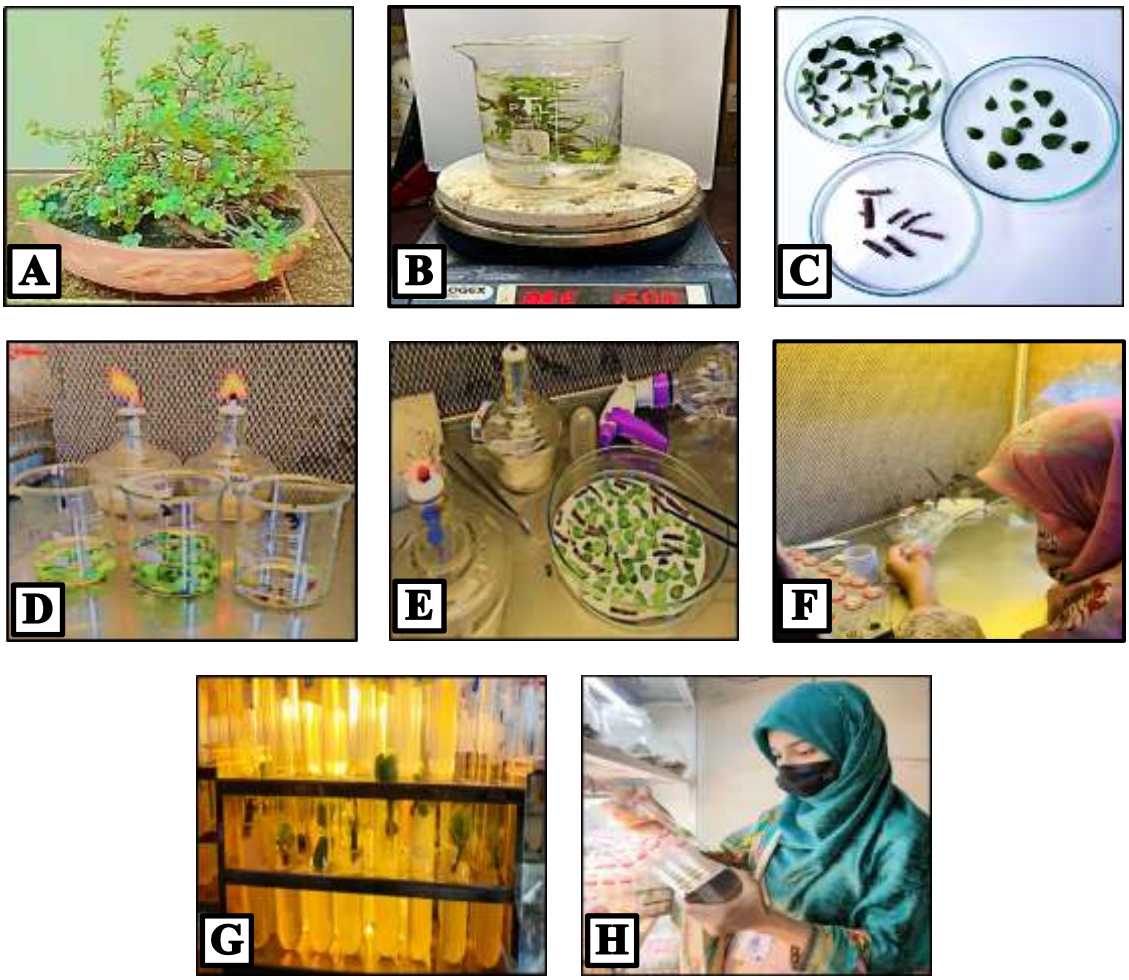


**Figure 1. Explant culture process: A) Crassula ovata Parent Plant B) Washing of isolated plant material. C) Cutting of explant; i. Leaf, ii. Shoot tip with two leaves iii. Stem. D) Surface sterilization of explant separately. E) Drying of explant. F) Inoculation on nutrient medium. G) Incubation of cultured test tubes. H) Inspection of cultures after few days.**

**Acclimatization of regenerated plantlets**

Plantlets with well-developed root systems were carefully separated and rinsed under running tap water

to remove any adhering gel. They were then transferred into pots containing a 1:1 mixture of peat moss and succulent soil, covered with plastic bags, and acclimatized in the culture room under controlled environmental conditions. Regular observations were made of the plantlets. Consequently, the plantlets were relocated to a greenhouse and their capability to according to the natural environment was observed. Once the plantlets had begun to grow and were stable in the soil, they were transferred to the natural environment to harden.

**Data collection and statistical analysis**

This study employed a completely randomized design (CRD), with treatments conducted in triplicate. Data on plantlet regeneration were recorded after four weeks of culture and articulated as mean ± SE from three separate studies. Moreover, Duncan's Multiple Range Test (DMRT) was performed using SPSS software.

## RESULTS

In the current research, the protocol for optimization of micropropagatıon of *C. ovata* was developed (Table 1).

