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Review Article

PHYTOESTROGEN ENHANCEMENT OF *PSORALEA CORYLIFOLIA* LINN. THROUGH SUSPENSION CULTURE

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ABSTRACT

Psoralea corylifolia Linn., an endangered medicinal plant of *Fabaceae* family, is rich in phytoestrogen genistein (4',5,7-trihydroxyisoflavone) and daidzein (4',7-dihydroxyisoflavone). They are plant derived xeno estrogen not generated within the endocrine system but protect against hormone-related disorders such as breast cancer, prostate cancers, osteoporosis and arthritis. Highest concentrations of phytoestrogen have been reported in *Glycine max.* The production of phytoestrogen is often low (less than1% dry weight) and depends greatly on the physiological and developmental stage of plant. Plant cell cultures provide a valuable platform for the production of phytoestrogen and high-value secondary metabolites of commercial interest. Cell suspension culture in presence of elicitors (abiotic and biotic) leads to higher yield of phytoestrogen (genistein and daidzein) in *Psoralea corylifolia* as reported by HPLC. Present review is the application of elicitors in tissue culture for phytoestrogen enhancement in *Psoralea corylifolia* and *Fabaceae* family. Elicitors are signalling molecule that induces the plant secondary metabolite by altering the transcriptional pathway.

Keywords*: HPLC, Genistein, Daidzein, Fabaceae, Suspension Culture, Phytoestrogen*

INTRODUCTION

Phytoestrogen are a class of flavonoid phenolic compounds. They are biologically active compounds produced by the members of the *Fabaceae* family and are used by humans as prime source of medicinally important compounds (Adlercreutz *et al*., 1997). Major phytoestrogen (genistein and daidzein) have been reported in *Glycine max* (Boue *et al*., 2003). They are simply waste of metabolic product which are released as a result of stress responses (abiotic and biotic stress) and dumped in to central vacuoles in plant cell (Zhao *et al*., 2005). They are synthesized by the phenylpropanoid metabolic pathway in which the amino acid phenylalanine is used to produce 4-coumaroyl-CoA combined with malonyl-CoA to yield the true backbone of flavonoids (Ververidis *et al*., 2007). They are termed as antioxidants because of their ability to trap singlet oxygen (Wand *et al*., 2001).

Genistein and daidzein appear to protect against hormone-related disorders such as breast cancer and prostate cancers, osteoporosis and arthritis (Alekel *et al*., 2000; Kim *et al.,* 2009). Studies on rodents have found genistein and daidzein to be useful in the treatment of leukemia in combination with certain other antileukemic drugs (Raynal *et al.,* 2008).

Psoralea corylifolia linn. is an endangered medicinal plant of *Fabaceae* family and an excellent sources for both genistein and daidzein. A comparative survey of leguminous plants determined that remarkably high concentrations of genistein and daidzein (over 2 g. kg^{-1} dry weight) were found in the leaves of *P. corylifolia* as campared *to Pueraria lobata* and *Glycine max* (Kaufman *et al*., 1997). A plant cell culture offers uniform secondary product synthesis by overcoming the effect of unforeseen climatic conditions and diseases in field grown plants (Pandey *et al.,* 2013). *In vitro* plant materials are one of the good sources for the production of secondary metabolite and in a variety of plant cell cultures; elicitors have increased production of phytoestrogen in large number of medicinal plants (Hussain e*t al*., 2012). A plant

cell, tissue, and organ culture has an inherent capacity to manufacture valuable chemical compounds as the parent plant does in nature (Ahmed and Baig, 2013).

