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# Phcog Rev. : Review Article

# Production of phytoestrogens by plant cell and tissue cultures: recent scenario and exciting prospects.

**Amit N. Shinde[1], Nutan Malpathak[1]* and Devanand P. Fulzele[2]**

[1]*Department of Botany, University of Pune, Pune- 411 007, India*

[2] *Plant Biotechnology and Secondary Products Section,*

*Nuclear Agriculture and Biotechnology Division, Bhabha Atomic Research Centre, Mumbai- 400 085, India*

***Author for Correspondence :** mpathak@unipune.ernet.in*

**ABSTRACT**

Phytoestrogens are group of plant derived compounds structurally and functionally mimic mammalian estrogen. Phytoestrogenic isoflavones, daidzein and genistein are associated with several health promoting properties against sex hormone related ailments and cancer. They are restricted to leguminoceae family and often subjected genotype and environment dependant variation in accumulation. Plant cell cultures have higher rate of metabolism and condensed biosynthetic rate, resulted in shorter period of time required for secondary metabolite production compared to intact plants. In present review we have summarized various studies carried out for production of isoflavones by *in vitro* cell cultures. Additionally several product optimization strategies including manipulation of physical and chemical parameters, elicitation, permeabilization and bioreactor application for mass cultivation were discussed with respect to isoflavonoids production. Advances in functional and structural genomics has resulted in identification and cloning of relevant genes from isoflavone biosynthetic pathway and regulatory systems. Metabolic engineering has improved productivity of plant cell cultures, intact plants and resulted in fortification of isoflavones in several legumes and non leguminous plants. This review highlights recent trends and future prospects of various strategies to direct higher than average productivity of phytoestrogenic isoflavones in plant cell cultures.

**KEY WORDS:** Plant cell cultures, phytoestrogens, elicitation, bioreactor, metabolic engineering

## INTRODUCTION

Phytoestrogens are group of plant derived compounds that structurally and functionally mimic mammalian estrogen (1). The major subtypes of phytoestrogens chiefly comprise isoflavones, lignans and coumestanes (Fig. 1) (2). Isoflavonoids mainly comprises daidzein, genistein, glycetine, formononetin and biochanin A (fig. 2). They mainly belong to flavonoid group, well known for UV protection, plant-microorganism, plant-insect pest interactions, growth and development in plants (3, 4).

Several investigations revealed the role of isoflavonoids as signal molecules in plant microorganism interaction including attraction of rhizobial bacteria for nitrogen fixing root nodule formation (5, 6, 7). Isoflavonoid daidzein and genistein are associated with induction of hypersensitive response against pathogen and demonstrated toxic effect to the several classes of oomycetic fungal pathogens (8). Various genetic approaches confirmed their role as preformed and inducible antimicrobial and anti insect compounds, associated with disease resistance in legumes (9, 10, 11, 12).

### *Health promoting and pharmacological properties of isoflavones (daidzein and genistein)*

Among various isoflavones, daidzein and genistein gained greater interest because of multidirectional health promoting properties. They share structural similarity with potent estrogen estradiol-17β (fig 2). Structural similarities particularly exist with the phenolic ring and the characteristic distance (11.5A°) between its 4'- and 7'- hydroxyl groups (13). As a result they can bind to estrogen receptors and sex hormone binding proteins and elicit both estrogenic and antiestrogenic properties. Various sex hormones related properties of daidzein and genistein are summarized in Table 1. The structural similarity was also observed with the tamoxifen, a synthetic anti-estrogen used as a chemopreventive agent in women with high risk of breast cancer (26).

Daidzein and genistein are associated with inhibition of protein tyrosine kinases and topoisomerase (27, 28, 29), inhibition of cell proliferation (30, 31), inhibition of angiogenesis (32, 33), apoptosis induction (21, 34, 35, 36) and antioxidant (37) properties. As a result they inhibit development of chemically induced cancers in bladder, stomach, lung, prostate, blood and have greater potential to be used as a cancer protective agent. Phytoestrogens are also reported to show anti-photodamaging activity against human skin carcinogenesis and ageing caused due to excessive light (38). Pre-clinical and clinical studies have shown lipid lowering effect and inhibition of low-density lipoprotein oxidation associated with these isoflavones (39).

