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Plant Systems: An Efficient Bio-manufacturer of r-hGH

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ABSTRACT

Recombinant human growth hormone (r-hGH) is a vital hormone that is being synthesised in a variety of animal systems such as rabbits, rats, monkeys etc and in microbial systems such as *E.coli*, yeast, *Pichia pastoris* etc. But plant systems are preferred over them. Production of human recombinant proteins in plant systems is on the rise due to its advantages in both production and *in vivo* action. r-hGH has been successfully produced in a variety of plants like tobacco, rice, maize, and soybean using multiple vectors. Various companies like Genentech and Eli Lilly have produced recombinant hGH which are being used in therapy for a number of hGH deficiency associated diseases like Turner's syndrome, Noonan's syndrome and chronic renal failures. This article aims to review the various plant systems involved in synthesis of r-hGH synthesis and the potential approaches towards the same.

Keywords: Recombinant hormone hGH, *E.coli*, yeast, *Pichia pastoris*, vectors.

INTRODUCTION

Biopharmaceuticals have revolutionized modern medicine and represent the fastest growing sector within the pharmaceutical industry (**Anne *et al.*, 2012**) for the treatment of diabetes, anaemia, hepatitis, cancer, cardiovascular diseases, and many others (**Leader *et al.*, 2008 and Woodnutt *et al.*, 2008**). Technology for enabling plants to bio-manufacture non-native proteins in commercially significant quantities has been available for just over 20 years **(Daveis, 2010).** Plant made pharmaceuticals is emerging as a promising approach to produce recombinant pharmaceuticals and have gained interest because of their advantages, such as low production costs, faster production process, simpler procedure of production and purification of proteins, product safety and ability of the plant cells to perform complex post-translational modifications etc. **(Thanavala *et al.*, 2005; Shin *et al.*, 2009; Xu *et al.*, 2014).** Unlike other expression systems, molecular farming of plants has different forms,

including cultivation of whole plants in fields, transient expression by agroinfiltration of plants or virus-infected plants, *in vitro* culture of plant tissues or organs and plant cell suspension culture (**Xu *et al.,* *2014)***.

Animal systems like transgenic rabbits **(Lipinski *et al.,* 2012*)*** and microbial systems such as *E.coli* **(Persson *et al.,* 2005)** have been used but have proven to elicit significant immunogenic responses **(Borrione *et al.,* 2012)**. Plant cell cultures combine the merits of whole plant systems with those of microbial and animal cell cultures, and already have an established track record for the production of valuable therapeutic secondary metabolites **(Hellwig *et al.,* 2004).** Several plant species (dicot, monocot, food and non-food, leafy crops, cereals and legumes) have been used for the expression of recombinant proteins **(Peters and Stoger, 2011).** Plant cell suspension cultures also offer greater control over the production environment, and simpler and cheaper downstream processing and purification for proteins secreted from the cells **(Sambrook *et al.,* 1989)**. Microalgae also represent attractive biotechnology platforms for the synthesis of recombinant therapeutic proteins and other high value products **(Gong *et al.,* 2011; Rasala and Mayfield, 2015)** along with computational methods which aim at improving hGH thermostability by using an empirical free energy function for scoring designed sequences, as it has limited stability in solution and poor oral absorption **(Filikov *et al.,* 2002).**

Adult growth hormone (GH) deficiency is a well-recognised clinical entity, causing abnormalities in substrate metabolism, bone remodelling and body composition, as well as physical, and psychosocial function **(Strobl and Thomas, 1994).** GH deficiency adults have reduced lean body mass and increased abdominal adiposity **(Gupta, 2011)** which are said to improve with GH treatment **(Maison *et al.,* 2004).** GH was first extracted from cadaveric pituitaries in the late 1950s **(Raben, 1962)** and are said to cause the Creutzfeldt–Jakob's disease, a dreaded complication of GH therapy obtained from the use of human pituitary, resulting in the death of patients **(Koch *et al.,* 1985).** With the increasing demand for hGH (human growth hormone), accompanied with the need to make this recombinant protein available to a wider population at a more reasonable cost, plants provide a feasible alternative to current production platforms (**Rabindran *et al.,* 2009**). Various findings have reported the expression of the hGH in a variety of transgenic plants.

