Phytoplastics Based on (PHB) Producing Transgenic Plants as an Alternative to Plastics: A Review

G. Siva Gokul[1], M. Pradeepkumar[2], S. Tina Deva Prarthana[3], V.E.Ida Christi[4]
{idachristi@psgpharma.ac.in[1]}

PSG College of Pharmacy[1234]

Abstract. In past several decades synthetic plastics were used which produced harmful effects on living-beings and environment. Bioplastics are a long chain of monomers joined together by ester bonds and are thus termed as polyesters. Polyhydroxyalkanoate (PHA) family is a thermoplastic polyester that is natural and its variants have properties similar to petroleum-based plastic products that show biodegradable properties. PHB (Polyhydroxybutarate) is a PHA (Polyhydroxyalkonate) family derivative which is an energy storage material in Microorganisms. 'Phytoplastic' is an innovative approach to produce PHB containing bioplastics from transgenic plants. Since PHA pathway already exists in some plants, upon introduction of 'Recombinant Technology Plasmid' concept, PHB yield in percentage has increased in plants. The altered genes encoding for 3-ketothiolase, acetoacetyl-CoA reductase was introduced with a binary Ti- plasmid through the in-planta method to the plant of interest. The main objective of this study was to provide a representative data of general various plants that can be modified to better PHB yield in transgenic plants.20 Methodology: The scrutinization of the transgenic plants was through the utilization of scientific articles like scholar, pubmed, springer, etc. The study reviews on alternative source for plastics from plant material. Here, plants such as maize, thale cress, rapeseed, Siberian oilseed, sugarcane, oil palm and tobacco are selected for this study and reviewed for their PHB biosynthesis. Thus, transgenic modification of plants shows increase in percentage yield of PHB content. Conclusion: Thus, we conclude that transgenic traits show increase in percentage yield of PHB content than in normal traits. Various plants can be modified to a better PHB yielding transgenic variety and can be commercialized to produce 'Phytoplastics'.

Keywords: Bioplastics, Polyhydroxyalkonate, Polyhydroxybutarate, transgenic plants.

1 Introduction

The development of biodegradable plastic substitutes is imperative, as the environmental risks associated with single-use plastic have become a global concern. Films for the packaging sector

ICSETPSD 2023, November 17-18, Coimbatore, India

DOI 10.4108/eai.17-11-2023.2342851

that resemble plastic and may break down into innocuous elements is imperative. Concern over the growing impact of non-biodegradable plastic trash is expanding. Many species of microbes naturally produce Polyhydroxyalkanoates (PHAs) and thus deemed as a potential alternative for conventional plastics [1]. The PHAs may undergo full biodegradation by a range of microorganisms in less than a year, in contrast to petroleum-derived polymers that require many decades to break down. Water and carbon dioxide are produced during this biodegradation and are released back into the environment [2]. Despite their apparent benefits and importance, plastics are not desired in the current scenario due to their non-biodegradable nature, disposal challenges, and other issues. It is estimated that in the seventy-five billion pounds of plastics manufactured annually, about forty percent end up in landfills; in marine environments thousands of tons are dumped annually; and burning plastics is both costly and risky due to the release of dangerous chemicals like hydrogen cyanide and hydrogen chloride. A number of significant drawbacks to recycling exist as well, such as the challenge of sifting through the diverse range of plastics and the restricted number of additional uses after recycling. Therefore, the replacement of the plastics from bio-degradable to degradable in order to produce environment-friendly products such as 'bioplastics' is a logical reality that would assist us to overcome the plethora of problems arising due to plastics [3].

Since bioplastics are made of long chains of monomers bound together by ester bonds, they are categorized as polyesters and come in many forms. The most widely used of them all is PHA (Polyhydroxyalkanoate), a class of renewable, biodegradable polymers with qualities akin to those of plastics [4]. 2 PHB is a member of the polyhydroxyalkanoate (PHA) family. It is a naturally occurring thermoplastic polyester with biodegradable qualities that is comparable to petroleum-based polymers. As a result, PHB is the focus of this report as a plastic substitute. PHB comes from a variety of sources, including plants, microorganisms, and seaweeds. Plants have been chosen as the subject of interest from a variety of sources because they have a number of advantages over other options. Although they don't normally synthesize PHB, plants do have the resources to do so. Plants are a safer alternative to microbes since although microbes can synthesize PHB; they also tend to break it down [5]. As a result, the required genes are taken from microorganisms, activated in plants, and genetically altered to produce the desired outcome. Once they are introduced to the market, the modified transgenic plants may provide PHB and eventually serve as a plastic substitute. Since the original demonstration of PHB around 20 years ago, at least 11 other plant species have been successfully developed to exhibit this trait.

