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# Recent Advances of Hormonal properties in Phloroglucinol and Melatonin for Plant Tissue Culture and its Applications: A Review

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**ABSTRACT: Plant tissue culture is a technique that is used for conserving the genetic material and increasing clonal regeneration of plants which is a tedious process while using standard methods. Moreover, not all plants can be successfully propagated using tissue culture and require novel hormones to achieve in-vitro propagation. However, there are a variety of hormones that are proposed to act as plant master regulators which can induce redifferentiation and can promote more efficient in -vitro growth of plants. Various studies showed certain regenerative hormonal-like properties using phloroglucinol and melatonin. Phloroglucinolis a naturally occurring secondary metabolite found in many plants that have growth-promoting properties such as increased root/shoot formation, somatic embryogenesis, reduces hyper-hydricity (accumulation of water), and improved recovery of cryopreserved plants. Furthermore, melatonin is a pleiotropic molecule known for its multifunctional properties which regulate functions like plant growth and development, including root architecture, leaf senescence, seed germination, fruit ripening, flowering time, and biomass production, and also fights oxidative stresses such as reactive oxygen species and NO to provide tolerance to the plants. This review focuses on the functions of melatonin and phloroglucinol in different aspects of plant tissue culture including growth, regeneration, and regulation in different plants.**
**Keywords: Somatic embryogenesis, Root formation, Hyper-Hydricity, Cryopreservation, Reactive oxygen species, Leaf senescence, Oxidative stress.**

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## INTRODUCTION

Plant tissue culture is also referred to as culturing of cells or tissues under sterile conditions to propagate different plant species and conserve their genetic resources on a large scale. However, many plants cannot successfully propagate using standard plant tissue culture techniques and require complex combinations of different media and hormones to improve in-vitro propagation. This will provide better and more efficient growth in plants. Several studies showed the potential of different hormones to achieve successful plant propagation. This compilation of studies illustrated the use of phloroglucinol and melatonin acting as growth-promoting regulators in plants (Table 1 and 2), as an efficient way to enhance the growth of plants under lab conditions. This research will also provide reasonable solutions for certain problems that restrict the current plant sciences research such as promoting secondary metabolite production, genetic conservation, and transformation studies.

**Significance and use of Phloroglucinol in plant tissue culture.** The compound Phloroglucinol (PG) has been used as an additional supplement with other plant growth regulators, yet the growth-promoting properties of this hormone in plants is still under. Phloroglucinol also known as 1,3,5-trihydroxy benzene or phloroglucin is a naturally occurring secondary metabolite extensively present in various plant families like Lauraceae, Cannabinacaeae, Rosaceae and some marine plants such as brown alga species (da Silva *et al.*, 2013). In the medicinal and pharmaceutical sector, PG possesses antibacterial, antioxidant, and anti-inflammatory bioactivities including anti-ulcer and cytotoxic effects against tumor cell lines (Abdel *et al.*, 2016; Singh *et al*., 2009, Singh and Sharma 2020). This compound was isolated from organic waste such as lemon peels and showed bactericidal properties against oral bacteria like *Streptococcus mutans*, and *Porphyromonas gingivalis* causing period on it is in the mouth which gives an edge in evaluating PG as an antimicrobial agent and exploring its use for plants. Moreover, various studies in plant tissue culture illustrated hyper-hydricity and poor recovery of cryopreserved tissue that gave negative results (da Silva *et al.*, 2013). Hyper-hydric stress illustrates different biochemical activities showing the oxidative stress and morphological abnormalities in the plant (da Silva *et al*., 2013). This hyper-hydric or humid environment in plant is linked to low production of enzymes responsible for lignin production. Hence, addition of phloroglucinol in the media can resolve hyper-hydricity by increasing the production of the enzymes and hence, increasing lignin synthesis in plants (Phan and Hegedus, 1986).