**Table 1. Surface sterilization of explants with different surface sterilizing agents.**

| Sr. No. | Sterilants | Concentration of sterilants | Time Duration (Min) | Incidence of contamination | Effect |
|---|---|---|---|---|---|
| **SHOOT TIP** | | | | | |
| 1a. | Ethanol ($C_2H_5OH$) | 60 | 2 | +++ | Negligible growth |
| 2a. | | 70 | 2.5 | - | Active and healthy growth |
| 1b. | Sodium hypochlorite (NaClO) | 8 | 8 | ++ | Minimum growth |
| 2b. | | 10 | 11 | - | Active and healthy growth |
| **STEM** | | | | | |
| 3a. | Ethanol ($C_2H_5OH$) | 60 | 2 | +++ | Negligible growth |
| 4a. | | 70 | 3.5 | - | Active and healthy growth |
| 3b. | Sodium hypochlorite (NaClO) | 8 | 10 | +++ | Negligible growth |
| 4b. | | 10 | 12 | - | Active and healthy growth |
| **LEAF** | | | | | |
| 5a. | Ethanol ($C_2H_5OH$) | 60 | 1 | +++ | Negligible growth |
| 6a. | | 70 | 2 | - | Active and healthy growth |
| 5b. | Sodium hypochlorite (NaClO) | 10 | 6.5 | ++ | Minimum growth |
| 6b. | | 10 | 9 | - | Active and healthy growth |

**++++(100%), +++(75%), ++(50%), +(25%), - (<5%)**

**Effect of root inducing PGRs on different explants growth**

MS medium was supplemented with three different PGRs 2-4 D, IAA and NAA individually, among auxins. Every concentration falls between 0.5 and 2.0 mg/l. The development of the explants was assessed using the quantity of root as well as root length and leaves. The effect of different concentrations of IAA, 2,4-D and NAA on proliferation activity was checked (Table 2).

**Table 2. Effect of auxins on *in vitro* regeneration of different explants of *C. ovata* on MS medium. (Culture after 4 weeks).**

| Treat-ment (mg/l) | Shoot Tip | | | Stem | | | Leaf | | |
|---|---|---|---|---|---|---|---|---|---|
| IAA | No. of Shoot (leaves) | No. of roots | Length of root(cm) | No. of Shoot (leaves) | No. of roots | Length of root(cm) | No. of Shoot (leaves) | No. of roots | Length of root(cm) |
| 0.5 | 14 ± 1.15c | 17 ± 0.58b | 1.99 ± 0.003c | 3 ± 0.57 | 2 ± 0.57 | 0.47± 0.03 | 0 ± 0 | 2 ± 0.57a | 2.21± 0.45a |
| 1.0 | 9 ± 1ab | 14 ± 0.58a | 1.08 ± 0.012b | 6 ± 0.57b | 5 ± 0.57 | 0.73 ± 0.08 | 0 ± 0 | 2 ± 0a | 2.1 ± 0.06a |
| 1.5 | 7 ± 0a | 21 ±1.15c | 1.03 ± 0.011a | 13 ± 1.15 | 9± 1.15 | 1.4 ± 0.06 | 0 ± 0 | 3 ± 0.57a | 2.91 ± 0.03a |
| 2.0 | 11 ± 1b | 13 ± 0.57a | 1.03 ± 0.009a | 8 ± 0 | 2 ± 0.57 | 0.36 ± 0.03 | 0 ± 0 | 3 ± 0a | 2.90 ± 0.11a |
| **2,4-D** | | | | | | | | | |
| 0.5 | 11± 1.15b | 13 ± 0c | 0.84 ± 0.0a | 7± 0.57a | 0 ± 0 | 0.0 ± 0.0 | 0 ± 0 | 2 ± 0a | 1.95 ± 0.27b |
| 1.0 | 8 ± 0.57ab | 7 ± 0.57a | 1.95± 0.104c | 10 ± 0.57b | 2 ± 0.57a | 0.62 ± 0.06b | 0 ± 0 | 3 ± 0.57a | 1.11 ± 0.21a |
| 1.5 | 7 ± 1.15a | 10 ± 1.15b | 1.21 ± 0.03b | 15 ± 1.15c | 11 ± 1.15c | 1.51 ± 0.11d | 0 ± 0 | 7 ± 0.57b | 2.46 ± 0.11c |
| 2.0 | 10 ± 1.15ab | 7 ± 0.57a | 1.93 ± 0.053c | 11 ± 0.57b | 7 ± 0.57b | 1.23 ± 0.18c | 0 ± 0 | 9 ± 0c | 3.08 ± 0.23d |
| **NAA** | | | | | | | | | |
| 0.5 | 9.00 ± 0c | 14 ± 0.57d | 1.16 ± 0.005 | 7 ± 1.15a | 0 ± 0 | 0.0 ± 0.0 | 0 ± 0 | 1 ± 0a | 0.90 ± 0.1a |
| 1.0 | 6 ± 0.57ab | 12 ± 0.57c | 0.51 ± 0.009 | 5 ± 0.57a | 2 ± 0a | 0.45 ± 0.03b | 0 ± 0 | 3 ± 0b | 1.1 ± 0.17b |
| 1.5 | 7 ± 0.57b | 10 ± 0.57b | 0.81 ± 0.006 | 15 ± 0.57b | 5 ± 1.15b | 0.49 ± 0.05b | 0 ± 0 | 10 ± 0.57c | 1.17 ± 0.05b |
| 2.0 | 5 ± 0.57a | 6 ± 0.57a | 1.23 ± 0.015 | 13 ± 1.15b | 12 ± 1.15c | 2.46 ± 0.27c | 0 ± 0 | 11 ± 0.57c | 1.93 ± 0.08c |