PHB offers several unique merits, such as providing a neutral carbon footprint and biodegradability, it also has the possibility to partially replace plastics composed of petroleum. This review aims to expound on the possibilities and potential of transgenic plants as a source of PHB and an alternative to plastics.

2 PHB BIOSYNTHESIS IN PLANTS

Since polyhydroxybutyrate (PHB) can replace plastics derived from petroleum, it is a polymer that is used as a bioplastic. It is a highly promising tool as a substitute for plastics because it is biodegradable, has a carbon footprint that is zero, and can be moulded and sculpted into the desired product [6]. Plants cannot synthesize PHB, but many bacteria can, and by transferring the necessary

bacterial genes to plants, it is possible to make plants produce PHB. About 20 years have passed since the first demonstration of transgenic plants' capacity to synthesize PHB, and at least 11 more plant species have been successfully developed to do the same. Transgenic plants are used to synthesize PHB efficiently because plants do not degrade the PHB that is produced, unlike bacteria or other microorganisms [7].

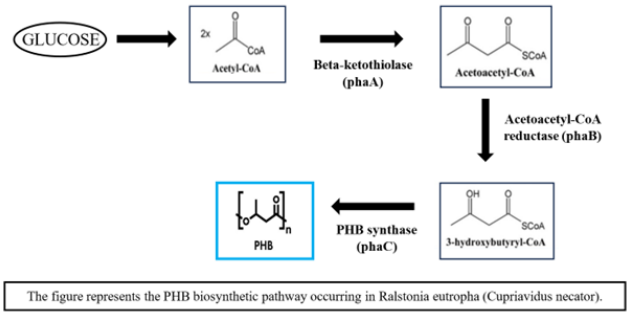

Fig. 1. The PHB biosynthetic pathway occuring in ralstonia eutropha (Cupravidus nector)

PHB is a 3-hydroxy butyrate polymer that creates linear polyester with a typical length of 103–104 units. Acetyl CoA is the first stage in the three-step process that makes up PHB biosynthesis, as shown in the schematic figure. To synthesize acetoacetyl CoA, two acetyl CoA molecules combine in step 1. To synthesize 3-hydroxyl butyryl-CoA, the keto group on the third carbon is oxidized and converted to a hydroxyl group in the second step. Polymerization using the enzyme PHB synthase is the third and last step. Acetyl CoA, the precursor of PHB, is widely distributed in plants, indicating that, with the right genes added, they can serve as viable bioplastic production platforms. Since chloroplasts are the organelle responsible for fatty acid synthesis, and uses acetyl Coenzyme A as a precursor, they have an especially high acetyl CoA flow [8]. There are several artificial sources of bioplastics, including plants, seaweeds, and microorganisms. The advent of numerous convenient ways for making high-quality products in huge amounts such as plant sugar conversion to plastic in crops and plastic development in microorganisms, is greatly aided by biotechnology. Biotechnology has produced bioplastics, which are more environmentally friendly and beneficial than traditional plastic. This review article concentrated on a few key plant sources that are utilized to produce PHB [9].

3 Methodology

The approach used to gather literature in the extensive literature survey, involves using keywords to search Google, PubMed, Science Direct, Royal Society of Chemistry, Springer, Wiley online library, American Chemical Society, Elsevier, and Google Scholar search engines.

4 PHB PRODUCTION IN CROPS

4.1 Zea mays

Zea mays is a member of the Poaceae family of plants. The engineering of PHB production in the maize stover served as the first example of bioplastic manufacture in a C4 crop. The PHB enzymes from Ralstonia eutropha were built in multi-gene transformation vectors, and several plant and viral promoters were used to drive their expression [10]. Among the promoters studied were the figwort mosaic virus (FMV), the maize chlorophyll a/b-binding gene promoter, the rice actin promoter (rACT), and the CaMV 35S promoter (cab-m5). To enhance transgenic expression, all promoter sequences were linked to the maize hsp70 intron. Polymer levels up to 5.73 percent DW were observed when the encoded enzymes were directed to the chloroplasts using the RbcS-TP from Arabidopsis [11]. It was demonstrated that while there was minimal development in the mesophyll chloroplasts, PHB granules were preferentially deposited in the chloroplasts of the bundle sheath cells that ring the vascular bundles. Based on this finding, it was postulated that the plastids of various cell types would have varied concentrations of acetyl-CoA, the substrate for PHB synthesis. Additionally, a link between elevated PHB accumulation levels and leaf chlorosis was discovered [12].