Plant Stress Mechanism

Plants have evolved a number of inducible defence mechanisms to respond both abiotic and biotic stress (Hammer schmidt, 2009). The plant defence system stimulates when it received extracellular or intracellular signal by the receptor present on plasma membrane binding of receptor and signal compound activate signal transduction cascade that leads to activation or *de novo* biosynthesis of transcription factors which regulate the expression of biosynthetic genes involved in isoflavonoid production (Zhao *et al*., 2005). Binding of the elicitor to plasma membrane receptor leads to changes in Ca^{2+} influx to the cytoplasm from extracellular and intracellular pools. Decrease in pH of the cytoplasm and activation of NADPH oxidases, protein phosphorylation patterns and protein kinase activation changes the cell wall structure (lignification) and in generates reactive oxygen species responsible for synthesis of JA and SA as secondary messengers and activation of genes that produce defence-related proteins, plant defence molecules like phytoalexins and other secondary compounds including alkaloids, phytoestrogen and isoflavonoids (Devergne *et al*., 1992).

Phytoestrogen

Phytoestrogen belongs to large group of naturally substituted phenolic compounds: coumestans prenyl flavonoid and isoflavones such as genistein (4', 5, 7-trihydroxyisoflavone) and daidzein (4',7-dihydroxyisoflavone) (Faith, 2005). They are plant derived xenoestrogen not generated within the endocrine system but protect against hormone-related disorders such as breast cancer, prostate cancers, osteoporosis and arthritis. Genistein (4', 5, 7 trihydroxyisoflavone) (Kaufman *et al*., 1997; Hsu *et al*., 2001) have anti-cancer properties and used in osteoporosis, attenuation of post-menopausal problems (Marini *et al.,* 2007; Liu and Siu, 2007). Daidzein (4', 7-dihydroxyisoflavone) induces protein synthesis, alkaline phosphatase activity, and DNA content in the osteoblasts which are the important cells in bone tissues formation and bone density (Sugimoto and Yamaguchi, 2000). These compounds have aromatic, anthelminthic, antibacterial and antifungal properties used as a diuretic, diaphoretic, laxative and stimulating agent (Vaidya, 2006). Phytoestrogens cannot be considered as nutrients lack of these in the diet does not produce any characteristic deficiency syndrome.

Mechanism of Action Genistein and Daidzein

Phytoestrogen have structural similarity with estradiol (17-β-estradiol) and exhibit estrogenic and antiestrogenic effects (Turner *et al.,* 2007). It modulates the concentration of endogenous estrogens by binding or inactivating the synthesis of sex hormone-binding globulin (SHBG) (Johnston, 2003). Genistein and Daidzein are aglycon of Genistin and daidzein glycosides and they are hydrolyzed by B-galactosidases enzyme in the small intestine (Decroos, 2005). Genistein and Daidzein present in plants of *Fabaceae* converted in to Equol (S) form dihydrodaidzein and O-desmethylangolensin by gut microflora (Bannwart, 1984). The amount of Equol excretion in urine is correlated with risk of breast cancer (Duncan, 2000).

Properties of Genistein

4', 5, 7-trihydroxyisoflavone and its chemical formula is $C_{15}H_{10}O_5$ Genistein inhibits the protein tyrosine kinase, topoisomerases activity and its treatment leads to apoptosis of rapidly proliferating cancerous cell (Constantinou and Huberman, 1995).

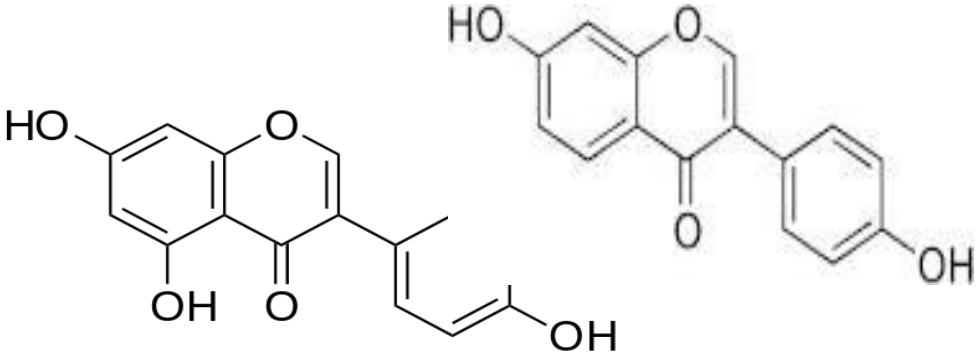
1. Genstein administrated orally was effective in cystic fibrosis because it restore the function of mutated protein (Hwang, 1997).
2. Topoisomerase II regulator of DNA replication is inhibited by genistein (Sinha, 1995).
3. Genistein blocks both PI and PIP kinases and hence reduces the IP3 concentration modulating signal transduction (Weber *et al*., 1997).
4. It stimulates the osteoblast mediated bone formation hence appear to be a very potent agent in prevention of osteoporosis (Potter *et al*., 1998).

