Popular Article

Popular Kheti

Volume -5, Issue-1 (January-March), 2017
Available online at www.popularkheti.com

eISSN: 2321-0001

Sugarcane for Economical Bioplastic Production

Manoj Baliram Pohare[1*], Sachin Ashok Bhor[1] and Pallavi Kisan Patil[2]
[1]The United Graduate School of Agricultural Sciences, [2]Graduate School of Science and Engineering
Ehime University, Matsuyama-790-8566, Japan
***Email of corresponding author: mpohare2007@gmail.com**

Plastics are the petroleum-based products and their demand in the market is continuously increasing. This increase in demand coupled with the depletion of existing oil resources, and a decline in the rate of their replacement has led to increases in the costs of fossil resources and energy. Despite of the several other disadvantages, plastic remains the most commonly used material in daily life. In order to avoid the hazardous effects of non-biodegradable plastic, an alternative ecofriendly and renewable bioplastic can be used. Sugarcane accounts for the largest value of production and holds an enviable position among all the commercial crops in India. By taking an advantage of Sugarcane as a resource for bioplastic production, we suggested the genetic modification of sugarcane with bacterial polyester, polyhydroxybutyrate (PHB). Here we proposed the possible strategy for economical, renewable and environment friendly bioplastic production in India.

Introduction

India is the second largest producer of the sugarcane in the world and it is mainly used for the sugar production. Sugarcane is highly efficient biomass producer. It is a C_4 plant that stores the sugar in the form of sucrose (Brown 1999). Sucrose, extracted and purified in specialized factories, is used as raw material in human food industries or is fermented to produce ethanol (Doelle and Doelle 1989). Ethanol is produced on a large scale by sugarcane industry. Sugarcane is the world's largest crop by production quantity (FAO 2015). It has inbuilt genetic containment features. Many cultivars are sterile under the usual commercial growing conditions, vegetatively propagated and do not persist without human cultivation. All this features makes the sugarcane as a unique transforming host (Anon 2004). The biochemical pathways of sugar assimilation in sugarcane can be used for the production of different valuable, economical and environment friendly products like biofibers, waxes, bioplastic (Lakshmanan et al 2006). The production of bioplastic has been tried in different microorganisms (Jehan et al 2016; Verlinden et al 2007) and plants (Poirier and Gruys 2002; Snell and Peoples 2002; Lossl et al 2003; Wrobel et al 2004). In this article we suggested the production of bacterial polyester, polyhydroxybutyrate (PHB), in the crop species sugarcane in India.

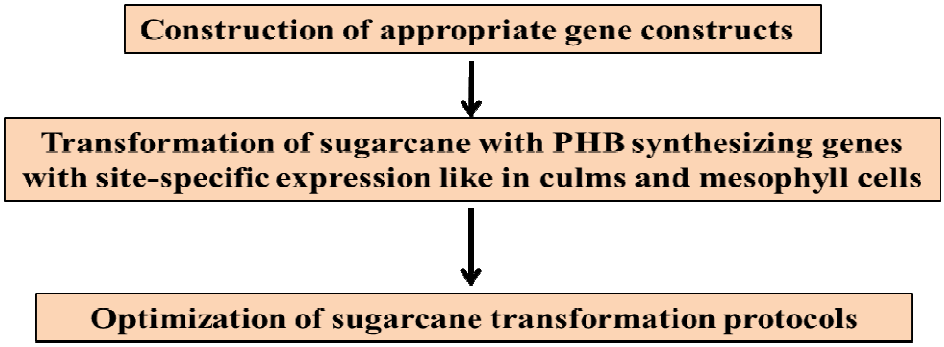
Current Scenario

Efforts are going on for the production of bioplasic in various plants. It is economically necessary to obtain more amount of bioplastic per dry weight of biomass against current achievement of 7 % in sugarcane (Bohlmann 2006; Somleva et al 2008). The PHB biosynthesis enzymes of *Ralstonia eutropha* [β-ketothiolase (phaA), acetoacetyl-reductase (phaB) and PHB synthase (phaC)] were expressed in the cytosol, mitochondria or plastids (Petrasovits et al 2007). These organelles are the site of high flux for the acetyl-CoA, which is the substrate for the production of bioplastic in bacteria. The high-level expression of above-mentioned gene will be responsible for the conversion of acetyl-CoA to bioplastic. *Ralstonia eutropha* is having a good capability for the production of PHB as per studies carried out (Petrasovits et al 2007). PHB accumulated in cytosolic lines at trace amounts, but was not detected in mitochondrial lines (Petrasovits et al 2007). In plastidic lines, the highest PHB accumulation is reported (Petrasovits et al 2007). The concentration of PHB in culm internodes of plastidic lines was substantially lower than in leaves (Petrasovits et al 2007).