4.2 Nicotiana tabacum

The Solanaceae family includes the plant species Nicotiana tabacum. The herbaceous plant of the species Nicotiana that is grown annually is also called as farmed tobacco. The ideal genetic construct of the tobacco plastid transformation for PHB production was constructed using the operon extension technique [13]. For use in this construct, the bacterial genes encoding the PHB pathway enzymes were selected due to their similarity to the codon usage and GC content of the tobacco plastome. By using regulatory elements that were known to induce significant amounts of plastidial recombinant protein production but exhibited minimal similarity to the host plastome, the expression of the transgenes was boosted [14]. A partial transcriptional unit containing the PHB pathway genes and a selected marker gene encoding spectinomycin resistance at the 5' and 3' 2 ends, respectively, was bordered by the host plant's psbA coding 2 sequence and 3' psbA untranslated region. This design eliminated the requirement for promoters to be added in order to drive transgenic expression because the transgenes could be introduced into the plastome as an extension of the psbA operon. This cultivar produced up to 18.8 percent DW PHB in a leaf tissue sample [15].

The plant's leaves are harvested for commercial purposes and processed into tobacco that is consumed by humans. Furthermore, there is more potential for PHB generation in these plants. The goal is to increase the 33translation effectiveness of the. This is done by the following steps:

4.2.1 Modifying codon usage for enhancing PHB production

By altering the codon use of PHB biosynthesis genes, the goal was to boost PHB output in transgenic tobacco plants. Two genes from the bacterium Ralstonia eutropha were examined: acetoacetyl-CoA reductase (PhaB) and polyhydroxyalkanoate synthase (PhaC). The plants with the

codon-optimized PhaB gene produced twice as much PHB as the control plants with the wild-type gene. The transgenic plants' leaves also showed higher PhaB expression, suggesting that the increased PhaB expression was a factor in the increased PHB synthesis [16].

4.2.2 Effectiveness of codon optimization on PhaB and PhaC genes

However, the PhaC gene's codon optimization had no discernible impact on PHB synthesis. This implies that PHB biosynthesis in tobacco leaves was more dependent on the effectiveness of the PhaB catalyzed process.

4.2.3 Experimental approach and validation

The scientists have developed several vectors that carried codon-optimized and wild-type PhaB and PhaC genes, and then they inserted the vectors into tobacco plants. They used RT-PCR to validate the expression of these genes and immunoblot analysis to quantify the synthesis of PhaB and PhaC proteins [17].

Overall, the study's findings show that transgenic tobacco plants can produce significantly more PHB when the PhaB gene's codons are optimized. As a result, this advances the creation of more economical and efficient processes for the production of PHB, a significant bioplastic with potential uses across numerous industries

4.2.4 Camelina Sativa

Camelina sativa is a species of plant in the Brassicaceae family. The production of poly-3-hydroxybutyrate (PHB) in the plastids of Camelina sativa seeds was investigated by comparing the amounts of polymer generated after transformation of plants with five distinct binary vectors carrying two combinations of five seed-specific promoters for transgene expression. Through altering the N-terminus of genes encoding PHB biosynthetic enzymes, a plastid targeting signal was encoded. PHB levels were measured up to 15% of the mature seed weight in single sacrificed T1 seeds carrying a genetic construct containing the oleosin and glycinin promoters [18]. A more detailed analysis of two of the best binary vectors' ability to produce PHB in a Camelina line bred for larger seed sizes resulted in lines with mature T2 seeds that had up to 15% polymer. The presence of distinct PHB granules in the seeds was demonstrated by transmission electron microscopy. A line with 13.7 percent PHB in T4 seeds was created after a few lines were passed over to the next generation. In terms of the commercialization of an oilseed-based platform for PHB synthesis, this study's polymer production levels in seeds are the highest that have been recorded to date [19].

Camelina sativa were genetically constructed via the PHB route of bacteria. There are two methods and they are:

- Localization of all three enzymes in cytoplasm in PHB pathway
- Localization of first two enzymes in cytosol and anchoring of third enzyme for polymerization to the cytosolic phase of ER.