Phloroglucinol with other growth hormones like IAA, resulted in 2 times growth of new rootsin the sugarcane plant and later, in the ex-vitro acclimatization phase, results recorded the formation of root hairs and high survival rate percentage (Gómez-Kosky *et al.,* 2021). Various researches have shown the use of PG in propagation of plants with *in vitro* tissue culturing of *J. curcas* where PG added with IBA promoted the growth frequency of roots up to 76.7% (Kumar *et al.,* 2010). PG in combination with IBA promoted root induction and with BA enhanced the shoot induction (Petti, 2020). Furthermore, regeneration from nodal plants of *W. coagulans Dunal* evaluated supplementation of 3.9uM PG with 2.2uM BA and 2.3uM Kn in MS media, which enhanced the propagation of nodal plants within the shoot region. In addition, a two-step method was used with pulse treatment for elongation of roots where 71.6uM choline chloride (CC) and 3.9 uM PG was given. The roots were transferred to the media and showed enhanced roots elongation (Jain *et al.,* 2011).

Studies also show the association of phloroglucinol with other growth hormones to enhance root and shoot induction to multiple folds (Gómez-Kosky *et al.,* 2021; Kumar *et al.,* 2010; Petti, 2020; Jain *et al.,* 2011). The effect of PG on direct rooting of apple rootstocks with different concentrations of PG were evaluated for 0, 0.5, 1.0 and 2 $mg \cdot L^{-1}$ concentrations respectively. Induction of a low concentration of phloroglucinol in root media resulted in early rooting with an 80% high rooting percentage after the third week of culturing whereas high concentrations of phloroglucinol up to 1 mM induced rooting up to 100% from the second week of culturing (Kim *et al.,* 2020). The broader applications of the use of Phloroglucinol are very limiting and yet to be explored as there are several drawbacks of using a high concentration of PG in the media where higher concentrations up to 2mM resulted in reduced rooting efficiency of 80%. Several studies also illustrated PG as a sterilizing agent in tissue culture media that will sterilize the media without the process of autoclaving (Cardoso and Teixeira da Silva 2012).

**Significance and use of Melatonin in plant tissue culture.** Melatonin (N-acetyl-methoxy tryptamine) is a ubiquitous molecule having pleiotropic actions with some multi-functional properties like growth and development in plants including root architecture, leaf senescence, seed germination, fruit ripening, flowering time, and biomass production, and also fights oxidative stresses such as reactive oxygen species and NO to provide tolerance to the plants. Melatonin was first discovered in the bovine pineal gland in the year 1958 and was known for its role as a neurotransmitter in animals (Bose and Howlader 2020). Melatonin contributes to regulating many physiological events such as cardiac rhythms, sleep, body temperature, mood, appetite, immunological systems in animals, and retina physiology in animals (Arnao and Hernández 2015; Tousi *et al.,* 2020). It was until 1995 that melatonin was discovered in higher plants by by Dubbels *et al.* (1995) and Hattori *et al.* (1995) and was called Phyto-melatonin (Arnao and Hernández 2015; Liu *et al.,* 2020). This discovery led to the growing study of this hormone in plants and its presence was reported in several variety vegetables, fruits, seeds, cereals, and medicinal herbs (Paredes *et al.*, 2009).

Further research on melatonin was focused to determine the physiological role in plants and as a result, melatonin was reported as a growth promoter and rooting agent followed by strong shreds of evidence (Zhang *et al.,* 2015). Apart from growth and development processes, melatonin was also seen to participate in biotic and abiotic response regulation in plants (Zhang and Zhang 2021). Melatonin being a plant growth regulator has come forward as a bio stimulant of choice for an eco-friendly and safe method to boost plant resistance against severe stress conditions. This is because the plant's natural defense system fails to give adequate protection against extreme conditions (Sharma *et al*., 2020). The amount of phyto-melatonin varies which makes extraction and quantification techniques difficult in different parts of the same plant (Ghosh, 2021). Several studies provided pieces of evidence suggesting that plants just do not synthesize their melatonin but also it can be stored in plant's certain parts such as fruits, dry seeds, etc. Melatonin is synthesized in intracellular organelles such as mitochondria and chloroplast. Regulation of circadian time management is one common function of melatonin in plants and animals (Reiter *et al*., 2015).

Melatonin has a very significant influence on improving root regeneration and growth and it has been observed that at low concentrations it promotes root growth whereas at high concentrations it inhibits the root growth (Liu *et al*., 2022). Exogenous melatonin as a growth stimulator shows a similar role as auxin as they share tryptophan as a precursor (Bhattacharya and Jha 2020). In horticulture, melatonin alleviates damages due to cold stress in many plants, one such example is tea, where melatonin enhanced cold stress tolerance by promoting redox homeostasis and anti-oxidant defenses in plants (Li *et al*., 2018).