### *Biosynthesis and distribution*

Isoflavonoids daidzein and genistein are synthesized from the phenyl propanoid pathway (PPP). First step towards the PPP is catalyzed by the enzyme phenylalanine ammonia lyase which catalyses formation of trans-cinnamic acid by L-deamination of phenyl alanine. Chalcone synthase is a key enzyme for flavonoid biosynthesis as it catalyzes the first committed step into flavonoid branch (40). Isoflavonoids are formed from ubiquitously present flavanones by migration of B-ring to 3-

position, followed by hydroxylation at the 2-position. This reaction is catalyzed in presence of NADPH and molecular oxygen by the cytochrome P450 enzyme CYP93C1 (2-hydroxyisoflavone synthase) also termed as isoflavone synthase (IFS) (41, 42). The resulted 2-hydroxyisoflavanone is unstable and undergoes dehydration to yield isoflavones daidzein and genistein. Isoflavone synthase is stereoselective and more restricted to leguminoceae family. Detail schematic representation of daidzein and genistein biosynthesis is shown in Fig. 3.

It was observed that, daidzein and genistein are present as three different types of glycosidic conjugates, linked either to glucose, malonyl or acetyl derivative of glucose (43). The glucosyl group is generally substituted on 7' or 4' position of aglycon skeleton, whereas malonyl group linked to 6" position of sugar moiety (44). These conjugates help in storage of their less soluble aglycons. Esterification of these compounds allows their transport to vacuoles where they may be used as substrates for vacuolar peroxidase in the peroxidase/phenolic/ ascorbate system (45, 46).

Isoflavonoid accumulation was found to be dependant on genotype and varies with the stress conditions (47, 48, 49). Genotype dependent variation in isoflavone accumulation was obtained among different species of *Genista*, *Psoralea* and *Pueraria* (50, 51, 52). However detailed study corroborated that environment had greater effect on isoflavone accumulation than genotype (53, 54, 55). With regards to health promoting effect of isoflavones, efforts have been made to determine the content of daidzein and genistein in various legumes (56, 57, 58), fruits and nuts (43), vegetables (59) and food products (60, 61). Soybean being a rich source, also analyzed with its processed products for phytoestrogenic isoflavones (62, 63). Profiling of flavonoids and isoflavonoids from various legume species was investigated in details (64, 65). Table 2, represents list of some highest daidzein and genistein accumulating plant species.

***Plant cell culture: alternative for secondary metabolite production***

Selection of elite genotypes is essential for secondary metabolites production through biotechnological application. It can be accomplished by chemoprofiling of selected genotypes through suitable quantitative approaches (HPLC/GC) and genetic diversity analysis. Micropropagation is a viable alternative for growing elite plants having similar principle constituents. This approach was successfully used for the clonal propagation of *Pueraria lobata* and *Genista* sps. accumulating higher levels of phytoestrogenic isoflavones (67, 68). However field cultivated plants often subjected to pathogen attack and variable climatic conditions resulted in reduced accumulation of secondary metabolites especially phenolic compounds (69, 70, 71, 72). The possibility of *in vitro* plant cell cultures for production of plant pharmaceuticals was studied in details as an alternate and complimentary method to whole plant extraction (73, 74, 75). Cell culture system has several advantages over conventional method of cultivation of plants, such as efficient production system irrespective of seasonal variation, also offering an advantage of product optimization by manipulating physical, chemical parameters and metabolic engineering. Additionally it was observed that time required for highest level accumulation of secondary products was reduced in plants cell cultures compared to its counterparts. Further it has to be noted that glycoside and ester derivatives of isoflavones found in plant tissues have not been successfully chemically synthesized (26, 76).

***Plant cell cultures for production of phytoestrogenic isoflavones***

***Callus and suspension cultures***

Callus cultures comprises undifferentiated plant cells established from different explants (root, stem, leaves etc.) on medium containing various combinations and concentrations of plant growth regulators especially auxins and cytokinins. Callus cultures subjected to liquid medium yield suspension cell cultures consist of single cell to cell aggregates of various sizes. Callus and suspension cell cultures have showed potential of accumulating wide range of secondary metabolites (77, 78, 79, 80, 81, 82, 83).

