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Cell suspension culture of yew: an alternative method of taxol production

Raheleh Gorzi*

Department of Horticultural Sciences, Faculty of Agriculture, University of Zanjan, Zanjan, Iran

Abstract

Taxol is one of the most important anti-cancer drugs in the world, which is obtained from the genus of the Taxus. Taxol, with its antimicrobial properties, causes the death of proliferating cells by preventing the formation of abnormally dividing spindles. Abnormal division stops DNA transcription in the G2 / M division of mitosis and thus causes the proliferation of proliferating cells. Due to the low amount of taxol in the tissues of yew and also the very low growth of this plant, meeting the therapeutic need for this drug is the most important issue facing scientists. Commercially, taxol synthesis seems unlikely due to limited resources and the possibility of over-consumption of a natural product that could be found in endangered yew species. Therefore, a suitable alternative method for taxol production should be used. Yew cell suspension culture is one of the most important alternatives for long-term and sustainable production of taxol. This review is about cell suspension culture in different yew species.

Keywords: taxol, anti-cancer drug, secondary metabolite, cell suspension culture, taxus

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Introduction

Taxol is a very important secondary metabolite that has a diterpenoid structure (Jordon and Wilson, 1995). This valuable substance called generic name paclitaxel is the most effective anti-cancer drug of plant origin (Liu et al., 2016). Today, cancer is one of the deadliest diseases that human beings have ever struggled with and is one of the most common causes of death in the world (Shoeb, 2006). Uterine, breast and ovarian cancers are among the most common cancers among women worldwide. In recent decades, medicinal Plants have been used as a source of natural compounds agents to cancer with minimal side effects. Today, chemotherapy is the most important and main method of cancer treatment. In this method, anti-cancer drugs are used alone or in combination with other drugs(Siegel and Jemal, 2012). During the 1960s, the National Cancer Institute (NCI) began research into the anticancer effects of medicinal Plants and eventually discovered the anticancer drug taxol(Itokawa and Lee, 2002). Clinical use of Taxol was approved by the FDA after a series of clinical trials in 1983-86(Wall, 1993) and has been used as the most effective drug for the treatment of breast and ovarian cancer and for the treatment of other cancers, including cancers Lung, bladder,

* *Corresponding Author*
E-mail Address: rahil.gorzi@gmail.com
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Fig. I. Taxus trees

intestines and stomach are used(Holmes et al., 1995. Suffness and Wall, 1995).

Taxol function

Taxol polymerizes tubulin and stops it from depolymerizing, thus stopping the mitosis division cycle (Hortuwitz, 1992). Taxol stops the transcription of DNA in the G2-M stage by forming an abnormal division spindle, causing cell death and preventing cell proliferation (Jennewein and Croteau, 2001). Taxol is a trademark of Paclitaxel, registered by Bristol-Myers squibb. All taxus species contain the valuable substance taxol, including T. baccata, T. brevifolia, T. cuspidata, T. Canadensis, T. chinensis, T. Canadensis, T. yunnanensis and Taxus x media, Taxus x hunnewelliana(Cope, 1998). Taxol was first found in the skin, roots and other parts of T. brevifolia in 1971(Wani et al., 1971).

Distribution

Yew(taxus) is a tree of the genus Taxus, order of the Taxales and the family of Taxaceae, which is distributed in temperate regions, especially in the Northern Hemisphere, Europe, Asia and North America(Bedi, 1996). Eight species of yew have been reported and so far 72 varieties have been introduced for yew , the difference of which is based on height, leaf colour and cold resistance (Itokawa and Lee, 2003). Fossil studies show that yew trees are 190 million years old and the oldest fossils belong to the Miocene and Pliocene periods. In later periods, yew trees were seen with beech and hornbeam trees(Mossadegh, 1993). Yew is a shade-loving tree and is resistant to low light environments and can perform its physiological activities even in low light. Yew leaves are narrow and long and are dark green and clear on the upper surface of the leaflet, but light green on the lower surface. The leaves are 2-3 cm long and 2-3 mm wide. The flowers of the plant are male and female, located on two separate bases (Mossadegh, 1993). Yew is a slow-growing but long-lived tree that has been observed in nature for 1000 to 1500 years(Fig I).