## APPLICATIONSOF PHLOROGLUCINOL IN PLANT TISSUE CULTURE

**Hyper-Hydricity.** In plant tissue culture, a problem arises of accumulation of excess water in which plant roots result in poor development and growth. This phenomenon is termed hyper-hydricity and is linked with reduced production of several enzymes responsible for the synthesis of lignin precursors and their polymerization due to humid environment, high illuminance, and high PGR levels (Kevers *et al.,* 2004). This causes stress conditions in plants which leads to several morphological changes in plants. These conditions develop several abnormalities in the plant such as a reduction in lignin oxidative stress (Rogers and Campbell 2004). The possible solution to this problem can be controlling the PGR levels or light conditions and changing the gelling agents.

Different enzymes like CoA ligase resulted in lesser levels and expressions in hyper-hydric tissues and explants. When phloridizin and PG were added to media for *Malus domestica* and *Helianthus* resulted in increased activity of lignin synthesis enzymes (Phan and Hegedus 1986). Another study was conducted showing identical results where different plant *Achyrocline flaccida* was used in which shoots were grown in a liquid medium with phloroglucinol resulted in better and increased lignification and improved xylem development (Ross and Castillo 2010).

**Growth and development of plants using Phloroglucinol.** There are several methods of tissue culturing protocols in different studies given in the literature that can be most effective in the micropropagation of plants. This effectiveness of growth and development is given using different combinations of chemicals such as PGRs-illuminance with several abiotic and biotic factors. Furthermore, the most effective way to achieve a considerable growth of plants is chemical manipulation in plant tissue culture, where new and novel chemicals can be used and these can become the alternative for currently used plant growth hormones. Different phenolic compounds reactions with other hormones are already being used in PTC to enhance cellular multiplication, callus formation, formation of adventitious shoots and roots, and promoting the development and proliferation of shoots upto certain folds. The most important reaction to phenolic compounds is mainly with IAA which includes regulation of IAA levels and oxidative catabolism of IAA can be the modification which resulted in the inactivation of auxins (Normanlya *et al*., 2004).

When the phenolic compounds are added externally, they act as alternatives for oxidative enzymes and prevent oxidative catabolism of auxins. The phenolic compounds act as alternates to several and wide range of plant hormones that are normally not used in plant tissue culture as their oxidation can cause several problems like the browning of media and necrosis of cells (Benson, 2000; Reis *et al.,* 2008).

**Effects of PG on shoot proliferation.** Shoot proliferation is the most important part of plant tissue culture where the development of shoots takes place by elongation of axillary buds. Due to its easy and simple protocol, it is an important development for mass-production of plants. Several studies illustrated the improved proliferation of shoots under lab conditions after induction of phloroglucinol. A study showed different linked interactions of PG and sucrose in shoot tips depending on their genotypes. PG when supplemented in media containing *Capsicum annuum* showed an increase in bud induction response by 18% (Kumar *et al*., 2005).

Another study revealed that the addition of PG for *Coccoloba uvifera* spp. has no effect on *in vitro* proliferation of shoots when compared to control, but significantly increased the shoot elongation with the combination of BAP (1.0 mg $L^{-1}$) and NAA (0.5 mg $L^{-1}$). The results recorded an increase in shoot length up to 8.2 cm within 4 weeks. The concentration of PG in this study revealed that lower and higher concentrations affected the morphometric traits in comparison to the control (Manokari *et al*., 2021).

**Effects of PG on cryopreservation.** The PG when added to media increased the recovery and survival rate of cryopreserved plants named as *Dendrobium nobile, Dendrobium protocorms* and *hybrid seeds, Cattleya walkeriana seeds, Oncidium flexuosum seeds,* and *Catasetum atratum seeds.* (Pereira *et al*., 2021; Vendrame and Faria 2011; Galdiano *et al*., 2012, 2017, 2013; Prenzier *et al*., 2018). Several other researchers reported a reduction in tissue browning by PG in culture media that was tested along with leaf segments of *Ficus carica* where PG provided an increased rate of morphogenesis with a high survival rate for cryopreserved samples (Kim *et al*., 2007).