Callus cultures were initiated and screened for daidzein accumulation in several *Psoralea* sps. with highest concentration (0.9680% dry wt) in *P. obtusifolia* (51). Interspecific variation was also reflected in isoflavones content among callus cultures of *Genista* sps. (50). Federici et al., reported production of isoflavone in 25 year old 40 callus strains of *Glycine max* with maximum of 46.3 mg $g^{-1}$ dry wt of isoflavones (84). In another study callus cultures of *Pueraria lobata* and *Maackia amurensis* showed elevated production of isoflavones compared to the parent plants (66, 85). Cell suspension cultures of *Genista* and *Glycine* were studied in details for production of isoflavones (76, 84). Suspension culture of *Genista tinctoria* accumulated 9414.7 mg / 100 g dry wt of genistein, so far highest amount of isoflavone reported from plant cell culture. *In vitro* cultures of different plants studied for isoflavone accumulation are summerized in Table 3. Initiation of callus cultures followed by selection of suitable clone for initiation of cell suspension is the most suitable strategy proposed for obtaining high isoflavone accumulating cultures.

***Organ cultures***

In many cases, production of secondary metabolites is restricted to differentiated tissues and product synthesis gets enhanced by morphogenesis (96, 97, 98, 99). Organ cultures offer an advantage of stable production over unorganized cultures and it was also observed that secondary metabolite profile of organ culture is similar to that of parent plants. Organ culture mainly comprise shoot, root cultures and *Agrobacterium* induced transformed cultures. Untransformed shoot and root cultures were studied for production of several classes of secondary metabolites (100, 101, 102, 103, 104).

*Agrobacterium rhizogenes* and *A. tumefaciens* have capacity of transferring T-DNA into plant cell. Successful integration of T DNA into plant genome resulted either into development of hairy roots or shooty teratomas. Hairy root cultures offers advantage over unorganized cultures in terms of genetic stability and faster growth independent of plant growth regulators (105, 74). *Agrobacterium* transformed cultures were extensively investigated for production of secondary

plant products of pharmaceutical value (106,107,108,109). Initiation and screening of *Psoralea* sps. hairy roots led to the identification of hairy root line (*P. Lachnostachys* 5) with maximum concentration of daidzein (0.679 % dry wt) (91). Abhayankar et al., investigated the metabolomic profile of the *Psoralea corylifolia* hairy root cultures. Difference in AFLP profile of hairy root clones suggested the independent nature of T-DNA integration in to plant genome which can also be correlated with variation in metabolic profile of the clones (92). In another study co-culture of shoots and hairy roots of G*enista tinctoria* produced high levels of daidzin and daidzein than intact plant (93). Additionally *Agrobacterium* induced cultures showed possibility of producing novel compounds that can not be found in the normal roots of the parent plant. Abhayankar et al., have reported accumulation of formononetin and its glycosides which were absent in untransformed roots (92). Similarly hairy root cultures of *Genista* selectively produced isoflavones isoliquiritigenin as a characteristic isoflavones (110). Organized cultures studied for production of isoflavones have been presented in Table 3.

***Strategy for enhancement of production***

Secondary product accumulation is a result of dynamic equilibrium between synthesis, transport, storage and degradation (111). Plant cell culture is a flexible system that can be easily manipulated to increase the product yields. Careful selection of productive cells and stimulation of biosynthetic activity using various methods is most studied approach to optimize product accumulation in plant cell cultures (112). Product optimization can be accomplished through (i) selection of elite clone; (ii) manipulation of medium and culture condition; (iii) elicitation and precursor feeding. Schematic representation of strategy for optimized isoflavones production by plant tissue cultures is presented in fig. 4.

Manipulation of physical and nutritional aspects in the culture is the most fundamental approach for increasing of culture productivity. Number of physical and chemical factors was investigated, showing influence on secondary metabolite accumulation. Manipulation of media constituents including plant growth regulators (113, 114), Sucrose (115, 116, 117), nitrate (118, 119, 120, 121) Phosphate (82, 122) had been shown to have influence on secondary metabolite production. Similarly manipulation in physical parameters such as light (123, 124, 125) and temperature (126) were shown to enhance the levels of secondary metabolites. In relation to this effect of media constituents were investigated for isoflavonoids production. Influence of coconut milk and casein hydrolysate was observed on cell growth and isoflavone accumulation in cell suspension cultures of *Pueraria lobata* (127). Similarly stimulatory effect of media constituents including nitrogen source on isoflavones accumulation was observeded in cell cultures of *Psoralea* and *Albizzia kalkora* (128, 129).