Taxol

Taxol is a type of alkaloid diterpene that is obtained from all different species of yew and is very important as an anti-cancer drug(Mihaljevic and Bjedow, 2002). Taxol with the scientific name of paclitaxel has a chemical formula of $C_{47}H_{51}NO_{14}$ and a molecular weight of 853.92 dalton, which has a melting point of 158-160 ° C(Jennwein and Croteau, 2001). All Taxanes are derivatives of diterpenoids, of which more than 350 kind have been identified in various yew species, the most important of which are taxol, 10-deacetylbaccatin III, baccatin III , and Taxinine. In general, the taxol synthesis pathway consists of nineteen enzymatic steps (Croteau et al., 2006). The first step in the reaction is to ringing of the geranylgeranyl diphosphate (GGPP) compound (Hezari et al., 1995).

Then, during a series of hydroxylation (Croteau et al., 2006) and acetylation(Walker and Croteau, 2000) reactions, very important intermediates of 10-deacetylbaccatin III and baccatin III are formed. After the formation of baccatin II, the α-Phenylalanine side chain is attached to it(Walker et al., 2004), and after several stages of enzymatic reactions(Onrubia et

al., 2013, Walker et al., 2002), the valuable substance of taxol is finally synthesized(Walker et al., 2002) (Fig II).