**Data are expressed as mean ± SE (Standard Error) from three independent experiments. Means followed by the same letter are not significantly different from each other at the 0.05 level according to Duncan's Multiple Range Test.**

Every IAA concentration showed development in every kind of explant, including leaves, shoot tips, and stems. All explants had an overall IAA content of 1.5 mg/l. The shooting and rooting responses peaked at 0.5, 1.5 mg/l at the shoot tip and 1.5 mg/l in the stem. There was no indication of shoot growth in the leaf

explant; it simply displayed roots. At doses of 1.5 and 2.0 mg/l, the best roots in the leaf was seen (Fig. 2, 5A-C).

At all 2, 4-D concentrations, growth was observed in the shoot tip, stem and leaf; the strongest activity for development shoot and root was recorded at 0.5 mg/l in the shoot tip and 1.5 mg/l in the stem. The leaf explant showed no signs of shoot growth, merely roots. The leaf showed the best rooting at a concentration of 2.0 mg/l (Fig. 3, 5D-F).

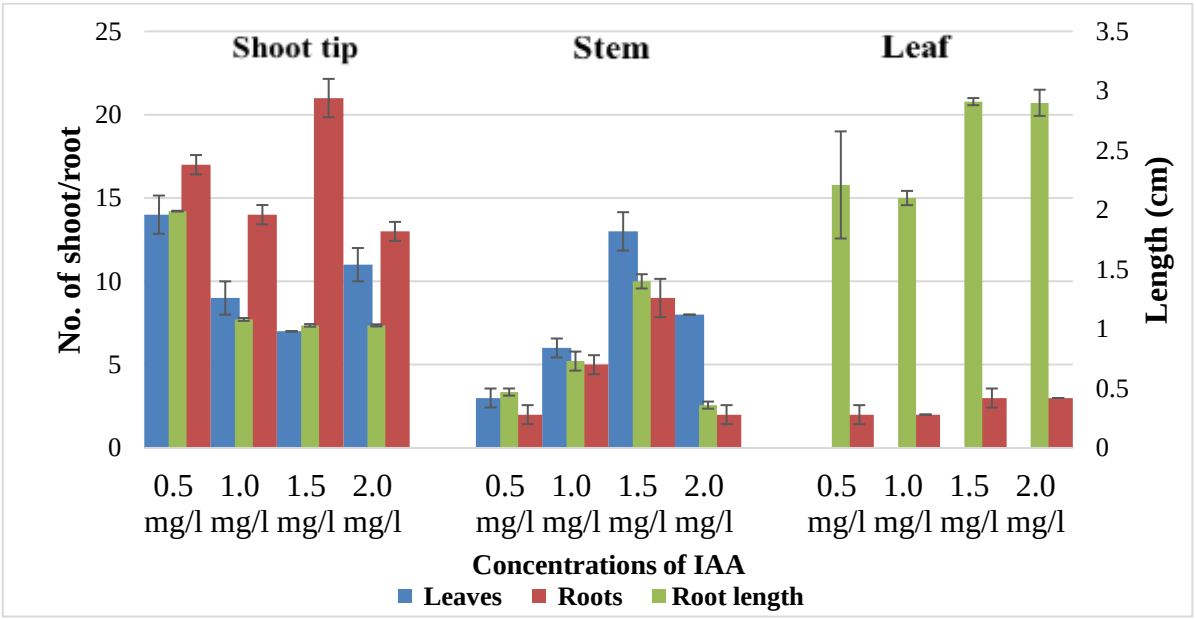


**Figure 2. Effect of IAA on *in vitro* regeneration of shoot tip, stem and leaf (culture after 4 weeks).**

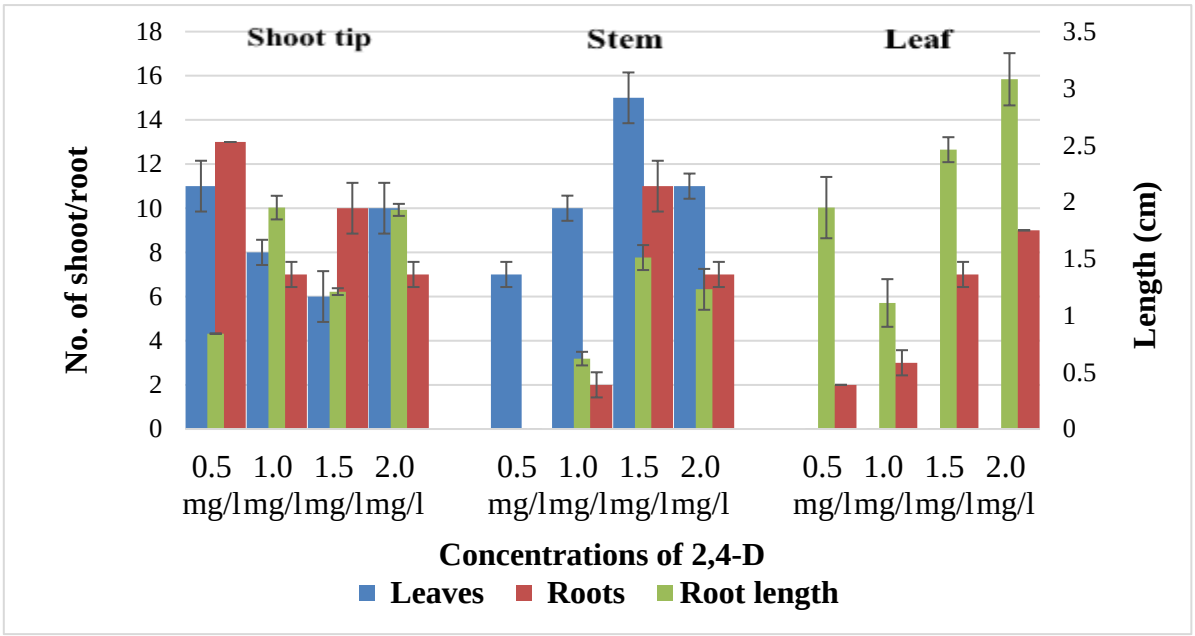


**Figure 3. Effect of 2,4-D on *in vitro* regeneration of shoot tip, stem and leaf (culture after 4 weeks).**

The explants grew at all NAA concentrations. The primary responses to 0.5 mg/l at the shoot tip and 2.0 mg/l in the stem were shooting and roots. The leaf explant showed no signs of shoot growth, merely roots.

The leaf exhibited the best rooting at a concentration of 2.0 mg/l (Fig. 4, 5G-I).

