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In vitro Cell Cultures of *Taxus* as a source of the Anti-Neoplastic Drug Taxol and Related Taxanes

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ABSTRACT

A variety of tissue explants, including green and red arils, seed material, youthful stems, and needles have been used to introduce the Callus cultures of Taxus cuspidata and Taxus canadensis. The best growth in defined media was shown in callus derived from stem segments. In order to prevent callus darking and subsequent growth regeneration, the culture medium was supplemented with reducing substances and phenol binding compounds. The different concentrations and ratios of 2, 4-D and kinetin were affected by cuspidata explant production for the growth of T. cuspidata explant. After 2 months in culture, T. cuspidata were extracted for taxol and hy HPLC analyzed. During the retention period, UV spectra, peak purity as assessed by a photodiode array spectroscopy and comparable to authentic taxol standard as well as an analysis of IH-NMR were shown for taxol presence (0.020 ± 0.005% of dry weight extracted). Suspension cultures of T. Cuspidata from the callus crops were established, subsequently immobilized on fibreglass mat, and maintained as immobilized crops over the course of a period of six months, cell crops also produced taxol up to 0.012 ± 0.007% of the dry weight extracted.

Keywords:

Callus cultures, Fibreglass mat, Growth regeneration, Photodiode array spectroscopy, *T. cuspidata*, Taxol

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INTRODUCTION

Currently, the *Taxus spp.* is the only source of taxol, an anti-neoplastic diterpen amide. There have been positive results in the treatment of advanced ovarian cancer in taxols, such as B16 melano ma, SRC MX-l mammary and CX-l colon xenografts [1]. Taxol (generic name paclitaxel) was originally derived from bark from *Taxus Brevifilia*, a slower growing yew native to the North-West Pacific region and is an effective antimitotic medication used in the treatment of a variety of carcinoma. Taxol, due to its binding affinity for tubulin, has been found to be a unique way to prevent cancer cell development by inhibiting the dissociation of microtubules [2].

The main limitation in the widespread use of this medication is its short supply because T. brevifolia, like other *Taxus spp*, produces extremely low Taxol amounts [3]. However, it is generalizably the labor intensive procedures required to remove this compound from the intact plant, in addition to environmental concerns. The growing use of new types of cancer and its early use during therapy have, during the last few years, led to intense efforts to meet taxol demand. Due to the complex structure of the molecule the complete synthesis of this compound on an industrial level was impractical. At present, a majority of the medicinal drugs are manufactured through a semi-synthesis from the natural precursor 10-deacetylbaccatin III, extracted primarily from leaves with a relatively good yew tissue yield. Half synthesis is also used to generate Taxotere, a Taximum-like synthetic analog. The semi-synthetic approach is subject to certain limitations depending on the extraction and isolation of precursors from distinct tissues, which depends on epigenetic and environmental factors, and can vary significantly in yield.

Plant cell cultivation is therefore considered a most promising approach to ensuring that Taxol and related compounds, commonly called taxanes, are supplied stably. Various efforts to produce cell crops from different *Taxus spp* have been made and different elicitors are used to increase production and release of taxol into a medium for cultivation. The availability and cost of this medication is still limited, mainly due to *Taxus spp's* recalcitrant behavior under *in vitro* conditions. Efforts were also

focused on isolation and cultivation of yew-associated endophytic fungi from taxol, but the rates of Taxol in comparison to tree were relatively small.

The only sources used in the market supply of Taxol and taxanes were *Taxus spp* and Endophytic Fungi. As a taxol-producing species, Hazel has been reported as bioprospection between angiosperms. Nonetheless, the option to extract taxol from these plants for the low recovered amount (1/10 the yew level), and for the need to remove the compound from the intact plant is not practical. It is not feasible. However, hazel found taxol was actually considered extracted not from hazel itself but from endophytic fungi within the hazel. Another important step in improving the production of this drug is knowledge of the biosynthetic pathway. By over-expressing selected genes in yew or other suitable hosts, regulation of taxol biosynthesis may potentially address the delivery problem.

The aim of this work was to establish experimental conditions for the development of hazel cell suspension crops (*Corylus avellana* species) and to test whether taxol and taxanes are manufactured. Hazel cell cultivation systems could be a potential Taxol source and associated compounds that cannot commercially be extracted from intact plants or chemical syntheses. The tissues *C. avellana* and T. baccata were distinguished in order to establish the cell cultures. The presence of taxanes was examined and their biological activity on a human cell line tested by cell culture media. The results showed that development of taxane in hazel and yew cell crops was similar, but hazel *in-vitro* cultivation, especially from seeds, was more productive than yew *in vitro.* In addition, elicitors induced the taxanes by hazel cells [4].