It was discovered that the ER technique produced polymers more steadily, with a PHB level of 10.2 percent for mature seed weight. These outcomes represent a major advancement toward commercial application. A novel construct called pMBXS763 was created for the ER method, in which the 4 Arabidopsis thaliana cytochrome B5 isoform D protein's c-terminus sequence was coupled with PHA synthase.

4.2.5 Saccharum officinarum

Saccharum officinarum is the scientific name of the plant species, which is a member of the Poaceae family. In chloroplasts, the synthesis of PHB was studied. The genes from Ralstonia eutropha encoding PHB biosynthetic enzymes and the selectable marker nptll were expressed using multiple single gene vectors in the first effort in order to modify sugarcane to produce PHB [20]. The transgenic expression was driven by the strong constitutive maize polyubiquitin promoter (ubi1), and the encoded enzymes were directed to the plastids via the pea RbcS-TP.

Particle bombardment was used to simultaneously deliver the single-gene vectors into sugarcane callus cultures [21]. The highest PHB content found was 1.88 percent DW, and about 20% of the 130 transgenic plants that were examined generated polymer at levels that could be detected by HPLC. Stems showed a noticeably decreased PHB concentration (up to 0.01 percent DW) [22]. In the plastids of bundle sheath cells of sugarcane leaves, PHB granules were identified by Nile blue staining, fluorescence microscopy, and TEM investigation; however, they were not visible in the mesophyll plastids.

4.2.6 Arabidopsis thaliana

Brassicaceae is the family to which Arabidopsis thaliana belongs to. The bacterial strain Alcaligenes eutrophus possesses three genes that code for the enzymes needed to catalyse the transformation of acetyl-CoA into poly[(R)-(-)-3-hydroxybutyrate] (PHB). To transfer these enzymes into the plastids of higher 2 plants, the genes were modified by the insertion of 2 DNA segments encoding a constitutive plant promoter, a poly(A) addition sequence, and a pea chloroplast transit peptide.

Through Agrobacterium-mediated transformation, each of the modified bacterial genes was inserted in Arabidopsis thaliana, and sexual crosses produced plants with all three genes. PHB was accumulated by these plants as 0.2–0.7-micron granules inside plastids, accounting for up to 14% of the dry weight [23]. Highly active mutant PHA synthase was responsible for the improvement in photosynthetic PHA production. Together with the wild type and mutant types, the NADPH-dependent acetoacetyl CoA reductase gene from Ralstonia eutropha was introduced into Arabidopsis thaliana. N149S and D171G are highly active mutant genes that express an 822–10 times growth in polymeric content in contrast to the wild type (T1). The yield of T2 progenes, which were developed, increased to 6.1 mg per g of dry cell weight. GC-MS investigations were conducted to further comply [24]. .

4.2.7 Brassica napus

The biological name of the plant species is Brassica napus which comes under the family Brassicaceae. Due to the fact that acetyl-CoA, the substrate essential for PHB synthesis' initial step, is widely present in fatty acid biosynthesis, brassica napus seeds might offer a more effective method for PHB synthesis [25]. To produce PHB, three enzymatic activities are required: PHB synthase, acetoacetyl-CoA reductase, and β-ketothiolase. Two specially engineered genes encoding these enzymes were engineered from the bacteria Ralstonia eutropha and placed in front of the Lesquerella fendleri oleate 12-hydroxylase promoter, which is specific to seeds. The gene cassettes were progressively transferred into a single multi-gene vector to convert Brassica napus.

The greatest amount of poly (β-hydroxybutyrate) that could be accumulated in leucoplasts from mature seeds was 7.7% of their fresh seed weight. The leucoplasts of these plants were found to be intact but distorted under an electron microscope, and they appeared to swell in response to the accumulation of polymers [26].

4.2.8 Elaeis guineensis

The African tree known as the oil palm (Elaeis guineensis), which belongs to the Arecaceae palm family, is mostly grown for its oil. This plant species shows promising potential in the production of PHB as an application for bioplastics through the following two steps:

- Introduction and gene transformation:

The process widely used to produce the biodegradable plastic known as polyhydroxybutyrate involved transforming oil palm embryogenic calli with bacterial DNA (PHB). The genes for the enzymes 3-ketothiolase (bktB), acetoacetyl-CoA reductase (phaB), and PHB synthase were inserted into the genetic material of oil palm calli (phaC). Furthermore, calli was also generated from the Escherichia coli threonine dehydratase (tdcB) gene. The promoter of maize ubiquitin regulated these genes [27].