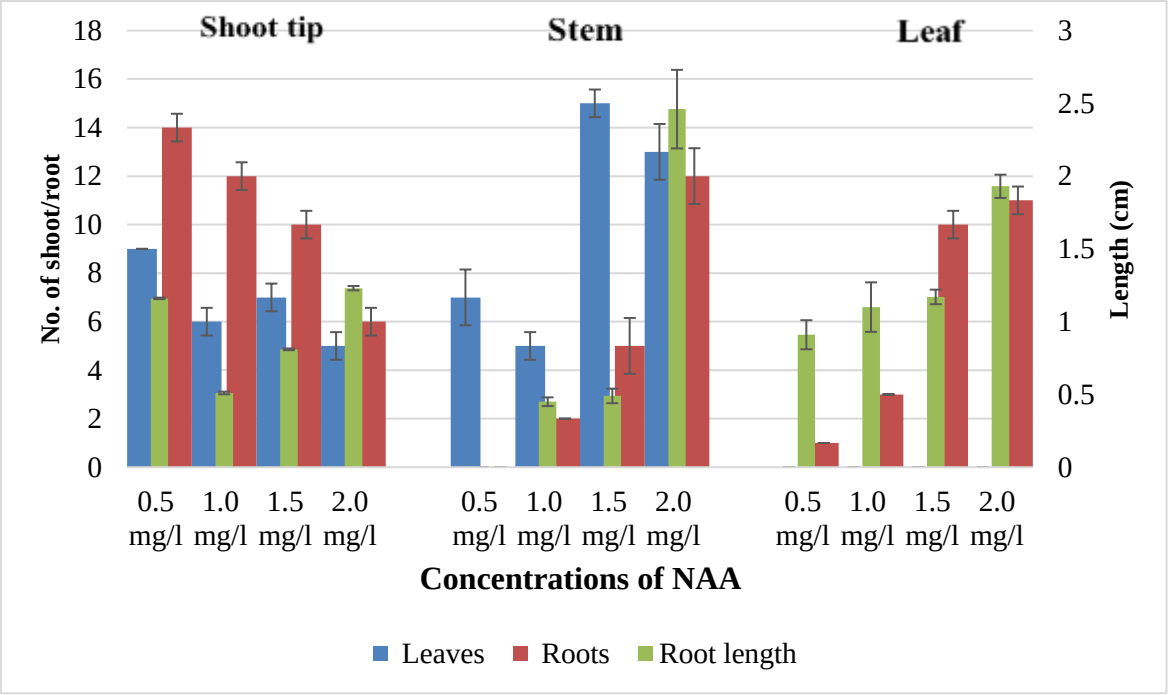


**Figure 4. Effect of NAA on *in vitro* regeneration of shoot tip, stem and leaf (culture after 4 weeks).**

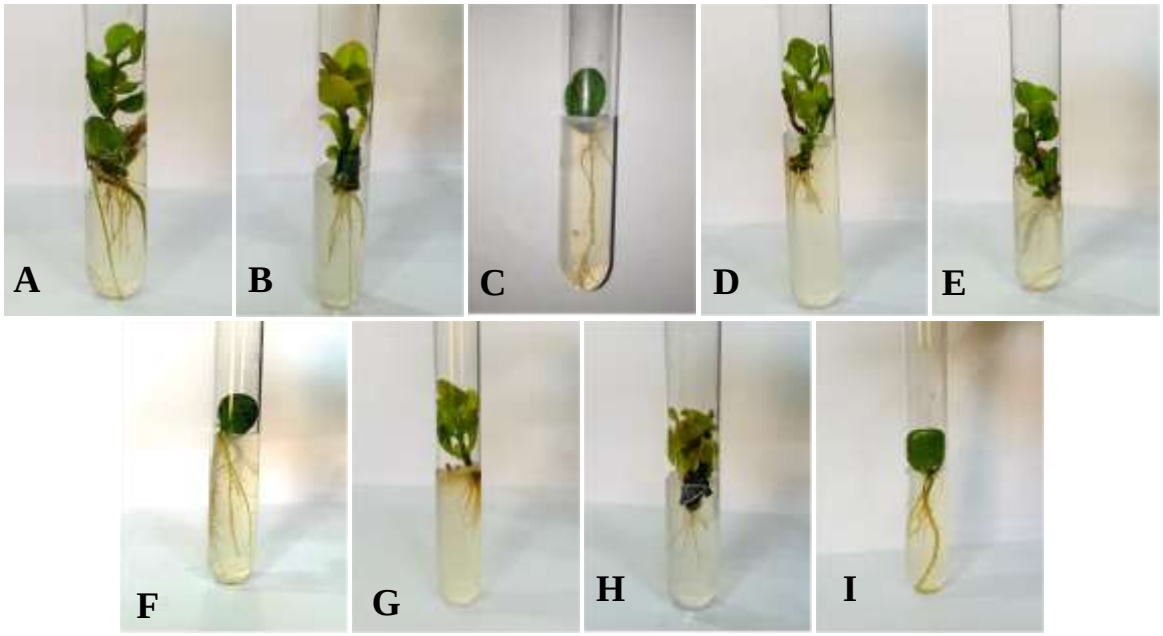


**Figure 5. Effect of root inducing PGRs on *in vitro* regeneration of different explants A) Shoot tip (0.5 mg/l IAA) B) Stem (1.5 mg/l IAA) C) Leaf (2.0 mg/l IAA) D) Shoot tip (0.5 mg/l 2,4-D) E) Stem**

**(1.5 mg/l 2,4-D) F) Leaf (2.0 mg/l 2,4-D) G) Shoot tip (0.5 mg/l NAA) H) Stem (2.0 mg/l NAA) I) Leaf (2.0 mg/l NAA) (Cultures after 4 weeks) (1.5X).**

**Effect of shoot inducing PGRs on different explants growth**

MS medium with three different PGRs combinations KIN, BAP and TDZ individually, among cytokinins. All combination ranges from 0.5-2.0 mg/l. Measurements and calculations of the explants' growth were made using roots as well as the length of the roots and number of leaves. The impact of varying BAP, KIN, and TDZ concentrations on regeneration activity was observed (Table 3).

**Table 3. Effect of cytokinins on *in vitro* regeneration of different explants of *C. ovata* on MS medium. (Culture after 4 weeks).**

| **Treatment (mg/l)** | **Shoot Tip** | | | **Stem** | | | **Leaf** | | |
|---|---|---|---|---|---|---|---|---|---|
| | No. of Shoot (leaves) | No. of roots | Length of root(cm) | No. of Shoot (leaves) | No. of roots | Length of root (cm) | No. of Shoot (leaves) | No. of roots | Length of root (cm) |
| **BAP** | | | | | | | | | |