**Effects on the initiation of root development.** Phloroglucinol acts as a potential hormone that promotes stimulation of callus induction and organogenesis in shoot and micro bulbs region with enhancement of root region. The concentrations can vary from 1-10 mg/L where the rate of induction ranges from 25-37% (Petti, 2020). Phloroglucinol acts as the root promoting hormone along with several other factors like auxin type and concentration, quality of shoots and roots, age, and temperature. Recent literature revealed that PG promoted rooting in different plant species like *Jatropha curcas* L. (Daud *et al.*, 2013) and apple cultivars (Dobránszki and Silva 2010). The growth of roots was significantly affected by induction of PG in plant media with a concentration of $p<0.05$ PG in *Carica papaya* spp (Al-Shara *et al*., 2020). The development of roots significantly increased up to 5 folds when IAA was induced with PG and auxin oxidative catabolism was decreased from 100% in control to 21% in PG-induced media. There were several hypothetical observations made by the researchers like IAA has a much higher role in inducing roots in explants than PG but PG enhances the activity and expression of IAA (Dobránszki and Silva 2010).

**Somatic embryogenesis.** From single cells, identical clones with similar genetic makeup can be achieved by the technique named somatic embryogenesis. There are several challenges to somatic embryogenesis such as somatic clones cannot always be obtained using regular plant growth hormones and manipulation of physiological conditions. Hence, the use of PG as potential PGR can induce somatic embryogenesis. When PG added to media it promoted SE development into plantlets and PG helped in increasing the proliferation rate of SE in many cases though some cases illustrated inhibitory effects by inhibiting rooting of *Prunus cerasus* shoots up to 50% when PG was added at 1.28mM concentration (Snir, 1983). The technique of somatic embryogenesis is the process of producing an embryo using somatic cells. This process of embryo production is explained by Strasburger in 1878 as a form of apomixis, also known as adventitious embryony (Merkle *et al*., 1995). Somatic embryogenesis is considered a model system to study various events and processes that occur during the growth and development of a plant, and not restricted to limiting factors when observed in variable conditions (Quiroz-Figueroa *et al*., 2006). The application of PG in somatic embryogenesis of plantshas shown a positive growth result as in the case of *Feijoa Sellowiana Berg*, the addition of PG and phloridzin gave rise to 3 replicates of 15-20 zygotic embryos in each treatment. The observation showed that the highest number of embryos were obtained at 79µM concentration of PG, and at 197.5µM, nearly 94% of the total explants produced somatic embryos but inhibits the germination of embryos when added in a higher concentration (Reis *et al*., 2008). In *Ornithogalum dubium*, 37.5% induction rate was obtained when 4mg/l PG is added to the media showing positive effects in callus induction within a concentration range of 1-4 mg/L (Petti, 2020). The addition of 2.5µM PG gave highest number of globular embryos and influences the change in the physiological process of endogenous cytokinin pool for *in vitro* development of *Tulbaghia simmleri*, (Kumari *et al*., 2018). When PG was used along with nutrient media for the somatic embryogenesis of *Lachenalia viridiflora* within a concentration range of 2.5-5µM, the highest percentage of embryo germination was obtained in addition to 5µM of PG with full strength MS media (Kumar *et al*., 2016). The positive influence of PG on the development and growth of somatic embryos in the case of different plant species has been observed and reported (Reis *et al*., 2008; Kaur *et al.*, 2018).

## APPLICATIONS OF MELATONIN IN PLANT TISSUE CULTURE

**Melatonin: Role in stimulating secondary metabolites production**:

In literature, melatonin has been reported to increase the secondary metabolites production in several plant species such as *Lepidium sativum, Vitis labrusca, Salvia rosmarinus*, and *Citrus aurantium*. In *Lepidium sativum*, exogenous melatonin treatment and UV-C radiation were used for the enhanced production of secondary metabolites i.e., polyphenolic compounds. The results revealed that for melatonin at 20 µM concentration among nine quantified compounds, the accumulation of secondary metabolites increased by almost three times (Ullah *et al.,* 2019). In *Vitis labrusca*, about 27 metabolites were observed which are known to be accumulated because of melatonin treatment. Furthermore, among the total of the present 464 metabolites, exogenous melatonin treatment significantly increased the production of 27 metabolites. The underlying reason lies in the fact that melatonin promotes the synthesis of ethylene which regulates certain metabolic pathways as well as plant hormone signal transduction and in turn regulates the expression of certain genes like VvLAR2, VvDRF, etc. and these genes promote the production of secondary metabolites production. In the grape berry, melatonin significantly changes the outline in terms of secondary metabolites production by encouraging the VvMYB-14 effectuated biosynthesis of ethylene. This VvMYB-14 engages in the MT signaling pathway which is ultimately responsible for the management of the secondary metabolites production (Ma *et al*., 2021).