VECTOR BASED TRANSFER OF GENE:

Nicotiana benthamiana is used for producing hGH, using plant virus-based expression vector. Such plants produced hGH (pphGH) increased the weight by ~17g in a hGH deficient animal (**Rabindran *et al.,* 2009**). After exposure to *Agrobacterium rhizogenes*, clonal root lines producing human growth hormone from *Nicotiana benthamiana* leaves can be created by infecting them with the tobacco mosaic virus-based vector - 30B. Using plant-virus based vectors, clonal root lines accumulated with hGH have been sustained for over a three year period in the absence of antibiotic selection, showing potential for commercial production of vaccines, antigens and therapeutic proteins in enclosed facilities (**Skarjinskaia *et al.,* 2008).**

hGH can also be expressed in plasmid vectors, pGH-Pi and pGH-PGi with selection markers for suppression of prolamin and glutenin gene expression (**Kuroda *et al.,* 2010)**. They can transform the competent cells of *Agrobacterium* by electroporation method and infect the somatic calli of dwarf rice seeds**.** The calli grown in greenhouses, yielded 470 µg of hGH /g dry weight of seed at the maximum level, approximately 0.7% of total

seed protein, in GH-PGi **(Kurita *et al*., 2002)**. The amount of hGH in our system may not be sufficient for a plant bioreactor, but in the study conducted by **Shigemitsu *et al*., (2012)**, the hGH expressed in transgenic rice seed was not degraded. Synthetic human growth hormone (shGH) is constructed on the basis of rice optimized codon usage and overlap PCR strategy **(Kim *et al*., 2008).** The shGH gene, can be fused with the signal sequence of rice amylase 3D (Ramy3D), and introduced into the plant expression vector containing HTP (Hygromycin phosphotransferase) as a selection marker for hygromycin B and the rice Ramy3D promoter expression system. The vector, pMYN449 was introduced into the rice calli via particle bombardment method. The transgenic calli harbouring the shGH gene was established in cell suspension cultures which was grown in darkness, in a shaking incubator. The shGH proteins accumulated in the transgenic rice cell suspension culture medium can be detected by Western blot analysis **(Sambrook *et al.*, 1989).** Similar results have been observed in other expression systems, transgenic tobacco cell cultures, soy, and maize **(Staub *et al.*, 2000).** The accumulation of shGH in the suspension culture medium of one of the transgenic rice callus lines reached maximum levels at 57 mg/L. The biological activity of shGH accumulated in the transgenic cell suspension medium was determined by measuring the induced proliferation of hGH-required Nb2 node lymphoma cells **(Tanaka *et al.*, 1980)** and the biological activity of shGH accumulated in the transgenic rice cell suspension culture was similar to that of the *E. coli* derived recombinant hGH **(Kim *et al*., 2008).**

OTHER METHODS OF GENE TRANSFER:

hGH was produced in genetically engineered soybean seeds by **Cunha *et al.*, (2011), u**tilizing the alpha prime (α′) subunit of β-conglycinin tissue-specific promoter from soybean and the α-Coixin signal peptide from Coix lacryma-jobi, they obtained transgenic soybean lines that expressed the mature form of hGH in the seeds. Expression levels of bioactive hGH was upto 2.9% of the total soluble seed protein content (corresponding to approximately 9 g/ kg) were measured in mature dry soybean seeds. The results of ultrastructural immunocytochemistry assays indicated that the recombinant hGH in seed cotyledonary cells was efficiently directed to protein storage vacuoles. Specific bioassays demonstrated that the hGH expressed in the soybean seeds was fully active. Other findings also reported hGH expression levels in tobacco, soy and maize seeds, which were 0.25, 0.16, 0.008 and 0.5% of those of total soluble protein respectively (**Staub *et al.*, 2000**, **Leite *et al.*, 2000**, **Russell *et al*., 2005).**

CONCLUSION

A major advantage of transgenic plants that are based on yeast or *E. coli* is their ability to perform most of the post-translational modifications (PTMs) that are required for the bioactivity and pharmacokinetics of recombinant therapeutic proteins. Furthermore, recent advances in the control of PTMs in transgenic plants have made it possible for plants to perform human-like modifications of recombinant proteins **(Gomord and Faye, 2004).**

The availability of unlimited biosynthetic hGH has promoted its potential usefulness for short term anabolic treatments in the postoperative phase, for major burns and injuries or in glucocorticoid treatment **(Girard, 1993)**, for the treatment of paediatric hypopituitary dwarfism and in children suffering from low levels of hGH **(Richmond and Rogol, 2016)**, in therapy to promote growth in Turner's syndrome **(Quigley *et al*., 2017)**, Prader-

Willi syndrome **(Grugni and Marzullo, 2016)**, Noonan syndrome **(Siklar *et al.*, 2016)** and chronic renal failure **(Bach and Hale, 2014).** Some of the most recent studies on hGH suggest that r-hGH treatment in young adults with obesity and non-alcoholic fatty liver disease may have benefits to reduce liver fat content, although larger studies are needed to confirm this effect **(Pan *et al.*, 2021).** In patients without comorbidities long-term GH treatment is safe and the risk of developing diabetes, cardiovascular disease, or tumours has not been reported **(Höybye *et al.*, 2020).**

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