Review Article

Properties of Daidzein

Daidzein 4', 7-Dihydroxyisoflavone and its chemical formula is $C_{15}H_{10}O_4$ (Kim *et al.*, 2010) -

1. Peroxisome proliferator activated receptors (PPAR) and their three isoforms, α, δ, and γ were transactivated by Daidzein (Dang, 2004).
2. Daidzein has biphasic effect on the cell growth of human colon-cancer (Guo *et al*., 2004).
3. The Free radicalquenching properties of daidzein prevented the formation of oxidized DNA, 8-hydroxy-2deoxy-guanosine in the cell (Ruiz-larrea *et al*., 1997).
4. Daidzein exhibits antihemolytic, antioxidative, antifugal, estrogenic and anti tumor properties along with cytotoxic effect against caspase 3 and TGF transforming growth factor beta 2 in cancer cell (Lee *et al*., 2003).



(1)

(II)

Chemical structure of (I) Daidzein and (II) Genistein

http://cerhr.niehs.nih.gov/chemicals/genisteinsoy/genistein/Genistein_Report_final.pdf

Structural Difference Between Daidzein and Genistein

Isoflavones	R1	R2	R3	R4	R5
Daidzein	H	H	H	H	H
Genistein	OH	H	H	H	H

Biosynthetic Pathways

Biosynthetic pathways in plant are the network of enzyme mediated and regulated chemical reaction leading to the manufacturing of both primary and secondary metabolites (Rates, 2001). The primary metabolite like sugar, aminoacid and fatty acid are needed for general growth and physiological development of plants. Whereas secondary metabolites such as alkaloids, glycosides, flavoniods, volatile oil etc are derived from primary metabolites and used against environmental stress (Dixon, 2001). They are of pharmaceutical importance. Organic reactions like Phosphorylation, Hydride, Transfer, Oxidation, Elimination, Acylation, Alkylation, Reduction, Codensation arrangement are responsible for biosynthetic pathways (Achnine *et al*., 2004). Phytoestrogen biosynthesis occurs in phenylpropaniod pathway. The Shikimic acid is a seven steps pathway in which carbohydrate biosynthesis of C6-C3 unit (phenyl propane derivative) are involved. It is an intermediate in producing tannin, flavones, Isoflavones, coumarin and vanillin. The shikimic acid pathway is present in plant, fungi and bacteria but it is not found in animal. The three amino acids, phenylalanine, tyrosine and tryptophan are essential nutrients in animal diet (Schmid and Amrhein, 1995).