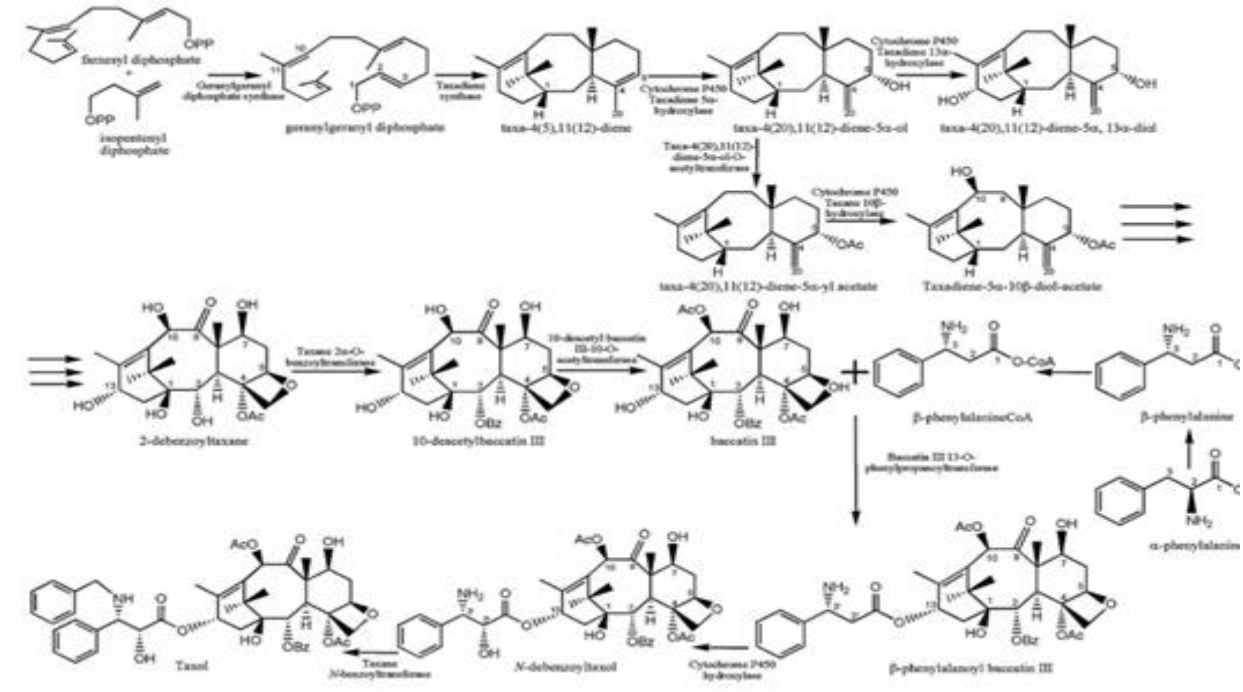

Fig. II. Biosynthetic path to Taxol

About 300 mg of taxol is obtained from each adult yew plant, while each patient needs 2.5 to 3 grams of medicine for treatment. Almost every cancer patient needs 6 yew trees during their treatment. Due to the increase in the number of patients and the lack of natural resources of the yew plant, the production of taxol from the plant as the only source of this valuable drug is not economical because to get one kilogram of taxol from the plant requires cutting down 2,000 - 2,500 of 200-year-old yew trees(Liu, 2016, Malik et al., 2011, Croteau et al., 2006 , Onrubia et al., 2013). The production of taxol from this natural source causes indiscriminate cutting and extinction of yew trees. Over the past decade, researchers have been looking for new ways to improve production conditions and reduce the price of this valuable drug to meet the needs of cancer patients and specialist clinics(Jennewein and Croteau, 2001). But the main problem is the small amount of taxol in the bark of the yew tree, the amount of taxol varies from 0.001 to 0.05 in different species of yew (Schippmann, 2001). For this reason, many attempts were made to replace other methods in the production of taxol. Other alternative methods, such as the semi-synthetic reaction of precursors such as baccatin III and 10-deacetylbaccatin III, which are present in larger quantities in the plant, as well as its production by biotechnological methods were considered(Stierle et al., 1995). In 1994, the first success in the production of taxol by chemical synthesis was achieved(Nicolau et al., 1994). However, the complexity of the taxol biosynthesis pathway, low yield and limited ability to perform chemical synthesis led to the consideration of an alternative method for the supply of this compound. Semi-synthetic taxol systems are one of these methods, which is done due to the precursors of bakatin and 10 bakatin in yew leaves. During the last two decades, biotechnology methods, especially yew cell culture, have been used in the production of taxol, which is one of the sustainable production

methods of this compound due to its independence from the environment(Zhong, 2002. Tabata, 2004).

Cell suspension culture

The most common plant cell culture systems are callus cultures, which are dedifferentiated cells. In a cell culture system, cells grow in a liquid culture medium and are constantly shaken. Cell culture should be subcultured periodically, which depends on the speed of cell growth (Mustafa et al., 2011). Continuous harvesting of the plant to produce taxols may not be a sufficient source to meet the clinical demands. so, interest in taxol production increased through culture of cell suspension. Cell cultures have a faster growth rate than whole plant cultures and show an increase in production compared to whole plants against the addition of treatments, which results in increased production in less time(Fett-Neto et al., 2004, Gandhi et al., 2015, Rao and Ravishankar, 2002). Due to the totipotency property in plants, it can be expected that all the compounds in the whole plant will be observed in its cell culture(Rao and Ravishankar, 2002). In cell cultures, it should be noted that the production of the active substance is influenced by two factors: production per cell and cell mass size, which in most cases, the optimal conditions for both factors are in conflict with the other to solve this problem. Two-stage culture is suggested that in the first stage, growth conditions are in favor of cell growth, and after obtaining the appropriate cell mass, the second stage begins, which is in favor of the production of the desired metabolite(Rao and Ravishankar, 2002, Zhong, 2002, Malik et al., 2011). Accumulation of drugs in large-scale suspension culture is considered as the best, sustainable and long-term alternative for taxol production(Lee et al., 2010). Many scientists prefer two-phase culture with the use of elicitor to increase productivity and maintain cell integrity.