Exogenous melatonin treatment of 50 µM in *Salvia rosmarinus* assisted them to overcome arsenic stress by building up their anti-oxidant machinery and their osmoregulation capacity which in turn improved the production of secondary metabolites in the herb. Rosemary is a medicinal plant that secretes essential oils that contain flavonoids which are helpful to treat various brain, blood, and heart-related diseases. On foliar exogenous melatonin treatment of 50µM, the percentage of the essential oil increased by 100% (Farouk *et al.,* 2019).

In *Citrus aurantium*, there is a variety of bioactive compounds production such as phenolic compounds, essential oils, and certain flavonoids which are known to have promising antiallergic, cardioprotective and vasodilatory effects. 15µM exogenous melatonin treatment was observed to give 1.5 times higher phenolic and flavonoid content in the plant whereas 1µM exogenous foliar treatment increased the yield of essential oils by 0.46% (Sarrou *et al*., 2015). In the past decades, the potential use of melatonin has shown extraordinary results in terms of regeneration and secondary metabolites production (Li *et al.*, 2020).

**Role of melatonin in cell differentiation.** After the meticulous analysis of the data related to this hormone's influence on photosynthesis, growth to cell differentiation process in a given plant, it is observed that it is a multifunctional hormone that affects various sets of aspects that combine individual benefits for plant growth. The most common problems faced by a plant during cell differentiation are photosynthesis, metal/chemical phytotoxicity, chlorophyll content, etc.

Experiments by Tousi *et al*. (2020), exogenous pre-treatment of melatonin alleviates cadmium phytotoxicity and improves growth in mallow plants. Cadmium accumulates in different parts of plants like roots, shoots and edible parts which leads to a lowering of the quality of plants by synthesis of Reactive Oxygen Species causing damage to plant membranes and destroying cellular organelles (Farouk *et al.,* 2019). To eliminate this problem, pre-treatments of melatonin are given exogenously reducing the accumulation when concentrations were scrutinized. This was due to the effects of melatonin during cadmium stress made to reduce the translocation factor of cadmium from roots to other parts of the plant. Results show that more cadmium is found in roots than in any other part of the plant. The assumptions by researchers illustrate that this is due to stimulation of carbohydrates metabolism and reduced cadmium-induced oxidative stress. When this hormone is provided exogenously at different concentrations of 15uM, 50uM & 100uM results were varying comparing to the control plant. However, the higher composition of hormones gave negative results by inhibiting the biochemical/physiological activities. 15uM & 50uM concentrations resulted in almost equal with little variation in shoot length, relative water content, and stomatal conductance (Tousi *et al.,* 2020). When ROS and RNS are formed and accumulated in plants it leads to electron leakage, lipid peroxidation, and membrane damage along with nucleic acid and protein damage (Kabiri *et al.*, 2018). NO is another signaling molecule that has several roles in physiological functions so they play a key role in responding to multiple abiotic stresses. The signaling pathway that is mediated directly by NO, melatonin has been observed to be involved in such signaling pathways.

One study was conducted on pea plants on the parts of the foliate application and seed soaking activity using melatonin hormone. Rather than seed soaking, foliate application of hormone enhanced/regulated the growth biomarkers like root growth, shoot growth, leaf area, and fresh/dry mass of plants (Yusuf *et al*., 2020). Results showed that this hormone influenced all mentioned biomarkers i.e., giving concentrations of 10µM, and 50µM only 100µM concentration gave better results in length, weight, and height of plant (Zhang *et al*., 2013). Coming to the chlorophyll content aspect, seeds soaked with melatonin for 100µM resulted to have better chlorophyll and carotenoid contents of pea plants. Along with these, nitrate reductase and carbonic anhydrase which are the most important photosynthetic enzymes are regulated and stimulated by the application of melatonin as foliar spray (Yusuf *et al.,* 2020). This enhances the process and leads to the growth and development of plants without delay. Net photosynthetic rate and stomatal conductance are also raised during analyzation of data collected (Yusuf *et al*., 2020).