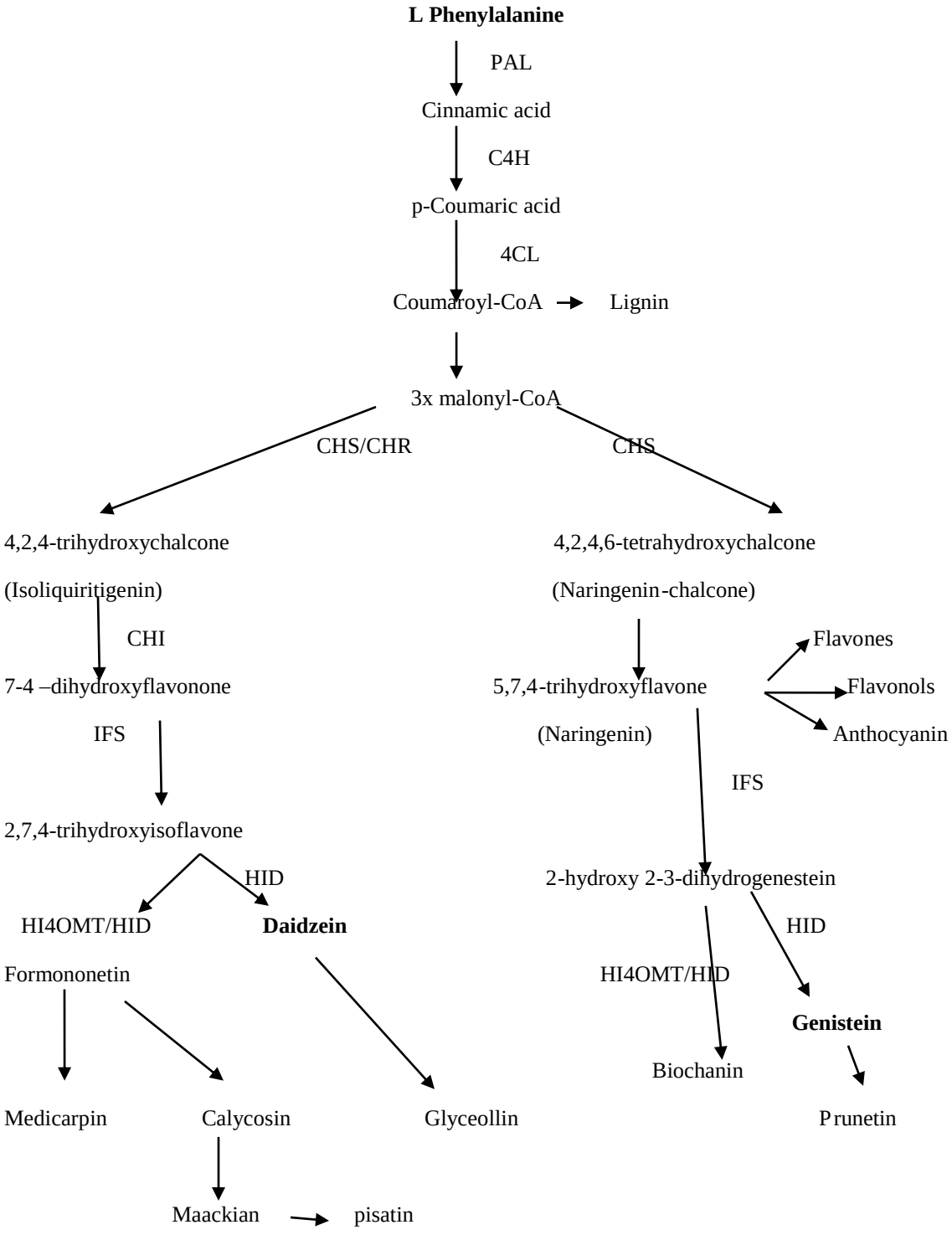


Figure 1: Phenylpropanoid pathway Isoflavonoid Branch (Mishra *et al.*, 2010)

PAL phenylalanine ammonialyase, *C4H* cinnamate 4-hydroxylase, *4CL* 4-coumaroyl CoA ligase, *CHS* chalcone synthase, *CHI* chalcone isomerases, *CHR* chalcone reductase, *IFS* isoflavone synthase, *HID* hydroxyisoflavanonedehydratases, *HI4'OMT* hydroxyisoflavanone, 4-O-methyltransferase

Isoflavone Synthase (IFS) Gene

IFS gene responsible for phenylpropanoid pathway in *Psoralea corylifolia linn.* have been identified and sequenced (Mishra *et al.*, 2010). IFS gene of *Psoralea corylifolia* (*PcIFS)* comprised of 1,563bp which encodes a polypeptide of 520 Amino acid residues. Gene was responsive to elicitors and leads to higher accumulation of phytoestrogen in *Psoralea corylifolia*. The biosynsthesis of isoflavonoids is catalyzed by P450 cytochrome having 2-hydroxyisoflavanone synthases (Akashi *et al*, 2005; Hashim *et al*.,1990).