- Resistance and Regeneration of Transformed Calli:

Numerous plantlets were regenerated from the altered calli, which were resistant to the herbicide Basta. The transgenes' steady insertion into the oil palm plants' genomes was verified by molecular investigations. It has previously been shown that plants can produce PHB, albeit the output was not very high. By introducing the PHB genes into the plastids of cotton and Arabidopsis plants, the output of PHB was increased. Similarly, enhanced polymer production was seen in oil palm plastids when the PHB and PHBV genes were expressed more [28]. The research demonstrated that transgenic oil palm plants that produced PHB were created by integrating the transgenes into the oil palm calli. All things considered in this review offers insightful information about the possibility of using genetic engineering to create biodegradable polymers in oil palm plants [29]

Plant	Plant Tissue	PHB-Total content	Reference and Notes
Zea Mays	Leaves	5.66% DW	PHB mostly in bundle sheath cell plastids [10]
Zea Mays	Suspension Culture/Peroxisome	0.2% FW	PHB biosynthesised in peroxisomes by adding bacterial gene in carboxy-terminal of target sequence.MW of 100 000 with PDI of 2.69 [30]
Nicotiama tabacum	Leaves	10-18.8%DW	Fertile transplastomic cultures that are modified with a synthetic operon psbA for PHB synthesis yield high quantities of bioplastic. [13].
Nicotiama tabacum	Leaves	0.09%DW	PHB synthesis rises when acetyl-CoA carboxylase is chemically inhibited [31]
Camelina Sativa	Seeds	19.9% DW in single seeds	Highest level reported in seed; high levels of PHB impaired survival of seedlings [32].
Saccharum officinarum	Leaves/Plastid	1.88% DW	PHB is present in all plastids, with the exception of thin-walled cortical cells in the stalk rind and mesophyll in leaves [20].

Arabidopsis thaliana	Leaves	14%DW	TP-Thio-1/TP-Red-1/TP-Syn-1 hybrid: the highest PHB concentration found in the leaves prior to senescence [23].Initial proof of PHB synthesis in plastids; three distinct constructs converted and then crossed to achieve the entire pathway; some chlorosis in leaves at elevated PHB concentration.
Brassica napus	Seeds	7.7%FW	The first instance of PHB synthesis in seeds [25].

Note: DW dry weight, FW Fresh weight

5 Discussion

The negative consequences of synthetic plastics on humans, animals, and the environment are well known to the public these days. Bioplastics, which are readily disposed of in the environment and biodegradable, are a popular substitute for synthetic plastics [33]. PHB offers several attractive benefits, including as biodegradability and perhaps a carbon footprint that is neutral, and it has the ability to partially replace plastics made of petroleum [34]. Microbial polyesters, or PHAs, are created by biochemical processes within microbial cells from acetyl3 -coenzyme A (acetyl-CoAs) by PHA synthase, an enzyme specific to its substrate that is found in the cytoplasm of the cell. Several bacteria are capable of synthesizing PHA from vegetable and animal fats [35]. Microorganisms that can convert triglycerides into PHA polymer chains and accumulate them as granules within the cytosol of bacterial cells should possess two primary characteristics: the ability to generate lipase, an enzyme that breaks down triglycerides to release long-chain fatty acids, and the ability to undergo β-oxidation, which converts the triglycerides into PHA chains [36]. In fact, enoyl-CoA is oxidised to (R)-3-hydroxyacyl-CoA when oils or lipids are utilised as the carbon substrate and are used through the fatty acid pathway. This catalysis is initiated by the (R)8-specific enoyl-CoA hydratase (PhaJ). The immediate precursor of PHA biosynthesis is (R) 3-hydroxyacyl CoA, which

also acts as a substrate3 for the enzyme PHA synthase (PhaC). By using biotechnology, we can alter the product to better meet our needs and raise its quality.[37]. The PHA market is predicted to be driven in the upcoming years by increased use of PHA in a variety of 3 application sectors, including high added-value industries like the biomedical and cosmetics sectors [38]. From 2020 to 2027, the PHA market is expected to increase at a pace of 7.60 3 percent, or 45.49 tonnes, in volume.The availability of feedstock is crucial for producing PHA from oily biomass on a worldwide scale [39]. A biodiesel facility with a capacity of 10 million gallons annually was predicted to be able to produce 20.9 tons of PHB.