RESULTS AND DISCUSSION

Callus derived from hazel seeds was precocious and fast-growing

Specific explants were used for developing callus cultures to optimize the initiation protocol for *in vitro* hazel cultivation. Calli were successfully induced with several concentrations and combinations of regulators of plant growth from stems, leaves and seeds grown on paper. The development of Callus was generally precocious and active in the 10th to 30th culture day and demonstrated a great variation in phytohormone concentration and explant type. Almost the combinations in phytohormones were used alone or in combination with Benzyl Adenine (BA) with a preference for 2, 4-dichlorogenoxyacetic acid (2,4-D). Seed-based callus induction was also elevated (more than 50%) in conjunction with BA for the use of naphthalene acetic acid (NAA). Stones and seeds with a maximum callus induction of 75 % were the best sources of explants [5]. However, over the periods of approximately two years, calli derived from grains remained white and friable (Figure. 1A), with a constant growth rate of 169 ± 15 mg per week. After 8-10 months after initial subculture, Calli stem and leaves decreased, leading to a slow-growing, brown callus, dying 1-2 months later. Part of the seed explants, cultivated in a medium supplemented with callus induction plant growth regulators, and was cultivated after several days of culture with somatic embryo formation at a maximum of 14-18 days in culture (Figure. 1B) [6]. Developing Somatic embryos obviously did not depend on the concentration/combination of a phytohormone; however, no embryo has been developed with medium without the use of phytohormones. *In vitro* plantlets formed in somatic embryos, transferred to the medium without the use of phytohormones, preserved in a phyto chamber [7].

Figureure 1(A). Callus from hazel seed.

Figureure 1(B). Somatic embryo from hazel seed.

The same type of *T. baccata* explant is used simultaneously as a positive control for establishing callus cultures. The development of Callus from yew explants usually displayed slower growth from the 30th to 60th cultivable day. Stems and leaves (Figure. 1C) were the most productives explants; the maximum callus induction was 76% and the average callus induction was 63%, respectively [8]. The calli derived from seed were lower productive, and were cultivated in NAA at a maximum of 52%. For approximately one year these calli remained pale and friable yellow, with a very slow growth rate, though stems and leafs were brown, died after ten-12 months of cultivation. In addition, the different combination of growth regulators has already been used for the production of *T. baccata* stems and leaves. Some attempts to grow yew seeds have already been made. Nevertheless, first seed was germinated *in vitro*, and for the establishment of callus cultures embryos or seedlings were used. Here, without waiting for germination, we directly transfer sterilized hazel and

yew seeds to the correct medium for callus induction. The new approach provided many benefits, including shortening the time taken to appear in the callus. In fact, seed crops reduced the risk of pollution compared with the crop produced from stems and leaves.

Figureure 1(C). Callus from yew leaf.

Hazel suspension cell cultures produced taxanes

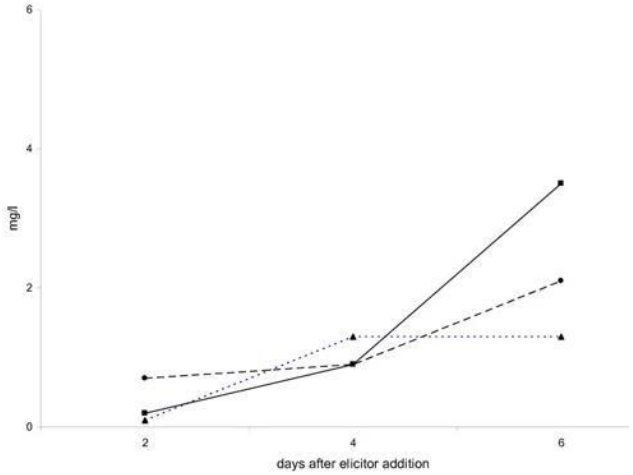
Cell culture of suspension of *C. avellana* selected white and friable calli seed, 2 months old, medium supplemented with 3 different hormonal concentrations and/or combinations. Cell cultures derived from stem calluses of *T. baccata* in contrast, a growing condition already proven to be suitable for the development of yew cell cultures, were prepared and grown at 2 mg/l of NAA [9]. With some variation, hazel and yew crops produced similar rates of taxanes and released in medium for 16 weeks, as shown by the ELISA study. While ELISA has not permitted the identification or quantification of the single taxane, it is commonly used for screening taxanes. No associations were found with hormone levels and/or combination and development of taxane. The main purpose of this study was to verify, however, if hazel was capable of producing taxanes in conditions controlled by *in vitro* culture and if this production is comparable to that of your cell culture. Further studies are needed to research key environmental factors influencing hazel cell taxa growth. Furthermore, for the development of these metabolites, the explant form from which the callus was obtained could also be important. Due to their increased productivity and faster growth, only seed-derived calli was used in the present study to classify cell culture.