Use of elicitors

Elicitors are compounds of biological and non-biological origin that stimulate secondary metabolites(Dornenburg and Knorr, 1995). Elicitors are biological stress agents and their use in low concentrations on the plant induces a stress response (real stress or good stress) leading to the production of secondary metabolites in plants (phenolic compounds, flavonoids, alkaloids and ...) And also protects the plant against living and non-living factors without affecting the efficiency of the plant(Vazquez-Hernandeza et al., 2019, Vargas-Hernandez et al., 2017, Cardenas-Manríquez et al., 2016). Elicitors are used to stimulate the production of plant metabolites(Mejía-Teniente et al., 2010). Researchers focus on the effects of an elicitor. Methyl jasmonate is an elicitor that researchers have studied extensively on its effect on taxol biosynthesis(Ketchum et al., 1999, Bonfill et al., 2006). So far, many different compounds have been used to induce taxol in cell culture. Improvement of taxol production in culture of T. wallichiana Zucc cell suspension has been reported with the addition of IAA-conjugates (IAAglycine, IAA-phenylalanine, IAA-alanine and IAAaspartic acid)(Jha et al., 1998). Phenylalanine and vanadyl sulfate were used in *Taxus baccata* L. cells(Brincat et al., 2002). A study by Zhong found that L-phenylalanine, as a precursor to the taxol side chain, had a significant effect on increasing taxol synthesis in the suspension culture(Zhang et al., 2002). Also, application of methyl jasmonate to *T. cuspidate* caused a different increase in the expression of genes in the taxol production pathway, which showed that the transcripts of the genes at the end of the pathway are much less than the transcripts of the genes at the beginning of the pathway. The importance of these genes was in metabolite engineering(Nims et al., 2006). Study on ethylene inhibitors such as silver nitrate and cobalt chloride, along with their combination in *Taxus spp*. suspension culture showed an increase in taxol production(Zhang and Wu, 2003). The use of three elicitors of chitosan, methyl jasmonate and silver on *T. chinensis* culture showed that the combined use of these three elicitors increased taxol to forty times control, ten times the use of silver alone, six times the use of chitosan alone and twice the use of only Of methyl jasmonate, which has been argued that different elicitor induce different defense responses(Zhang et al., 2000 , Sabater-Jara and Pedreño, 2013). Many factors affect the production of taxol, including: Inducing factors, addition time, culture days and culture stage,

different concentrations of elicitors have been shown to affect cell culture and taxol production(Linden and Phisalaphong, 2000). Khosroshahi et al (2006) tried to improve the production of taxol by combining different induction factors in the suspension culture of *Taxus baccata*. They developed suspension culture using Gamborg's B5 medium with various inducers such as vandyl sulfate, cobalt chloride, silver nitrate ammonium citrate, phenylalanine, methyl jasmonate, salicylic acid and fungal elicitor(Khosroushahi et al., 2006). In another study, the ability of dimethylsulfoxide (DMSO) to induce the synthesis and release of taxanes in the cell suspension culture of *Taxus baccata* was investigated and showed that addition of 5% DMSO had the best performance of taxanes in both sections(Kajani et al., 2012).

Production of taxol in bioreactors

With the advancement of biotechnology, semi- or total synthesis of taxol has been done by culturing endophytes and in cell suspension culture. Taxol production in bioreactors is continuous and abundant and is also considered as an excellent alternative method for long-term production of taxol without destroying the plant in its natural habitat(Lee et al., 2010). Several types of bioreactors are designed to plant cells culture in large volumes(Roberts and Shuler, 1997). In a study, Stirred, Airlift and Wave bioreactors were used for the production of taxol and baccatin III from cultured cells with calcium alginate. The highest amount of taxol and baccatin III produced was related to Stirred bioreactor, followed by Wave and Airlift bioreactors(Bentebibel et al., 2005). Today, for commercial production of Taxol, bioreactors with a volume of more than 75,000 liters are used by Phyton Biotech, ESCAgenetic, Samyang Genex, Nattermann (Germany)(Frense, 2007. Kolewe et al., 2008).

Metabolic engineering

Simulation of the taxol-producing gene has also been conceived but is still ongoing. Large-scale biological process engineering of plant cells is a cost-effective method and the responsiveness of various factors studied has further highlighted the application of this method. Endophytic fungi of the species Taxus have also been extensively studied for taxol production, however, the stable production of these microorganisms has not yet been proven and also the purification of intact tissues compared to plant cell culture is probably long and It is expensive(Cusido et al., 2002).

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