

## Renewable Energy Resources, Biofuel Plants and Genetic Manipulations

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### Abstract

Energy used in every aspect of our daily life is a necessary element and can be transformed into different forms. Energy resources are divided into renewable and non-renewable. Since non-renewable energy sources are fossil fuels (coal, oil, natural gas), which will run out and cause global warming, nowadays, renewable energy sources are preferred as an alternative to these sources. Renewable energy sources are used to produce continuous energy such as biomass, solar, marine, geothermal, wind energy. Today, fuels such as bioethanol, biodiesel, and biogas are obtained by using biomass. These types of fuel called biofuels are produced using plants, plant wastes, and algae. In this review article, renewable energy, biofuel plants, their properties and genetic interventions on these plants are included.

*Keywords:* Biofuels, biofuel crops, genetic manipulations, and renewable energy.

### 1. Introduction

Technological developments, industrialization and the increase in the world population rapidly enhance the demand for energy. Energy, which is the basic input in production, is a necessary element for increasing the welfare level of societies and is used in almost every field of daily life. In summary, energy, which is defined as the ability to do work, can be found in different types such as mechanical, heat, electrical, chemical and nuclear, can be converted to another with appropriate methods and classified in different ways. Energy resources are generally classified according to their use and recyclability. The classification made according to their use, energy resources are divided into renewable and non-renewable. Moreover, they are classified as primary and secondary energy sources according to their convertibility [1]. Most of the world's energy needs are met by fossil fuels. Increasing energy demand, uncertain climate change, depletion of fossil fuels, and the problem of global warming are driving energy use towards clean and renewable energy today [2]. Renewable energy sources meet 14% of the total world energy need. Renewable energy sources (biomass, solar, marine, geothermal, wind power, etc.) are sources used to produce continuous energy [3]. Biomass energy is an energy source obtained from plant and animal origin substances containing

carbohydrate compounds. Today, fuels such as bioethanol, biodiesel, and biogas are obtained by using biomass energy sources. Bioethanol and biodiesel are a type of fuels produced using various plant or animal oils. Biogas, on the other hand, is mainly methane and carbon dioxide gas, which is formed as a result of fermentation of organic materials (plant and animal wastes, urban and industrial wastes) in an oxygen-free environment [4]. Biomass energy is generally plant-based energy sources in solid, liquid, and gaseous form that are produced from agricultural products widely available in nature by various physical, chemical and biological methods have commercial properties and have standardized basic and certain properties [5].

### 2. Plants Used in Biofuel Production and Their Properties

Biofuels have become increasingly important in feeding the global fuel market, as renewable energy plays a crucial role in partially replacing traditional fossil fuel. Engines running on biofuels emit significantly fewer particles, hydrocarbons, and fewer greenhouse gas emissions than engines running on conventional fossil fuels. Compared to fossil fuels, biofuels are environmentally friendly due to their carbon renewables and they can reduce the global warming factor by reducing net carbon emissions to the atmosphere [6]. Biofuels are grouped as biodiesel, bioethanol, biomethanol, biogas, biodimethyl ether, and bio-oil. The most preferred biofuels today are bioethanol and biodiesel. In the production of

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bioethanol, plants such as barley, corn, potatoes, sweet potatoes, sugar sorghum, sugar cane, sugar beet and wheat are used as raw materials. In the production of biodiesel, rapeseed, flax, cotton, safflower, olive, peanut, sesame, flax and soybean plants are used as raw materials. The production of biogas are used animal, food industry, agriculture, fruit, vegetable, oil industry and wastewater treatment sludge and slaughterhouse wastes [5].

Plants contain 40-60% cellulose, 20-40% hemicellulose and 10-25% lignin. Cellulose is the most familiar form of carbon and is a biopolymer of glucose. Hemicellulose is a short, highly branched sugar chain containing 5 and 6 carbon sugars and uronic acid. Lignin is sugar-free [5]. Bioethanol is one of the high-octane biofuels obtained from plants whose origin is sugar and starch. Bioethanol is the product that comes out as a result of the fermentation process applied after the starch in agricultural products is converted into sugar. Bioethanol is a fuel that can often be mixed with gasoline [4]. Some plants are used in bioethanol production and their properties.

Branch Millet (*Panicum virgatum L.*) is a perennial and warm climate plant. Can be used for a long time like 10 years. Since it is a perennial plant, it is a very economical plant as there will be no costs of preparing the soil, seeds, and planting every year. It is a species that has been used in bioethanol production in recent years [7].

Sugar beet (*Beta vulgaris L.*) is a 2-year agricultural plant belonging to the spinach family, from which sugar is obtained from the root part. Vegetative organs develop in the first year and generative organs in the second year. Seeds are adjacent. Its height varies between 85-180 cm depending on the climate, place and type in which it is grown. It meets 30% of the world sugar production. The structure of sugar beet consists of 4.5% chemically bound water, 4.5% cell tissue, 90.95% sap. The composition of the juice consists of 15.18% sugar, 1.0-1.6% non-sugar organic substances, 0.8% inorganic salts [8].