***Elicitation***

Secondary metabolites, especially phytoalexins are produced by plants as a defense response against pathogenic infection and insect attack (130). Hence elicitation has effective application in induction of secondary metabolite production in plant tissue culture. Elicitation can be accomplished by addition abiotic elicitor (metal ions, inorganic compounds) and biotic elicitors (fungal, bacterial, viral and plant cell wall extract) to cell cultures (130,131). Influence of elicitors on plant secondary metabolite production was elucidated in several *in vitro* systems (132, 133, 134, 135). Similarly influence of signal transducers such as salicylic acid, methyl (98, 136, 137) and jasmonic acid jasmonate (138, 139) are investigated on secondary metabolites production.

Light and elicitor induced signal transduction pathway with respect to phenylpropanoid response was studied in soybean (140). Elicitors induced phenylpropanoid pathway and accumulation of isoflavonoids including daidzein and genistein was observed in several plant sps. and cell cultures (141, 142, 143, 144, 145, 146). It was also observed that isoflavonoids are constitutively expressed in tissues, but further induced in response to pathogen attack (147). *In vitro* cultures of several plant sps. were studied for isoflavonoids accumulation and products release upon challenged with biotic and abiotic elicitors. Hairy roots of *Psoralea lachnostachys* showed daidzein accumulation upon treatment with chitosan, $CuSO_4$ in dark and light conditions (128). Hairy root cultures of *Pueraria lobata* showed variation in growth and isoflavone production under influence of various rare earth elements (148). Similar influence of elicitation on isoflavones accumulation was demonstrated in cell cultures of *Cicer arietinum, Glycine max*, *Albizzia kalkora* (47, 129, 149). Collectively it was observed elicitation promoted isoflavones accumulation and product release without affecting culture viability. On this basis it can be anticipated that isoflavones biosynthesis in plant cell culture could be efficiently regulated through elicitation.

***Precursor feeding and permeabilization***

Precursor feeding concept is useful in cases where precursor molecules likely to get incorporated in to product directly or indirectly through degradative metabolism. Attempt to increase the production of desired molecules by supplying precursor have been effective in case of *Solanum lyratum* and *Cistanche salsa* (150, 151). In case of isoflavonoids biosynthesis, first step towards the phenyl propanoid pathway is catalyzed by the enzyme phenyl alanine amino lyase (PAL), the key enzyme that catalyses anti-elimination of ammonia from phenylalanine to yield cinnamic acid and its derivatives (152). Considering this phenylalanine supplementation expected to results in increased metabolic flux through phenyl propanoid biosynthetic pathway. Production of anthocyanin and phenylethanoid glycosides was enhanced by feeding phenyl alanine and related precursors to the cell cultures of strawberry and *Cistanche deserticola* respectively (153, 154). However parameters such as concentration, time of addition of precursor and feedback inhibition are critical and need to be considered for application of precursor feeding strategy.

In plant cell cultures most of the time secondary metabolites are stored within the cell vacuole and becomes a major limiting factor for continuous operation mode. Secretion of metabolites into medium either naturally or by artificially