| 0.5 | 8± 0.57b | 0 | 0 | 11 ± 0.57c | 0 | 0 | --- | --- | --- |
| 1.0 | 10± 0.57c | 0 | 0 | 5 ± 0.57b | 0 | 0 | --- | --- | --- |
| 1.5 | 6 ± 0a | 0 | 0 | --- | --- | --- | --- | --- | --- |
| 2.0 | 5 ± 0a | 0 | 0 | --- | --- | --- | --- | --- | --- |
| **TDZ** | | | | | | | | | |
| 0.5 | 4± 0.57b | 0 | 0 | --- | --- | --- | --- | --- | --- |
| 1.0 | 3± 0ab | 0 | 0 | --- | --- | --- | --- | --- | --- |
| 1.5 | 2± 0a | 0 | 0 | --- | --- | --- | --- | --- | --- |
| 2.0 | 13± 0.57c | 0 | 0 | --- | --- | --- | --- | --- | --- |
| **KIN** | | | | | | | | | |
| 0.5 | 4 ± 0.57a | 3 ± 0a | 0.566± 0.0a | 2 ± 0.57a | 0 | 0 | --- | --- | --- |
| 1.0 | 6 ± 0.57b | 7 ± 0.57b | 0.6367 ± 0.015b | 5± 0.57b | 0 | 0 | --- | --- | --- |
| 1.5 | 9 ± 0c | 10 ± 0d | 0.68± 0.0c | 8 ± 0.57c | 0 | 0 | --- | --- | --- |
| 2.0 | 10 ± 0.57c | 9 ± 0c | 0.66± 0.0bc | 11 ± 0.57b | 2± 0.57b | 0.46 ± 0.06b | --- | --- | --- |

**Data are expressed as mean ± SE (Standard Error) from three independent experiments. Means followed by the same letter are not significantly different from each other at the 0.05 level according to Duncan's Multiple Range Test.**

While leaf explants showed a negative reaction, shoot tips at all BAP concentrations showed growth. The shoot tip's maximum shooting response was observed at 1.0 mg/l. Stem showed growth in two concentrations, 0.5 mg/l was more effective where neither of the explants showed any indication of root development (Fig. 6, 9A-B).