When melatonin hormone was applied to soybean seeds by soaking them with a coagulating agent with different concentrations of hormone-like 10µM, 50µM, 100µM, 200µM, it was observed that at the end of the 3$^{rd}$ and the 4$^{th}$ day, seeds are germinated which are treated with 50µM and 100µM concentration of hormone (Wei *et al.,* 2015). Apart from this, it was also noted that the leaf size of plants with melatonin treated is larger than control plants. This entitles the effect of hormone signaling on photosynthesis and growth factor stimulation in plant development. After the fifth week, plants are observed to be taller than controls and a trifoliate leaf is appeared to signify the hormone present in the inputs for plant growth and development (Wei *et al.,* 2015). After analyzing the agronomic traits of soybean, it revealed that the size and number of pods and seeds are more/better than control plants during the field test. The positive results showed that hormone did not affect the aspect of test weight (Wei *et al.,* 2015). Among the above-mentioned concentrations, 200µM of hormone didn't provide desired results and inhibited some of the mechanisms from occurring.

**Stress tolerant activity.** A plant faces several stresses depending on its physical or physiological or biological deficiencies affecting the growth and developmental phases. Regarding these aspects, there are some experiments and research available playing an evidential role in the usage of melatonin hormone preparing the plant and culture to be tolerant of certain stresses of the plant.

Experiments were done on basil (*Ocimum basilicum* L.) under salt stress, despite not affecting the root growth and shoot growth, but have shown enhanced results in growth, and antioxidant activity with the exogenous application of 10µM melatonin (Bahcesular *et al*., 2020). Experiments conducted on cucumber seeds with 100µM of melatonin resulted in a better seed germination rate than control plants (Zhang *et al.*, 2013). Moreover, it was stated that 150µM of NaCl along with 1% melatonin also gave the expected rate of seed germination in cucumber seeds (Zhang *et al*., 2013). This enhanced the assimilation rate under salt stress water provided to the plants and salinity did not affect the growth and germination activities. The same conditions are repeated with the chilling stress (Posmyk *et al.,* 2008). It was recorded that use of melatonin also resulted in a better germination rate, seedling growth, and vigor index under the cadmium stress (Nabaei and Amooaghaie 2019). Fewer studies showed that cadmium or other metal stresses make the plant weak in assimilation and developmental phases, but in the case of Catharanthus, results were a contrast in nature with no defective qualities roseus (Nabaei and Amooaghaie 2019).

Researchers recently recognized this hormone as a bio-stimulant and plant signaling regulator (Liang *et al*., 2018) based on the study and research conducted in a few experiments on *Catharanthus roseus* and *Moringa oleifera*. Under abiotic stress, this hormone regulates the signaling pathways of proteins to enhance the growth of roots, shoot, and even in flowering stages also in *Moringa oleifera* (Sadak *et al.,* 2020). Especially, under drought conditions, in cucumber plants, exogenous application of 100µM hormone in plant raised the root-shoot growth ratio along with the enhanced root development later (Zhang *et al.,* 2014). Increased levels of ROS and RNS inside a cell are one of the primary responses to stress. Abiotic stresses make plants perform down-regulation of growth factors and upregulation of suppression factors; this results in plant death gradually. But the use of melatonin hormone between the concentrations of 50µM-100µM boosts the plant growth gene regulation like psa A, F, G, H, and others for photosynthesis. It could also regulate the ATPase activity for plant development. This is possible only when different combinations of NaCl and $H_2O$ are collectively presented to plants (Wei *et al.*, 2015).

**Growth regulatory properties of melatonin in higher plants.** One study revealed the role of melatonin as a growth promoter in etiolated lupin (*Lupinus albus*) where it shows growth-promoting effects of hypocotyls at micromolar concentrations and inhibitory effects at higher concentrations. Melatonin is 63% similar to indole-3-acetic acid which makes it an auxin-like hormone (Hernandez-Ruiz *et al.,* 2004). In red cabbage (*Brassica oleracea rubrum*) and mustard, the concentration-dependent action of melatonin is there ranging from promoting to inhibitory effect (Posmyk *et al*., 2008; Chen *et al*., 2009).