Taxane production in hazel cell cultures was not due to fungus contamination

In a fungal-specific substratum, samples of calluses and cell culture media held in sterile conditions for 6 months at least were grown to decide if taxans contained in hazel cultures might be attributable to endophytic fungi. No hyphal growth was observed in microscopically examined plates containing a callus or medium for a period of 2 months. This finding suggested that, rather than as a consequence of endophytic fungal contamination, taxans collected from hazel culture were to be due to hazel metabolism.

Taxanes produced in hazel cell cultures was increased through elicitation

Seed derived calli suspension cultures in hazel were obtained by methyljasmonate or chitosan + mechanical jasmonate. The medium was collected and analyzed by HPLC from these cell cultures 2, 4, and 6 days following publication. Maximum taxanes including 10-deacetyl-baccatin III and 10-deacetyl-taxol, 7-xylosyltaxol were increased after 6 elicitation days (Figure. 2). Taxanes of this kind were applied after six days. Methyljasmonate plus chitosan treatment was more effective than that of methyljasmonate alone in increasing taxane production [10]. For yew cells, similar effects were recorded. Such data suggest that longer elicitation cycles might be an excellent means of achieving increased taxol production and related hazel cell cultivation compounds.



Figureure 2. Taxane production after elicitation.

CONCLUSION

Taxol findings were highly documented in certain hazel varieties and gave hope for the use of new taxol species. Nevertheless, some and perhaps all taxol in hazel is assumed to be derived from endophytic fungi in hazel, rather than formed by the metabolism of hazel itself. Here it has been shown that hazel can develop taxol in the absence of micro-organisms, such as endophytic fungus, when it is present in aseptic conditions. Then it can be assumed that hazel has the metabolic route to taxol development most definitely. Therefore, further biochemical studies may be investigated for identifying, until now considered as a particular metabolism of *Taxus spp* among higher plants, the enzymes involved in the biosynthesis of Taxol in hazel. Characterization of these enzymes is also an important step towards bioengineering the development of hazel crops.

REFERENCES

1. Dhedhi NA, Ashraf H, Ansari NB, et al. Self-medication among people visiting outpatient clinics of a Tertiary care hospital, Karachi. J Family Med Primary Care 2021;10:773.

2. Malik S, Cusidó RM, Mirjalili MH, et al. Production of the anticancer drug Taxol in Taxus baccata suspension cultures: A review. Process Biochem 2011;46(1):23–34.
3. Expósito O, Bonfill M, Moyano E, et al. Biotechnological production of Taxol and related taxoids: Current state and prospects. Anti-Cancer Agents Med Chem 2009;9(1):109–121.
4. Mendoza A, Ishihara Y, Baran PS. Scalable enantioselective total synthesis of taxanes. Nat Chem 2011;4(1):21–25.
5. Gallego A, Malik S, Yousefzadi M, et al. Taxol from Corylus avellana: Paving the way for a new source of this anti-cancer drug. Plant Cell Tissue Organ Cult 2017;129(1):1–16.
6. Osuna L, Tapia N, Cusidó R, et al. Taxane production induced by methyl jasmonate in free and immobilized cell cultures of Mexican yew (Taxus globosa Schltdl). Acta Physiol Plant 2015;37(10): 199.
7. Guan Y, Li SG, Fan XF, et al. Application of somatic embryogenesis in woody plants. Front Plant Sci 2016;7:938.
8. Akinyosoye ST, Adetumbi JA, Amusa OD, et al. (2014). Effect of seed size on in vitro seed germination, seedling growth, embryogenic callus induction and plantlet regeneration from embryo of maize (Zea mays L.) seed. Niger J Genet 2014;28(2):1-7.
9. Arumugam T, Ayyanar M, Koil Pillai YJ, et al. Phytochemical screening and antibacterial activity of leaf and callus extracts of Centella asiatica. Bangladesh Journal of Pharmacology 2011;6(1):855.
10. Onrubia M, Moyano E, Bonfill M, et al. Coronatine, a more powerful elicitor for inducing taxane biosynthesis in Taxus media cell cultures than methyl jasmonate. J Plant Physiol 2013;170(2): 211–219.
11. Gallego A, Imseng N, Bonfill M, et al. Development of a hazel cell culture-based paclitaxel and baccatin III production process on a benchtop scale. J Biotechnol 2015;195:93–102.