6 Conclusion

In conclusion this review summarizes the potential of plants such as Zea mays, Nicotiana tabacum, Camelina sativa, Saccharum officinarum, Arabidopsis thaliana, Brassica napus and Elaeis guineensis as efficient synthesizers of phytoplastics. Furthermore, it can be concluded that these plants when genetically engineered and converted into transgenic species, do not degrade the synthesized PHB and are a more suitable medium to produce and commercialize PHB as phytoplastics. Phytoplastics thus synthesized are bio-degradable and are more suitable to the environment. Furthermore, a crop that produces polymers has been successfully commercialised if a higher product yield (¿10 percent DW PHB) and a decrease in agronomic penalties are achieved. Therefore, it is possible that in the near future, phytoplastics may act as alternatives to harmful plastics that are currently in use and establish the new standard, making them an inventive and sensible replacement for plastics. The development of these novel biomaterials is largely made possible by biotechnology, which also enables the materials' eventual transfer to full technological maturity and commercial accessibility. The primary forces behind the continued advancement of the bioplastics under discussion are the value-adding of waste and the proactive management of vital raw materials, while biotechnological techniques present growing prospects for the plastics industry as a whole. To improve the PHB yield in transgenic plants and commercialize them, this analysis finds several plants that can undergo genetic modification to give a greater PHB generating type .

References

[1] Somleva MN, Peoples OP, Snell KD. PHA Bioplastics, Biochemicals, and Energy from Crops. Plant Biotechnology Journal. 2013 Jan;11(2):233–252. Available from: http://dx.doi.org/10.1111/pbi.12039.

[2] Ranganadhareddy A. A Review on Biotechnological Approaches for the Production of Polyhydroxyalkanoates. Journal of Biochemical Technology. 2022;14(2):12–17. Available from: http://dx.doi.org/10.51847/hxh14vrhor.

[3] Angus HJF, Bourne EJ, Searle F, Weigel H. Complexes between polyhydroxy-compounds and inorganic oxy-acids. Tungstate complexes on sugars and other cyclic polyhydroxy-compounds. Tetrahedron Letters. 1964 Jan;5(1):55–60. Available from: http://dx.doi.org/10.1016/s0040-4039(01)89322-9.

[4] M A. Polyhydroxyalkanoates (PHAs) Database: Genomics of Polyhydroxyalkanoates (PHAs) Biosynthesis. Journal of Computer Science & Systems Biology. 2018;11(06). Available from: http://dx.doi.org/10.4172/jcsb.1000290.

[5] M A. Polyhydroxyalkanoates (PHAs) Database: Genomics of Polyhydroxyalkanoates (PHAs) Biosynthesis. Journal of Computer Science & Systems Biology. 2018;11(06). Available from: http://dx.doi.org/10.4172/jcsb.1000290.

[6] Montero G, Coronado MA, García C, Campbell HE, Montes DG, Torres R, et al. Wheat Straw Open Burning: Emissions and Impact on Climate Change. Global Wheat Production. 2018 Aug. Available from: http://dx.doi.org/10.5772/intechopen.76031.

[7] Anjum A, Zuber M, Zia KM, Noreen A, Anjum MN, Tabasum S. Microbial production of polyhydroxyalkanoates (PHAs) and its copolymers: A review of recent advancements. International Journal of Biological Macromolecules. 2016 Aug;89:161–174. Available from: http://dx.doi.org/10.1016/j.ijbiomac.2016.04.069.

[8] Zimmermann L, Dombrowski A, Völker C, Wagner M. Are bioplastics and plant-based materials safer than conventional plastics? In vitro toxicity and chemical composition. Environment International. 2020 Dec;145:106066. Available from: http://dx.doi.org/10.1016/j.envint.2020.106066.

[9] Srivastava N, Gupta VK, Pati R, Kumar Gaur R. Genetically by Modified Crops: An Overview. Biotechnology(Faisalabad). 2011 Feb;10(2):136–148. Available from: http://dx.doi.org/10.3923/biotech.2011.136.148.

[10] Zhu Y, Feuer G, Day SL, Wrzesinski S, Planelles V. Multigene Lentiviral Vectors Based on Differential Splicing and Translational Control. Molecular Therapy. 2001 Oct;4(4):375–382. Available from: http://dx.doi.org/10.1006/mthe.2001.0469.

[11] Dobrogojski J, Spychalski M, Luciński R, Borek S. Transgenic plants as a source of polyhydroxyalkanoates. Acta Physiologiae Plantarum. 2018 Aug;40(9). Available from: http://dx.doi.org/10.1007/s11738-018-2742-4.