***Psoralea corylifolia* Linn**

Psoralea corylifolia linn. is an endangered medicinal plant of *Fabaceae* family commonly known as Babchi and Bakuchi (Oudhiya, 2001). It grows as winter season weed annual erect herb 30 -180 cm high, leaves broadly elliptic, incisodentate flower yellow or bluish purple, in dense axillary long-peduncle heads pods small 3.5 - 4.5 mm x 2.0 - 3.0 mm, ovoid- oblong, dark chocolate to almost black seed one, smooth, adhering to the pericarp (Uikey *et al.*, 2010)**.** It is distributed in India and China. Especially in the semi arid region of Rajasthan and Eastern districts of Punjab, Himalayas, Bundelkhand, Bengal, Bihar, Karnataka (Agrawal *et al*., 2006). Mechanical dormancy is found in *Psoralea corylifolia* because its seed coats or other coverings are too hard to allow the embryo to expand during germination (Baskin and Baskin, 1999).

Elicitors

Elicitors are low molecular weight compound enhances the secondary metabolite synthesis and stress response signalling in plant cell culture (Radman *et al*., 2004). Stress responses involve reversible phosphorylaton, ion fluxes, (Ca2þ, Kþ, Hþ), Salicyclic Acid (SA) or oxylipins such as Jasmonic acid (JA), Ethylene, Reactive Oxygen species (ROS), transcription factors and promoter elements (Stratmann, 2003). On the basis of nature elicitors can be divided in the abiotic and biotic elicitor. Abiotic elicitors are physical factors, chemical compound which disturb the membrane integrity where as biotic elicitors have biological origin, derived from the pathogen or from the plant itself (Sharma *et al.*, 2011).

Table 1: Type of Elicitors Used in Phytoestrogen Enhancement in the family, *Fabaceae*

S. no	Plant	Culture system	Elicitors		Phytoestrogen	References
			Abiotic	Biotic		
1.	*Psoralea corylifolia*	Callus culture	Salicylic acid	Yeast extract, Chitosan,	Genistein and daidzein	(Shinde *et al.*, 2009)
		Hairy root culture	Cd^{2+}		Phytoestrogens	(Satdive *et al*., 2014)
2	*Pueraria lobata*	Callus culture	Methyl jasmonate		Genistein and daidzein	(Krawczyk and Thiem, 2010).
3.	*Pueraria tuberosa*	Callus culture	Ethrel solution		Puerarin, Genistein, Daidzein	(Goyal and Ramawat, 2008)
4	*Glycine max L.*	Field grown plant	Salicylic acid Methyl jasmonate	*Aspergillus niger Rhizopus oligosporus*	Daidzein Glycitein	(Saini *et al*., 2013)
5.	*Medicago truncatula*	Callus culture		Yeast extract	Phytoestrogens	(Broeckling *et al*., 2005).

Elicitors and Suspension Culture

Elicitors act as switch for increasing the yield of genistein and daidzein (Gaid *et al*., 2011). Elicitation by abiotic elicitors heavy metal ions (Ag^{2+}, Cd^{2+}) and biotic elicitors polysaccharides (yeast extract and chitosan), plant response-signaling compounds (methyl jasmonate and salicylic acid) induces genistein and daidzein accumulation in plant cell cultures (Dicosmo *et al*., 1985; Poulev *et al*., 2003). HPLC analysis exhibited the enhanced genistein and daidzein concentration in *Psoralea corylifolia* cell suspension culture by elicitation (Shinde *et al*., 2009). Although abiotic elicitors have received less interest (Radman *et al*., 2003; Zhao *et al*., 2010).