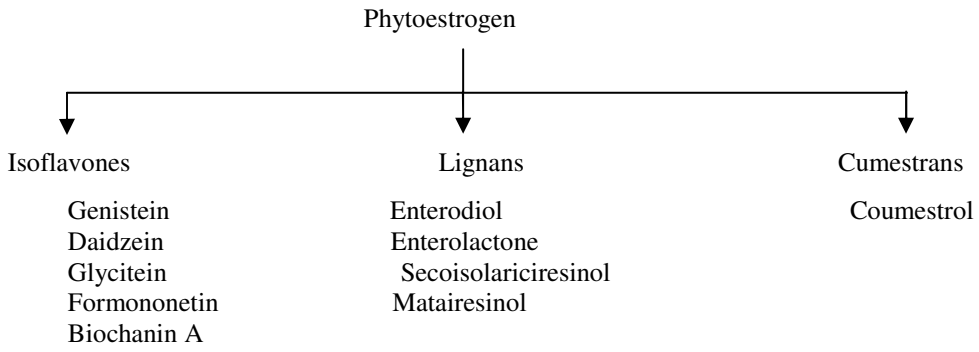
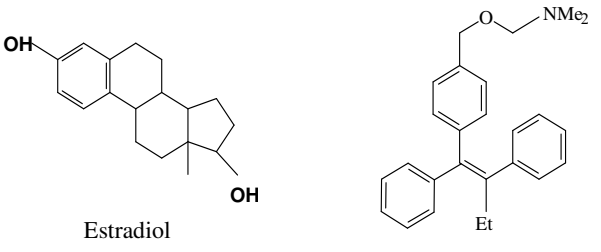
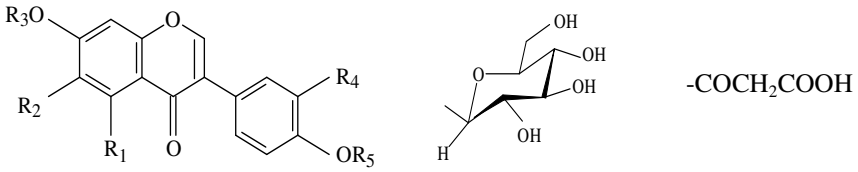



***Figure 1:*** *Schematic presentation of phytoestrogens subtypes. (Modified from ref no. 2)*




Isoflavone

| Isoflavone | R1 | R2 | R3 | R4 | R5 |
|---|---|---|---|---|---|
| Daidzein | H | H | H | H | H |
| Genistein | 0H | H | H | H | H |
| Glycitein | H | 0Me | H | H | H |
| Formononetin | H | H | H | H | Me |
| Biochanin A | 0H | H | H | H | Me |

***Figure 2:*** *Structural interpretation of isoflavones, estradiol and tamoxifen.*

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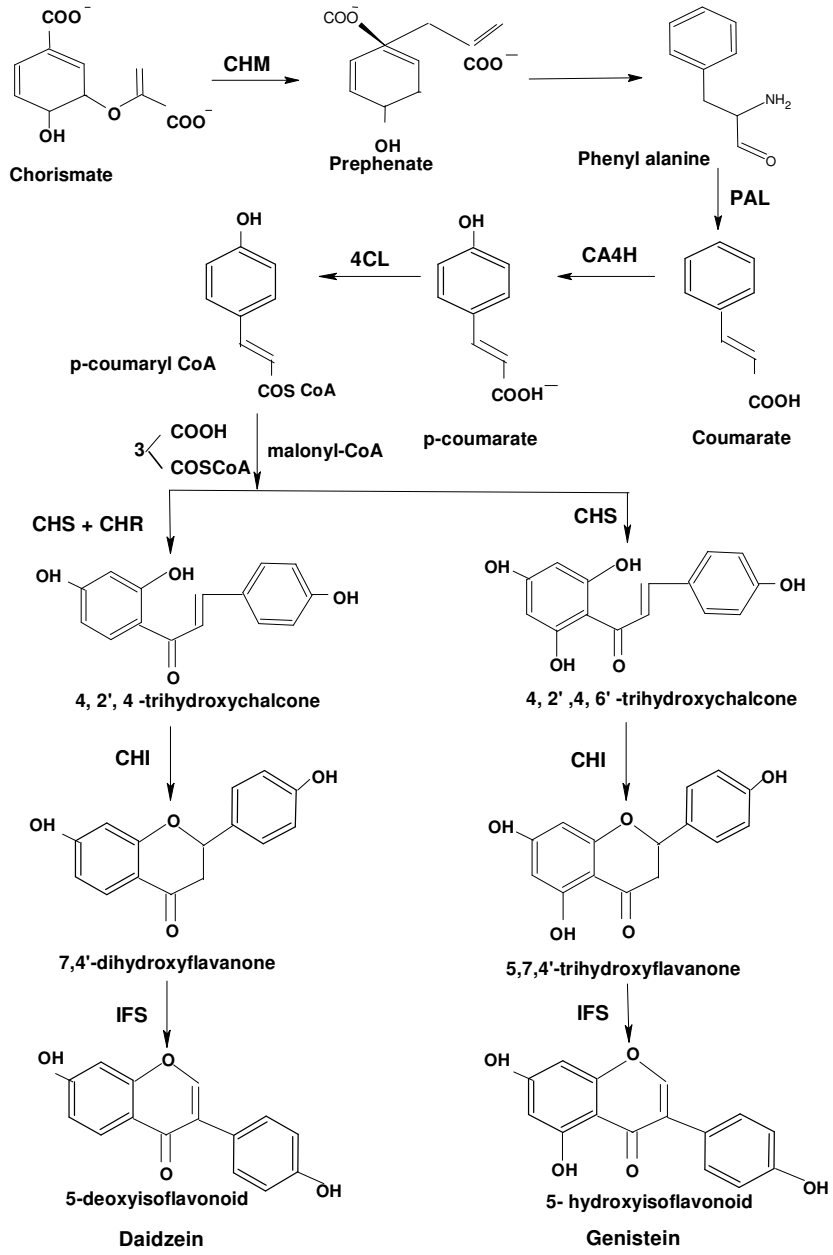