[12] Wang P, Xiong X, Fu P, Wu G, Liu F. Marker gene excision in transgenic Brassica napus via Agrobacterium-mediated Cre/lox transient expression system. Oil Crop Science. 2020 May;5(2):58–65. Available from: http://dx.doi.org/10.1016/j.ocsci.2020.06.003.

[13] Bohmert-Tatarev K, McAvoy S, Daughtry S, Peoples OP, Snell KD. High Levels of Bioplastic Are Produced in Fertile Transplastomic Tobacco Plants Engineered with a Synthetic Operon for the Production of Polyhydroxybutyrate. Plant Physiology. 2011 Feb;155(4):1690–1708. Available from: http://dx.doi.org/10.1104/pp.110.169581.

[14] Sharma A, Sohi BS, Chandra S. SN Based Forest Fire Detection and Early Warning System. International Journal of Innovative Technology and Exploring Engineering. 2019 Jul;8(9):209–214. Available from: http://dx.doi.org/10.35940/ijitee.h6733.078919.

[15] Rawat A, Mali RR. Phytochemical Properties and Pharmcological Activities of Nicotiana Tabacum: A Review. Indian Journal of Pharmaceutical and Biological Research. 2013 Jun;1(02):74–82. Available from: http://dx.doi.org/10.30750/ijpbr.1.2.9.

[16] NAKASHITA H, ARAI Y, YOSHIOKA K, FUKUI T, DOI Y, USAMI R, et al. Production of Biodegradable Polyester by a Transgenic Tobacco. Bioscience, Biotechnology, and Biochemistry. 1999 Jan;63(5):870–874. Available from: http://dx.doi.org/10.1271/bbb.63.870.

[17] WANG Y. Synthesis of medium-chain-length-polyhydroxyalkanoates in tobacco via chloroplast ge-netic engineering. Chinese Science Bulletin. 2005;50(11):1113. Available from: http://dx.doi.org/10.1360/982005-130.

[18] Malik MR, Yang W, Patterson N, Tang J, Wellinghoff RL, Preuss ML, et al. Production of high levels of poly-3-hydroxybutyrate in plastids of Camelina sativa seeds. Plant Biotechnology Journal. 2014 Nov;13(5):675–688. Available from: http://dx.doi.org/10.1111/pbi.12290.

[19] Barbante A, Irons S, Hawes C, Frigerio L, Vitale A, Pedrazzini E. Anchorage to the cytosolic face of the endoplasmic reticulum membrane: a new strategy to stabilize a cytosolic recombinant antigen in plants. Plant Biotechnology Journal. 2008 Jul;6(6):560–575. Available from: http://dx.doi.org/10.1111/j.1467-7652.2008.00342.x.

[20] Danne T, Nimri R, Battelino T, Bergenstal RM, Close KL, DeVries JH, et al. International Consensus on Use of Continuous Glucose Monitoring. Diabetes Care. 2017 Nov;40(12):1631–1640. Available from: http://dx.doi.org/10.2337/dc17-1600.

[21] McQualter RB, Petrasovits LA, Gebbie LK, Schweitzer D, Blackman DM, Chrysanthopoulos P, et al. The use of an acetoacetyl-CoA synthase in place of a β-ketothiolase enhances poly-3-hydroxybutyrate production in sugarcane mesophyll cells. Plant Biotechnology Journal. 2014 Dec;13(5):700–707. Available from: http://dx.doi.org/10.1111/pbi.12298.

[22] Menzel G, Harloff HJ, Jung C. Expression of bacterial poly(3-hydroxybutyrate) synthesis genes in hairy roots of sugar beet (Beta vulgaris L.). Applied Microbiology and Biotechnology. 2003 Jan;60(5):571–576. Available from: http://dx.doi.org/10.1007/s00253-002-1152-z.

[23] Nawrath C, Poirier Y, Somerville C. Targeting of the polyhydroxybutyrate biosynthetic pathway to the plastids of Arabidopsis thaliana results in high levels of polymer accumulation. Proceedings of the National Academy of Sciences. 1994 Dec;91(26):12760–12764. Available from: http://dx.doi.org/10.1073/pnas.91.26.12760.

[24] Miller ND, Williams DF. On the biodegradation of poly-β-hydroxybutyrate (PHB) homopolymer and poly-β-hydroxybutyrate-hydroxyvalerate copolymers. Biomaterials. 1987 Mar;8(2):129–137. Available from: http://dx.doi.org/10.1016/0142-9612(87)90102-5.