Another effect of melatonin on organogenesis was seen as a histogenesis inducer and later it was confirmed in cucumber (*Cucumis sativus*) (Zhang *et al.,* 2013). The effect of melatonin in the formation of adventitious roots is considerable where melatonin in combination with auxins such as indole-3-acetic acid (IAA) and indole-3-butyric acid (IBA) shows potential effect on rooting and is a topic of interest (Pacurar *et al*., 2014). Melatonin treatment altered the levels of abscisic acid (ABA) and gibberellins (GA). In saline conditions, melatonin provoked GA biosynthetic genes (GA20ox and GA30x) in cucumber seedlings. This promoted salt inhibited the germination process due to the high-level activation of GAs as GA4 (Zhang *et al*., 2014). Melatonin also upregulated ABA catabolism genes (two CYP707 monooxygenases) and down regulated a key enzyme in the biosynthesis of ABA called 9-cis-epoxy carotenoid dioxygenase (NCED). This decreased ABA levels rapidly under salt stress during seed germination (Zhang *et al.,* 2014). A study was done to see the effect of exogenous melatonin treatment on tomatoes to understand the difference in the quality of the fruit, ethylene metabolism, and post-harvest ripening proved that 501M melatonin for 2hrs brings a considerable change in fruit ripening parameters such as ethylene signaling, lycopene levels, fruit softening, flavor, and biosynthesis of enzymes as compared to untreated tomatoes (Sun *et al*., 2015).

## CONCLUSION AND FUTURE SCOPE

Studies showed that phloroglucinol can be used as a cryoprotectant that increases the recovery and survival rate of the cryopreserved plant and can be beneficial for long-term storage of plantlets, synthetic seeds, and explants (Pereira *et al.,* 2021) whereas, the primary role of phyto-melatonin in plants is to provide a barrier and defensive strategies against the oxidative stresses in extreme conditions. The role of melatonin signals in the microbial-root interface can be the emerging field that is yet to be explored and studied along with different metabolic pathways like ion transport, related to the growth and development of plant species, and different nutrient availability for plants. Another aspect that is yet to be covered is, behavior and expression of these hormones for secondary metabolites of the desired plant. At present, there are limited plant species that are used for research purposes in plant tissue culture. There are several different studies in the literature (Table 1, 2) that revealed phloroglucinol and melatonin can be used as growth hormones when added at different concentrations but still their potential as growth regulators is unclear. Furthermore, these compounds have shown (Table 1, 2) the potential of enhancing the roots and shoot elongation, promoting somatic embryogenesis, used as cryoprotectants, stress-tolerant, cellular differentiation, and can be used as growth regulators if added in particular concentrations along with other hormones. The compounds PG and melatonin also open up new avenues in increasing the mass production of different plant species and can also have an edge in improving the genetic stability of genetically modified plants and this can lead to higher chances of survival of different plant species that are genetically modified or unsuccessful to regenerate using tissue culture. However, the pathways and mechanisms followed in plants are yet to be fully explored, this review paper outlines the recent trends and research that are done for phloroglucinol and melatonin in different plant species which will give researchers a better understanding to use them in their future experimental researches.