***Figure 3: Schematic representation of biosynthesis of daidzein and genistein.***

*[PAL: Phenylalanine ammonia lyase, CHS: Chalcone synthase, CHR: Chalcone reductase, CHI: Chalcone isomerase, IFS: Isoflavone synthase, CHM: Chorismate mutase, CA4H: Cinnamic Acid 4-Hydroxylase, 4CL: 4-Coumarate:CoA ligase ].*

***Table 1: Summary of pharmacological activities of daidzein and genistein***
*(on the basis of epidemiological studies, in vitro, animal models and human trials).*

| Activity/Mode of Action | References |
|---|---|
| **Antiestrognic properties;** | |
| Modulators of production and bioavailability of steroidal hormone. | 14, 15, 16, 17, 18 |
| Alter sex hormone receptors. | |
| Stimulatory effect on synthesis of hepatic sex hormone binding globulin | 19 |
| Apoptosis and inhibition of cell proliferation | 20, 21 |
| **Estrogenic properties;** | |
| Prevention of post menopausal ailments and osteoporosis | 22, 23 |
| Alternate to hormone replacement therapy | 24, 25 |

***Table 2: Phytoestrogens content in several plant sps. (µg/100gm):***

| Plant name | Daidzein | Genistein |
|---|---|---|
| *Glycine max* ("Santa rosa") | 56,000 | 84,100 |
| *Glycine max* (Chapman) | 41,300 | 46,400 |
| *Phaseolus vulgaris* | 28.2 | 158.0 |
| *Cajanus cajan* | 14.6 | 737.0 |
| *Cicer arietinum* | 34.2 | 69.3 |
| *Pisum sativum* | 7.9 | 22.8 |
| *Trigonella foenumgraecum* | 10.2 | 9.8 |
| *Vigna mungo* | 6.9 | Tr |
| *Arachis hypogea* | 49.7 | 82.7 |
| *Pueraria lobata* (root) | 185,000 | 12,600 |
| *Trifolium pratense* | 12,200 | 4,010 |
| *Psoralea obtusifolia* [1] | 6,07,200 | Na |
| *Genista sagittalis* [2] | 16,33,600 | 3,100 |
| Maackia amurensis [3] | Tr | 1,920 |

*Tr: Traces, Na: Not analysed (Table modified from 50, 51, 56, 66)*

***Table 3: Plant cell cultures studied for estrogenic isoflavone production.***

| Type of culture | References |
|---|---|
| **Unorganised culture system** | |
| **Callus culture:** | |
| *Psoralea sp.* | 51 |
| *Maackia amurensis* | 66 |
| *Glycine max* | 84 |
| *Genista sp* | 50 |
| *Pueraria lobata* | 85, 86 |
| **Suspension culture:** | |
| *Glycine max* | 84 |
| *Genista tinctoria* | 76 |
| *Cicer arietinum* | 87 |
| *Lupinus* sps. | 88 |
| *Pueraria lobata* | 89, 90 |
| **Organized culture system** | |
| **Hairy root cultures:** | |
| *Psoralea sp.* | |
| *Psoralea corylifolia* | 91 |
| *Pueraria lobata* | 92 |
| *Lupinus sps.* | 94 |
| *Lupinus mutabilis* | 88 |
| | 95 |