Strategy

Therefore, we would like to suggest some strategies for producing PHB in mitochondria and mesophyll cell plastids, and for increasing PHB yield in culms. Additionally, work should be carried out for gene expression in the stem of sugarcane as it occupies the huge volume of sugarcane.

Flow chart



Methodology

A) Construction of Appropriate Gene Constructs

Use of various expression systems with different promoters: It is found that the expression of gene can be reduced due to the use of promoter of dicotyledonous *spp*. It is necessary to compare the efficiency of different promoters, including the rice and maize polyubiquitin, Cavendish banana streak badnavirus promoter and chlorophyll A/B binding protein promoter, which can result in selection of proper gene construct. The highest expression system can be used for further research after sufficient screening.

Use of advanced vector systems in PAct vectors with Rep activator protein: For high-level expression of transgene, production of maximum number of transgene mRNA is favorable. The use of a virus-based episomal amplification technology as a plant bioreactor platform exploits the process of Rep-mediated rolling circle replication for the high-level amplification of virus-based episomes in plants and subsequent expression of heterologous proteins (Pirlo 2007).

Use of tightly regulated inducible promoter: The above practice can help us to know the effect of available acetyl-CoA flux and its impact over production at various stages of growth cycle. This will also help to reason out the factors inhibiting PHB production in mitochondria and cytosol. After studying the impact of PHB on various stages of growth (controlled by its inducible expression), one needs to select a proper growth stage of sugarcane for most efficient bioplastic production.

Vacuole directed accumulation of PHB polymer: As we know, vacuole is the organelle, which occupies maximum volume after nucleus in cell. The accumulation of PHB may be having adverse effect on cell metabolism and this can be avoided by targeting the product to vacuoles. Work has been done for identifying signal peptide, which is targeted only to vacuole (Chrispeels et al 1992). Proteins which are targeted to vacuole can be studied in *silico* and the conserved potential target motif can be identified with the help of available ESTs present at NCBI database. Candidate protein includes proteases (aspartic proteases), proteinase inhibitors (cystatins, trypsin-inhibitor proteins) and vacuolar sorting proteins. Legumain protein from sugarcane contains a motif that is highly conserved across their homologues known to locate to the vacuole (Jackson et al 2007). This conserved domain is responsible for vacuolar localization and can be engineered so that PHB can also be targeted to vacuole.

B) Standardization of Sugarcane Plastid Transformation

The successful transformation of sugarcane chloroplasts will greatly impact the feasibility of producing transgenic plants with bioplastic. The incorporation of a single copy of the transgene into the chloroplast genome can result in very high transgene expression. This is because each sugarcane cell contains 10 to 100 plastids, which in turn will have 10 to 100 copies of the genome.

C) Improving the PHB Accumulation in Culm and Mitochondria

Use of enzyme and substrate enrichment in mitochondria: It is found that, there is very less or no production of PHB in mitochondria (Petrasovits et al 2007). The above practice can help to show that the proteins are targeted correctly and are functional following post-targeting processing.

Identification of appropriate cultivar of sugarcane as plant material: There is a variation in PHB accumulation in culm region due to different types of cells present. According to reports in the previous literatures, it is found that the cells containing more inter-nodal fiber content accumulates more PHB. Therefore, it is necessary to select the transforming germplasm having high culm internode fiber content. This cell types varies from 5–40 % among different cultivars.

Conclusion

From past three decades, a significant development has been made to engineer PHB-producing plants that can provide economical, renewable bioplastics and biofuels. Also, by taking the advantage of Sugarcane as one of the biggest biomass producer crop, here we suggest to exploit this crop for PHB production under Indian conditions and thereby sustainable bioplastic production. Still, challenges in PHB-transgenic plant production and increasing their efficiency are needed to be addressed in the near future. In context to Indian situations, the commercialization of such technology will open the new doors for future bio-industry.