[25] Houmiel KL, Slater S, Broyles D, Casagrande L, Colburn S, Gonzalez K, et al. Poly(β-hydroxybutyrate) production in oilseed leukoplasts of Brassica napus. Planta. 1999 Oct;209(4):547–550. Available from: http://dx.doi.org/10.1007/s004250050760.

[26] Slater S, Mitsky TA, Houmiel KL, Hao M, Reiser SE, Taylor NB, et al. Metabolic engineering of Arabidopsis and Brassica for poly(3-hydroxybutyrate- co-3-hydroxyvalerate)

copolymer production. Nature Biotechnology. 1999 Oct;17(10):1011–1016. Available from: http://dx.doi.org/10.1038/13711.

[27] AKDAĞ HA, DUZYER GEBİZLİ S, HOCKENBERGER A. PRODUCTION AND CHARACTERIZATION OF PHB, PHBV ELECTROSPUN FIBERS AND THEIR BLENDS. Tekstil ve Mühendis. 2023 Sep;30(131):171–179. Available from: http://dx.doi.org/10.7216/teksmuh.1268253.

[28] Huang ES, O'Grady MJ, Basu A, Winn AN, John PM, Lee J, et al.. The Cost-Effectiveness of Continuous Glucose Monitoring in Type 1 Diabetes. American Diabetes Association; 2010. Available from: https://doi.org/10.2337/dc09-2042.

[29] Xuanyu L, Qingyun W, Shujun L, Songquan S. Advances in the Genetic Transformation of Sorghum bicolor. CHINESE BULLETIN OF BOTANY. 2011 Nov;46(2):216–223. Available from: http://dx.doi.org/10.3724/sp.j.1259.2011.00216.

[30] M A. Polyhydroxyalkanoates (PHAs) Database: Genomics of Polyhydroxyalkanoates (PHAs) Biosynthesis. Journal of Computer Science & Systems Biology. 2018;11(06). Available from: http://dx.doi.org/10.4172/jcsb.1000290.

[31] Colombo JP, Bachmann C. From Enzyme toEnzyme & Protein. Enzyme. 1992;46(6):275–275. Available from: http://dx.doi.org/10.1159/000468803.

[32] Patent survey on process biochemistry: A process for producing microbial fat or oil having lowered unsaponifiable matter content international patent application 2004/033698 to Suntory limited. Process Biochemistry. 2005 Mar;40(3–4):1487–1492. Available from: http://dx.doi.org/10.1016/s0032-9592(04)00196-7.

[33] Biotechnology Advances. 1997 Jan;15(1):293. Available from: http://dx.doi.org/10.1016/s0734-9750(97)88617-x.

[34] Reinforced Plastics. 2004 Jan;48(1):40. Available from: http://dx.doi.org/10.1016/s0034-3617(04)00112-2.

[35] Dobrogojski J, Spychalski M, Luciński R, Borek S. Transgenic plants as a source of polyhydroxyalkanoates. Acta Physiologiae Plantarum. 2018 Aug;40(9). Available from: http://dx.doi.org/10.1007/s11738-018-2742-4.

[36] Schnell JA, Treyvaud-Amiguet V, Arnason JT, Johnson DA. Expression of polyhydroxybutyric acid as a model for metabolic engineering of soybean seed coats. Transgenic Research. 2011 Dec;21(4):895–899. Available from: http://dx.doi.org/10.1007/s11248-011-9575-x.

[37] Snell KD, Peoples OP. Polyhydroxyalkanoate Polymers and Their Production in Transgenic Plants. Metabolic Engineering. 2002 Jan;4(1):29–40. Available from: http://dx.doi.org/10.1006/mben.2001.0214.

[38] Polonsky WH, Hessler D, Ruedy KJ, Beck RW. The Impact of Continuous Glucose Monitoring on Markers of Quality of Life in Adults With Type 1 Diabetes: Further Findings From the DIAMOND Randomized Clinical Trial. American Diabetes Association; 2017. Available from: https://doi.org/10.2337/dc17-0133.

[39] Snell KD, Singh V, Brumbley SM. Production of novel biopolymers in plants: recent technological advances and future prospects. Current Opinion in Biotechnology. 2015 Apr;32:68–75. Available from: http://dx.doi.org/10.1016/j.copbio.2014.11.005.