| | |
|---|---|
| **Shoot cultures:** | |
| *Genista tinctoria* | 76 |
| *Genista sp.* | 67 |
| *Genista sp.* (Co-culture system) | 93 |
| *Pueraria lobata* | 68 |

induced treatment, offers better removal of product and biomass reuse. Various methods have been studied for product release from plant cell cultures including permeabilization with dimethylsulfoxide (DMSO), Tween 80, Change of external pH, ultrasonics and temperature treatment (155, 156). Chemical permeabilization has been studied for the release of isoflavones form *Glycine max*, *Pueraria montana, Genista tinctoria* and *Cicer arietinum* (76, 142, 157, 158). In addition to this elicitor induced product release was observed in *Psoralea* sps., *Pueraria lobata and P. Montana* (128, 148, 158). Product optimization strategy coupled with permeabilization offers collective advantage of enhanced production and leaching out of product for effective reuse of biomass.

***Metabolic engineering***

Secondary metabolites isolated from plants provide an excellent source for pharmaceutically important compounds. Although many times, low overall production makes extraction process expensive and less suitable to industrial application. Metabolic engineering provided a promising strategy for improved productivity in plant tissue culture and intact plants (159, 160). Genetic engineering has two basic approaches mainly, combination of properties from different organisms to single organism and incorporation of specific regulatory mechanism (73).

Phenyl propanoid pathway is the extensively studied pathway leading to the synthesis of flavonoids, coumarins, lignins etc. Advances in structural and functional genomics have led to the identification of various genes coding for key enzymes, transcription regulation factors and DNA binding protein (161). Isoflavone synthase (IFS) is a key enzyme catalyzing conversion of flavanones to isoflavonoids and restricted to leguminoceae family only. IFS encoding cDNA has been cloned from soybean and other plant species which accumulated these isoflavones and more complex phytoalexins such as glyceollin (41, 42). Expression of IFS in non leguminous plants was studied as an innovative strategy for production of these bioactive compounds in diverse group of plant systems.

***Genetic manipulation in legumes and non leguminous plant system***

The ability to increase or reduce the expression of particular genes in transgenic plants raises many possilities to alter phenylpropanoids both quantitatively and qualitatively. Several efforts have been made for engineering non leguminous plants by introducing IFS in their genome. IFS have been introduced into *Arabidopsis thaliana*, corn and tobacco (41, 162). Expression of IFS in *A. thaliana* resulted into accumulation of glucose-rhamnose-genistein, rhamnose-genistein instead of free genistein where as over expression of chalcone isomerase in *Arabidopsis* expressing IFS leads to three fold increase in flavonols without increase in genistein conjugates (163). In another study, Liu et al, successfully demonstrated genistein accumulation in tobacco, lettuce and petunia as effect of overexpression and antisense suppression of genes for key enzymes (164).

Influence of C1 and R transcription factors on activation of phenylpropanoid genes was well demonstrated in *Trifolium repens* and *Pisum sativum* (165). Yu et al., reported that expression of C1 and R transcription factor in soy IFS expressing BMS cells of maize led to low levels of genistein accumulation compared to control (162). Expression of maize C1 and R transcription factors in soybean seed resulted in increased levels of daidzein with reduction in total genistein levels (166). Suppression of competent branch pathway may direct metabolic flux towords accumulation of molecule of interest. Isoflavone synthase converts naringenin to genistein whereas flavonone 3- hydroxylase further metabolize it to dihydroflavanol. Liu et al., studied the expression of IFS in tt6/tt3 *Arabidopsis* mutant which is impaired in expression of F3H and DFR from flavonoids/ anthocyanin branch. A higher level of genistein was recorded compared to wild type *Arabidopsis* expressing IFS only (163). Similar attempt was carried out by co-suppression of F3H, which blocked the anthocyanin pathway in C1 and R expressing soybean and resulted in higher levels of isoflavones especially genistein (166). In spite of success obtained through metabolic engineering, outcome of the various studies showed factors such as limitation in expression of introduced genes, enzyme activity, precursor pool and partitioning of metabolic flux between flavonol and isoflavone pathway, are the major constraint in obtaining isoflavones in diverse group of plant species.