Only the shoot tip showed growth at all TDZ doses, but the stem and leaf explants responded negatively. In the shoot tip, where there was no indication of root growth, the good response of shooting was seen at 2.0 mg/l (Fig. 7, 9C).

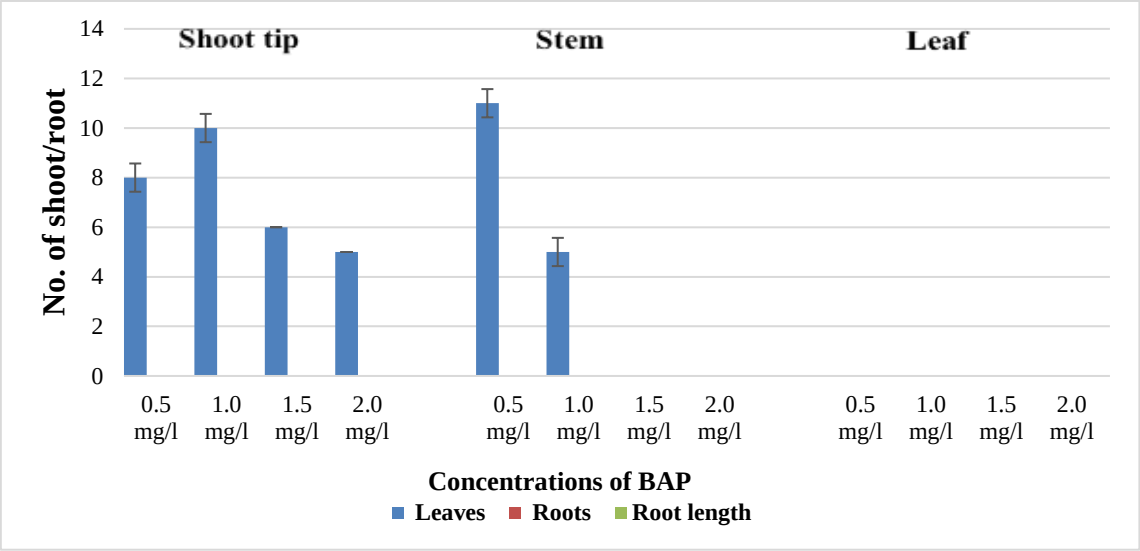


**Figure 6. Effect of BAP on *in vitro* regeneration of shoot tip and stem (culture after 4 weeks).**

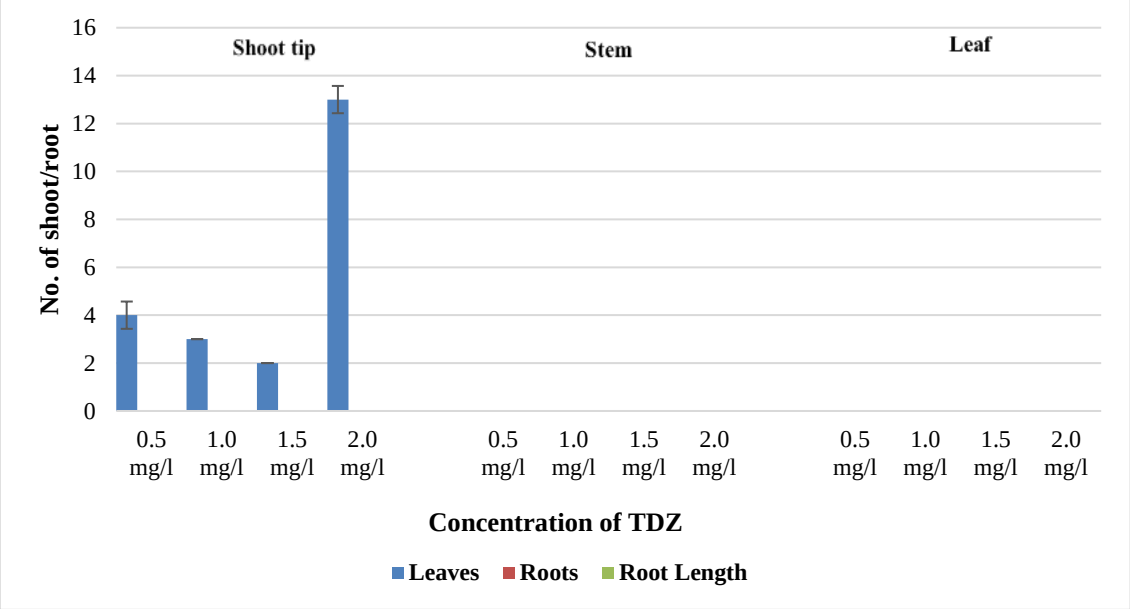


**Figure 7. Effect of TDZ on *in vitro* regeneration of shoot tip (culture after 4 weeks).**

While leaf explants responded negatively to all KIN concentrations, shoot tip and stem explants demonstrated growth. The optimal shooting and rooting response at the shoot tip was demonstrated by the 1.5 and 2.0 mg/l. Stem showed effective shooting response in all concentrations. At a dosage of 2.0 mg/l,

the greatest leaves number was seen, along with roots (Fig. 8, 9D-F).