Precursor feeding of phenylalanine at 0.5 mm concentration in *Psoralea corylifolia* enhanced the production of daidzen (1.99% dry wt) and genistein (0.22% dry wt) in cell suspension culture. Biotic elicitors yeast extract (100 mg^{-ml}) increased the production of daidzein (2.21% dry wt) and genistein (0.293 % dry wt). Salicyclic acid at 1mm stimulated the accumulation of daidzein (3.4% dry wt) and genistein (0.41 % dry wt) after 2 days' elicitor. In case of ploy amine spermine (100 mm) resulted in higher accumulation of daidzein (3.2% dry wt) and genistein (0.475% dry wt) (Shinde *et al*., 2009). Psoralen content was ~3 mg g^{-1} dry weight in suspension grown hairy roots much higher than in solid grown hairy roots in MS+4µM BAP (Baskaran and Jayabalan, 2009). Yeast extract enhanced shikimic acid, a precursor of phenyl propanoid pathway in cell cultures of *Medicago truncatula* at late stationary (Broeckling *et al*., 2005).

Table: 2. Plants of *Fabaceae* and their Major Phytochemical Content (Bansal *et al*., 2014)

S.no	Plant	Phyochemicals	References
1	*Clitoria ternatea*	Flavonoids	(Kumari, 2013)
2	*Cyclopia subternata*	Flavonoids	(Kokotkiewicz *et al*., 2012)
3	*Genista tinctoria*	Isoflavonoids	(Luczkiewicz *et al*., 2003)
4	*Glycyrrhiza echinata*	Flavonoids	(Ayabe *et al*.,1986)
5	*G.glabra*	Flavonoids	(Asada *et al*., 2000)
6	*G.uralensis*	Flavonoids	(Guo *et al*., 2012)
7	*Indigofera cordifolia*	Apigenin,kaempferol,quercetin	(Upman *et al*., 2011)
8	*Medicago truncatula*	Flavonoids,Isoflavonoids	(Farang *et al*., 2007)
9	*Psoralea corylifolia*	Isoflavoniods,Isoflavones	(Shinde *et al*.,2009)
10	*Pueraria lobata*	Isoflavone	(Theim *et al*., 2003)
11.	*Pueraria tuberosa*	Isoflavoniods	(Vaisnav *et al*., 2006)
12	*Pueraria candollei*	Phytoestrogen	(Danphitsanuparn *et al*., 2012)
13	*Sutherlandia frutescens*	GABA	(Phulukdaree *et al*., 2011)

Filter sterilized ethrel solution at 100 µM leads to 14 folds higher accumulation of isoflavones (puerarin, genistein, daidzein) in cell suspension culture of *Pueraria tuberosa* (Goyal and Ramawat, 2008). In cell

suspension culture enriched with sucrose at 3% stimulated the accumulation of isoflavones daidzein and genistein as compared to glucose, fructose and maltose (Shinde *et al*., 2009). Methyl jasmonate (MJ) at 0,100 and 200 µM stimulated isoflavones accumulation in *Pueraria lobata* plant cell cultures *in vitro* after 7 days of elicitation (Krawczyk and Thiem, 2010). Different biotic elicitors yeast extract and chitosan have increased the synthesis of psoralen four to seven-fold in 16-day old cell cultures of *Psoralea corylifolia* Linn. over control cells (Ahmed and Baig, 2013). Anthocyanin concentration was increased to 0.03 µg mg^{-1} in 18 days' culture as compared to that in the unelicitated cells by salicylic acid as an elicitor in *Vitis vinifera* suspension cultures (Saw *et al*., 2010). Cd^{2+} ion at 8 µ M was most effective for phytoestrogens induction in hairy root culture of *Psoralea corylifolia* (Satdive *et al*., 2014). Ag^{2+} ion at 25µM was effective for tanshinone production in *Salvi miltiorrhiza* cell suspension cultures (Zhao *et al*., 2010).

CONCLUSION

Elicitor based approach in *Psoralea corylifolia* Linn suspension culture is a new developing technology for increasing the yield of phytoestrogen and other secondary metabolite product synthesis by overcoming the effect of unforeseen climatic condition. Plants are tremendous source of medicinally important compound and reliable source of drugs, flavour, fragrances, dyes and pigments. Many of the drugs and food supplements are synthetic modification of naturally obtained substances. Both abiotic and biotic elicitors alter the biosynthetic pathway to enhance the phytoestrogen accumulation in suspension culture. This elicitor based approach is a new hope for conserving the endangered medicinal plants in their natural environment.

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