***Bioreactor strategy***

Plants cells are sensitive to the shear stresses because of their large size and the relatively inflexible cell wall and also show typical characteristics such as slow growth, steaky behavior and therefore require special attention for large scale cultivation. Large scale cultivation of plants cells also requires continuous monitoring of different parameters such as temperature, pressure, agitation, speed, foaming, pH, aeration rate and conductivity. Bioreactors are specialized vessels fitted with various controlling units for adjusting pH, dissolved oxygen, gas flow rate, temperature, cell density etc. enabling easy cultivation of cells in large volume. They are classified as a mechanically agitated bioreactors and pneumatically agitated bioreactors on the basis of mode of agitation. Though most of the studies in bioreactors based on batch culture mode, another set up called continuous culture system was also successfully used for plant cell cultures. In this case, constant inflow of fresh medium is balanced by constant efflux of spent medium and cells as a result all culture parameters remain constant for prolonged period of

time offering balanced growth and production (167). Plant cell cultures can also be operated under fed-batch or perfusion modes for obtaining high biomass densities (168, 169).

Bioreactor cultivation of organ cultures (hairy roots, shoots) was studied extensively for bioproduction of valuable secondary metabolites. Several types of reactors were designed and studied for hairy root growth and secondary metabolite production such as rotating drum, wave, stirred tank, bubble column, air lift and different types of gas-phase reactors (170, 171, 172, 173, 174, 175). Performance of hairy root cultures in various bioreactors may vary from plant to plant however stirred tank and trickle bed bioreactors was most suitable for large scale cultivation (109). Additional strategies including integrated hairy root growth-product recovery system, co-culture system was successfully studied in bioreactors for production of secondary metabolites including isoflavones (93, 176).

Bioreactor cultivation of cell cultures from different plant species was studied for isoflavonoids production. Ames and Worden studied a magnetofluidized bed bioreactor for continuous production of daidzein and genistein from immobilized soybean cultures. In the set up, immobilized cells concentrations reached over 10g of dry tissue/lit of biocatalyst and daidzein and genistein concentrations ranged from 10-200 µg/g of dry soybean tissue (177). In another study, suspension cultures of *Pueraria lobata* and *Glycine max* was cultivated in 5 and 10 lit stirred tank bioreactor respectively for isoflavone production (84, 90). Co-culture of hairy roots and shoots of *Genista tinctoria* were studied in prototype basket-bubble bioreactor for improved growth and phytoestrogens production. It was observed that hairy root cultures produce large amount of isoliquiritigenin whereas shoots accumulated several fold higher levels of daidzein (1647.5 mg/100g DW) and genistein (6941.5mg/100g DW) along with derivatives (93). The investigation showed that cell cultures of *Glycine max*, *Genista* and *Psoralea* has potential of bioreactor cultivation and offering an attractive alternative for production of phytoestrogenic isoflavones.

***Conclusion and Perspective***

Plant cultures were studied for enhanced production of secondary metabolites of commercial interests. Higher levels of phytoestrogenic isoflavones detected in *in vitro* cultures of *Glycine max, Genista* and *Psoralea* than field grown plants suggest the possible implementation for large scale cultivation and isoflavone production. Further studies with respect to media constituent manipulation, precursor feeding and specialized techniques such as permeabilization can be effectively employed to optimize yield and product recovery. Since daidzein and genistein are plant defense molecules (phytoalexins), elicitation could be an efficient strategy for increasing production of isoflavones by *in vitro* cultures. However application of these techniques may vary from plant to plant and requires detail investigation to determine most suitable strategy.

Genetic manipulation by *Agrobacterium* mediated transformation in *Psoralea* and *Genista* showed tendency of hyper accumulation of isoflavones along with novel metabolites. This opens an avenue for obtaining clones suitable for large scale cultivation. Bioreactor application provides controlled culture conditions for production at commercially feasible scale while maintaining the biosynthetic potential of the cultures. Combinatorial genomic approach strategy involving over-expression of genes encoding key enzymes and co suppression strategy collectively enhances isoflavone levels in legumes as well as non leguminous plants. Identification and characterization of additional components that couples integration of cell physiological, biochemical and genetic approaches could be of advantageous for promoting biosynthesis of phytoestrogenic isoflavones in plant cell cultures.

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