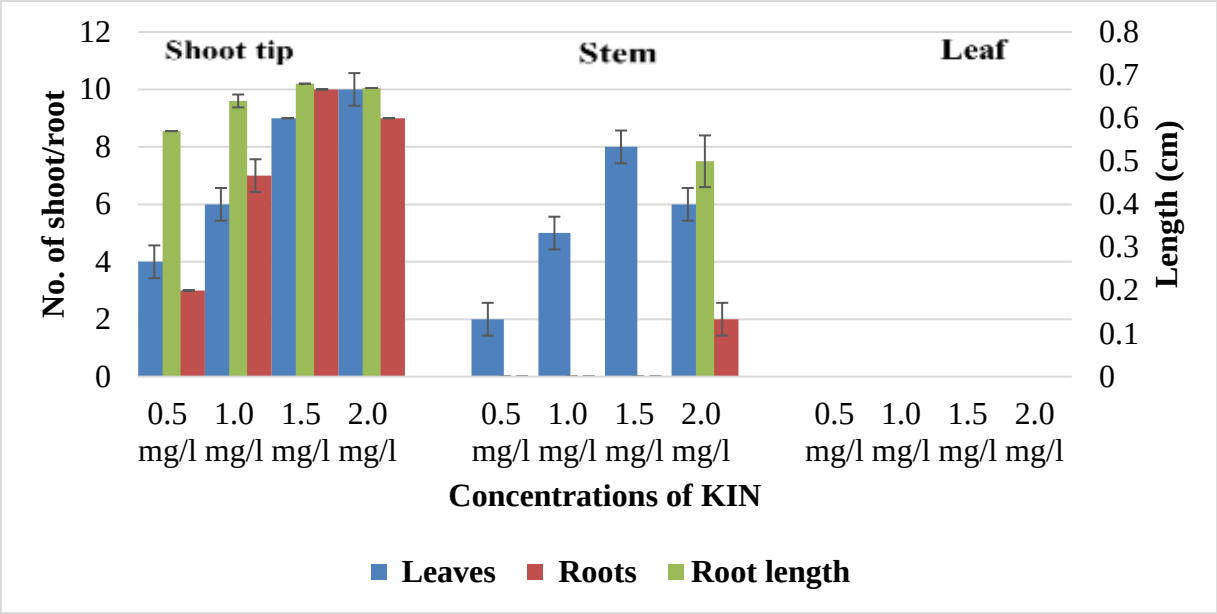


**Figure 8. Effect of KIN on *in vitro* regeneration of shoot tip and stem (culture after 4 weeks).**

**Acclimatization of Plantlets**

In pots covered with plastic bags, the plantlets with established roots were moved to 50% succulent soil (sand, potting soil, perlite 2:2:1) and 50% peat moss to acclimate in the culture chamber in a controlled atmosphere. The pots were supplied with sustainable moisture. Regular observations were made of the plantlets (Fig. 9G-I). The plantlets were then moved to a greenhouse, where their capacity to adjust to the surroundings was observed. After ten weeks, when the plantlets had settled in the soil and started to grow, they were transferred to a natural environment for hardening.