References

Anon. 2004. The Biology and Ecology of Sugarcane (*Saccharum* spp. Hybrids) in Australia, Canberra: Australian Government, Department of Health and Aging, Office of the Gene Technology Regulator, pp. 22–25.

Bohlmann GM. 2006. Polyhydroxyalkanoate production in crops. *In*: Feedstocks for the Future: Renewables for the Production of Chemicals and Materials (Bozell JJ, Patel MK, eds), Washington, DC: Oxford University Press, pp. 253–270.

Brown RH. 1999. Agronomic implications of C_4 photosynthesis, *In:* R.F. Sage, R.K. Monson (Eds.), C_4 Plant Biology, Academic Press, San Diego, pp. 473–507.

Chrispeels MJ, Raikhel NV. 1992. Short peptide domains target proteins to plant vacuoles. *Cell*, (68), 613–616.

Crop production. 2015. Food and Agriculture Organization of the United Nations.

Doelle MB, Doelle H. 1989. Ethanol production from sugar cane syrup using *Zymomonas mobilis. J. Biotechnol.*, (11), 25–36.

Jackson MA, Rae AL, Casu RE, Grof CPL, Bonnett GD, Maclean DJ. 2007. A bioinformatic approach to the identification of a conserved domain in a sugarcane legumain that directs GFP to the lytic vacuole. Functional Plant Biology, (34), 633–644.

Jehan Mohamed El-Mohamedy Hawas, Tarek El-Said El-Banna, Elsayed Belal Abdelmonteleb Belal and Ahmed AhmedAbd El-Aziz. 2016. Production of Bioplastic from some selected Bacterial strains. *Int. J. Curr. Microbiol. App. Sci.*, (5), 10-22.

Lakshmanan P, Geijskes RJ, Wang L, Elliott A, Grof CP, Berding N, Smith GR. 2006. Developmental and hormonal regulation of direct shoot organogenesis and somatic embryogenesis in sugarcane (*Saccharum* spp. interspecific hybrids) leaf culture. *Plant Cell Rep.*, (25), 1007–1015.

Lossl A, Eibl C, Harloff HJ, Jung C, Koop H. 2003. Polyester synthesis in transplastomic tobacco (Nicotiana tabacum L.): significant contents of polyhydroxybutyrate are associated with growth reduction. *Plant Cell Rep.,* (21), 891–899.

Palaniswamy H, Syamaladevi DP, Chakravarthi M, Philip A, Petchiyappan A, Narayanan S. 2016. Vacuolar targeting of r-proteins in sugarcane leads to higher levels of purifiable commercially equivalent recombinant proteins in cane juice. *Plant Biotechnol. J.*, (14), 791–807.

Petrasovits LA, Purnell MP, Nielsen LK, Brumbley SM. 2007. Production of polyhydroxybutyrate in sugarcane. *Plant Biotechnol. J.*, (5), 162–172.

Pirlo SD. 2007. Identification of viral-based replicating vectors suitable for development of a sugarcane bioreactor (Doctoral thesis) submitted to Institute of Health and Biomedical Innovations, Queensland University of Technology.

Poirier Y, Gruys KJ. 2002. Production of polyhydroxyalkanoates in transgenic plants. In Biopolymers (Steinbüchel, A., ed.), pp. 401– 435.

Snell KD, Peoples OP. 2002. Polyhydroxyalkanoate polymers and their production in transgenic plants. *Metab. Eng.*, (4), 29–40.

Somleva MN, Snell KD, Beaulieu JJ, Peoples OP, Garrison BR, Patterson NA. 2008. Production of polyhydroxybutyrate in switchgrass, a value-added co-product in an important lignocellulosic biomass crop. *Plant Biotechnol. J.*, (6), 663–678.

Verlinden RAJ, Hill DJ, Kenward MA, Williams CD, Radecka I. 2007. Bacterial synthesis of biodegradable polyhydroxyalkanoates. Journal of Applied Microbiology, (102), 1437–1449.

Wrobel M, Zebrowski J, Szopa J. 2004. Polyhydroxybutyrate synthesis in transgenic flax. *J. Biotechnol.*, (107), 41–54.