**Table 1: Studies and role of phloroglucinol in different plant species.**

| ***Plant Species (Family)*** | **Eudicot/ Monocot** | **Phloroglucinol Concentration + Hormones in MS media (mM/uM/mg/L)** | **Biological function of Phloroglucinol** | **References** |
|---|---|---|---|---|
| *Diospyros crassiflora (Ebenaceae)* | Monocot | 396.5 mM PG + 14.2 mM indole-3- butyric acid | Increased no. of roots/ auxin like property during root initiation | Tchouga *et al*. (2020) |
| *Musa accuminata Cv. Grand Naine (Musaceae)* | Monocot | 200 uM Phloroglucinol | Acts as cytokinin/auxin, *in vitro* regeneration of plantlets, enhanced roots/shoots | Londe *et al*. (2017) |
| *Carica papaya* L. *var. Maradol Roja (Caricaceae)* | Eudicot | 79 µM Phloroglucinol + 9.8 µM indole-3- butyric acid | New roots formation, increased no. of roots with zeolite treatment, roots elongation | Pérez *et al*. (2015) |
| *Vitex negund* L. *(Verbenaceae)* | Eudicot | 20 mg/L $AgNO_3$+ 100 mg/L Phloroglucinol | Maximum shoot proliferation, induction of high no. of shoots | Stephen *et al*. (2010) |
| *Saccharum spp. cv C90-469 (Poaceae)* | Monocot | 20 mg/L Phloroglucinol +1.3 mg/L indole-3- acetic acid | Elongation/Formation of new roots, auxin like property in root growth | Gómez-Kosky *et al*. (2021) |
| *Hedychium coronarium (Zingiberaceae)* | Monocot | 1.0 $mgL^{-1}$ Phlorogluciol | Regeneration of multiple shoots from *in vitro* regenerated shoots | Verma *et al*. (2013) |
| *Rosa damascena Mill. (Rosaceae)* | Eudicot | 100 mg $L^{-1}$ Phloroglucinol with other supplements | Increase multiplication rate of plant, shoot proliferation | Salekjalali *et al*. (2012) |
| *Aristolochia tagala (Aristolochiaceae)* | Eudicot | 10 µM Phloroglucinol | Prevent tissue browning, regeneration of shoot bud | Rmeya *et al*. (2013) |
| *Juglans regia* L. *(Juglandaceae)* | Eudicot | 0.4mM Phloroglucinol | Micro-shoot proliferation, enhancement of callus | Yegizbayeva *et al*. (2021) |
| *Withania coagulans (Solanaceae)* | Eudicot | 0.5 mg $L^{-1}$ Phloroglucinol | Enhances number of shoots and roots in explant | Dehvari - Nagan *et al*. (2021) |
| *Jatropha curcas* L. *(Euphorbiaceae)* | Eudicot | 100 mg $L^{-1}$ Phloroglucinol | Induces the roots of the explant | Boonyanan & Ketudat-Cairns, (2021) |
| *Decalepis hamiltoni (Apocynaceae)* | Eudicot | 200mM Phloroglucinol | Enhanced secondary shoots formation | Gururaj *et al.* (2021) |

**Table 2: Studies and role of melatonin in different plant species.**

| *Plant Species (Family)* | Eudicot/ Monocot | Melatonin Concentration + Hormones in MS media (mM/uM/mg/L) | Biological function of Melatonin | References |
|---|---|---|---|---|
| *Fragaria ananassa (Rosaceae)* | Eudicot | 100 µM / L melatonin | Increase in biomass and reduce toxic effects of cadmium | Wu *et al.* (2021) |
| *Malva parviflora (Malvaceae)* | Eudicot | 15 µM / L melatonn | Significant increase in growth, photosyntheti c pigments | Tousi *et al.* (2020) |
| *Cucumis sativus (Cucurbitaceae)* | Eudicot | 100 µM / L melatonin | Enhance germination percentage under water stress | Bose and Howlader (2020) |
| *Solanum lycopersicum (Solanaceae)* | Eudicot | 100 µM / L melatonin | Inhibits cadmium translocation and enhances plant tolerance by regulating sulphur uptake and assimilation | Hasan *et al.* (2019) |
| *Withanias omnifera (Solanaceae)* | Eudicot | 600 µM / L melatonin | Maximum adventitious root frequency | Adil *et al.* (2015) |
| *Citrullus lanatus (Cucurbitace)* | Eudicot | 100 µM / L melatonin | Plant host resistance and and pathogen suppression | Mandal *et al.* (2018) |
| *Arabidopsis thaliana (Braddicaceae)* | Eudicot | 20 µM / L melatonin | Delayed leaf senescence | Erland and Murch *et al.* (2015) |
| *Zea mays (Poaceae)* | Monocot | 100 µM / L melatonin | Induction of root length and increase in ATP synthase activity | Turk and Genisel (2020) |
| *Gossypium hirsutum (Malyaceae)* | Eudicot | 20 µM / L melatonin | Promotes seed germination under salt stress | Chen *et al.* (2021) |
| *Santalum album (Santalaceae)* | Eudicot | 1 µM / L melatonin | Enhances nitrogen metabolism and haustorium development and enhances growth | Meng *et al.* (2021) |
| *Festuca pratensis (Poaceae)* | Monocot | 50 µM / L melatonin | Degrade polycyclic aromatic hydrocarbons in rhizosphere | Rostami *et al.* (2021) |

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