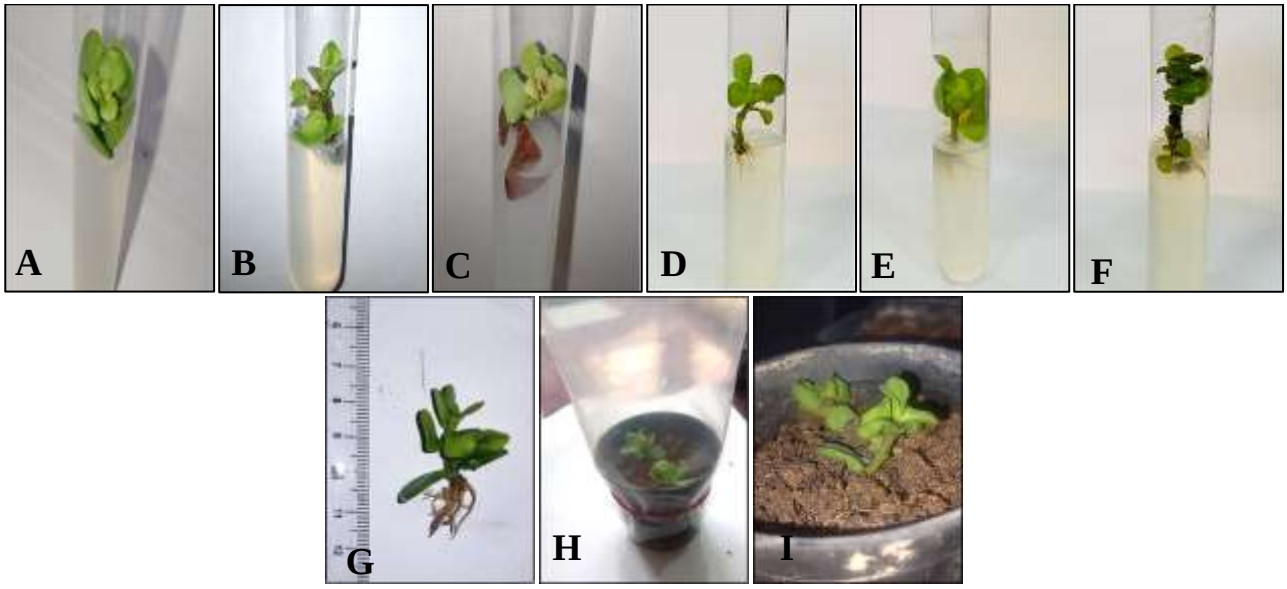


**Figure 9. Effect of shoot inducing PGRs on *in vitro* regeneration of different explants A) Shoot tip (1.0 mg/l BAP) B) Stem (0.5 mg/l BAP) C) Shoot tip (2.0 mg/l TDZ) D) Shoot tip (1.5 mg/l KIN) E) Shoot tip (2.0 mg/l KIN) F) Stem (2.0 mg/l KIN) (Cultures after 4 weeks) (1.5X). G) Acclimatization**

**of regenerated plantlets, before transferred to the soil (2X) H) Pot covered with polythene bag (1.5X) I) Pot uncovered (2X).**

## DISCUSSION

The present research was planned to investigate *in vitro* response of different explants of *Crassula ovata* (Mill.) Druce. The main goal was to boost output, and in order to bound the import of costly *C. ovata* by micropropagation, an effective regeneration system must be established [7]. As a result, parameters influencing regeneration explant type, and PGRs in culture media were examined.

Appropriate explant selection is crucial during the initial stage of micropropagation [30, 31]. While no comprehensive studies have been conducted on the effect of explant source in the micropropagation of *C. ovata*, research on other plant species highlights the critical role of explant origin in ensuring successful in vitro multiplication and preventing culture decline [32]. In order to replicate the mother plant's leaves, shoot tips, and stems, a variety of types of explants were selected for the present research. According to the findings, the shoot tip produced the most growth, and there were far more leaves and roots than in the leaf and stem explant.

Different types of explants were cultured under aseptic conditions in this study. 70% ethanol and 10% bleach were used to sterilize the explant's surface, which helped to create wholesome, contamination-free cultures. This outcome is consistent with earlier research that showed that sodium hypochlorite and ethanol were effective surface sterilizers [4, 15, 33].

Various concentrations of plant growth regulators were incorporated into the MS medium to investigate their effect on the shoot and root induction. These compounds are growth regulators: KIN, BAP TDZ, IAA, NAA and 2,4-D. Cell elongation and root growth depend on auxins [13, 34]. When IAA and 2,4-D were added separately to MS medium, the best root induction was seen. All explants showed impressive development from both PGRs. Cytokinins boosts the production of shoots and cell differentiation [19, 31]. When MS medium was used with BAP and KIN separately, significant shoot induction was seen. The shoot tip and stem of both PGRs exhibited notable growth.

With the exception of TDZ, all plant growth regulators were essential to the development and proliferation of *C. ovata* during micropropagation. Thidiazuron is utilized as cytokinins and is a significant regulator of plant growth. Every culture that received TDZ as a supplement became brown. It could be due to the oxidation of phenolics in tissue culture, which can cause tissues and the growth medium to brown and prevent tissues from growing in the media. It might slow down cell division and explant regeneration, which would ultimately source of death of grown tissues [31, 35, 36].

Under yellow light, the highest development was shown by explant. After three weeks of treatment with both light yellow and whitish, the explants showed encouraging shoot induction under the yellow light. Yellow light was believed to help explants accumulate bioactive flavonoids, which could result in faster development [37].

Bae and Yong (2007) found that their plants had a 95% survival rate when they used composite soil, which is a 1:1 mixture of sand and peat moss [34]. Ahmed *et al*. (2014) found that the survival rate of *C. ovata* was 90 percent when they employed succulent soil, which is a blend of soil, sand and perlite (2:2:1) [15]. But in the current work, acclimatization was accomplished by moving many healthy regenerated plantlets into tiny pots with a 1:1 ratio of succulent soil to peat moss, where *C. ovata* survival is 92%.

## CONCLUSION

It was concluded from study that number of factors were involved in the regeneration of *C. ovata* (Mill.) Druce. Different strengths of PGRs provided different results depending on the nature of their activity. The remarkable outcomes were observed on different strengths of IAA, 2,4-D and KIN. Shoot tip was the most responsive explant to regenerate shooting and rooting among other explants. The usage of *in vitro* propagation technique of *C. ovata* is significant and practical approach in addressing the plant's slow growth and promoting the mass production of disease free and healthy plants at commercial scale. The present study will also beneficial for the economy of country to benefit both horticulture and Plant conservation efforts. The developed protocol can be utilized further for secondary metabolite production and also for the elicitation of beneficial compounds under optimized culture conditions for their large-scale production.

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