Sweet Sorghum is a plant of the Monocotyledon class belonging to the Gramine family. 113 types available. It is a typical annual C4 plant and is widely grown in the world. It is preferred because of its high photosynthetic efficiency, it can be grown easily in any climate conditions, and it does not require excessive irrigation and fertilizer. It has high adaptability to different climates and can grow in low-quality soils [4]. Biodiesel is an alternative fuel similar to diesel that raw material is oil crops, waste oils, and animal fats, used alone or by adding it to the diesel at a certain rate. It is obtained as a result of a short-chain alcohol (ethanol, methanol) reaction of oils obtained from plants with high oil content such as sunflower, safflower, rapeseed, soybean with the help of a catalyst. Biodiesel and glycerin as a by-product are produced by methanol transesterification reaction from vegetable oils. The pure glycerin obtained can be converted into products with added value, some of pharmaceutical industry,

paper industry, soap industry, lubrication industry, cosmetics industry [5].

Some plants are used as raw materials in biodiesel production and their properties: Colza (*Brassica napus*) is an oil plant containing three main unsaturated fatty acids (oleic acid, linoleic acid, and linolenic acid) with oilseeds. The content and abundance of fatty acids are necessary for the nutrition and processing of seeds [9]. Rapeseed oil is the only oil with the best fatty acid composition among vegetable oils, with a low content of saturated fat (7%), moderate polyunsaturated fat (32%), and a high content of monounsaturated fat (61%). Rapeseed oil has the highest unsaturated fat content among the vegetable oils available. The 86% of the biodiesel produced in the world is obtained from the rapeseed. Rapeseed is a very good rotation crop. Making use of the vacant land and preventing winter erosion is an important feature. It is a market-ready product that is easy to cultivate and does not require any extra investment. Production costs in rapeseed agriculture are less than many other products [4].

Safflower (*Carthamus tinctorius L.*) is an annual and herbaceous oil plant belonging to the Compositae family. Due to its resistance to cold and heat, it is one of the alternative plants that can be evaluated in dry agricultural areas, and in irrigated agricultural areas with its resistance to weeds and salinity. It contains 13-46% oil in its seeds, approximately 90% of this oil consists of unsaturated fatty acids (linoleic and oleic) [10]. Safflower seeds contain 32-34% carbohydrates, 14-15% protein, 5-8% moisture, and ash. Cartharmin obtained from its flowers is used as a dye raw material. Its oilseed is used for biodiesel production and its pulp is used for animal feed. Compared to other oil crops, safflower is a species that adapts to regions where winters are cool and summers are dry. It is an alternative plant that can be used in arid agricultural lands due to its resistance to drought and cold [11].

Soybean (*Glycine max L.*) is a one-year cultured oil plant belonging to the legume family. Soybean is a good crop rotation plant and thanks to the *Bradyrhizobium japonicum* bacteria in its roots, it allows the free nitrogen in the air to bind to the soil and increases the fertility of the soil highly thanks to the organic matter it leaves [4]. The remaining 60-65% meal pulp after oil extraction can be used as the main protein source in the feed rations of farm and poultry animals. Soybean oil includes essential fatty acids known as linolenic, oleic, and linoleic acids [12].

### 3. Biofuel Crops and Genetic Interventions

Plants are not been domesticated for biofuel production. The best, most efficient way to turn plants into biofuel feedstocks is biotechnological. Biofuel raw material sources are divided into three generations. The first-generation raw materials are grain and sugar-

containing crops for bioethanol, and oilseed crops for biodiesel. Second generation biofuel raw materials are obtained from lignocellulosic products or straw waste. The problem encountered in the use of lignocellulosic raw materials is the removal of lignin. Acid and heat should be applied to remove lignin, but this is not always sufficient. Therefore, interventions should be made to increase cellulose synthesis by transgenic reduction or modification of lignin content. As third generation biofuel raw materials, cyanobacteria, algae and microalgae are used. Genetic manipulation is necessary to overcome problems such as light penetration, carbon assimilation, photoinhibition. Genetic interventions are needed to increase the oil content of oilseed crops used in biodiesel production [13].

Manipulations were made on the fatty acid desaturase 2 gene in order to increase the oil content of the rapeseed plant. FAD2 is a gene that affects oleic, linolenic and linolenic acids. A genomic editing system based on CRISPR/Cas9 technology was used to mutate all copies of BnaFAD2 to create new varieties of alleles at oleic acid and other acid levels. Mutants were found in two targeting sites, and phenotypic variation in mutants was systematically evaluated. The oleic acid content in the seeds of mutant plants increased significantly, exceeding the highest 80% compared to the wild type (66.43%), while the linolenic and linoleic acid contents decreased. Different types of mutations in BnaFAD2 alleles were found to have varying effects on oleic acid levels, suggesting the possibility of interfering with fatty acid levels by mutation at the specific locus of a gene [14].

The raw material of more than half of the world's fuel ethanol production is sucrose. It is obtained from sugar cane and sugar beet. In species with sugar content, the amount of sugar has not been increased for several decades. Genes have been cloned for bacterial isomerase enzymes that do not metabolize sucrose by plants but convert it into sugars digested by humans. It was thought that the sucrose isomerase (SI) expression pattern could also provide an important source of beneficial sugar and overcome the peak of sugar yield in plants. Addition of an SI gene adapted for vacuolar compartmentalization resulted in an increase in total stored sugar. Isomaltose, a sugar that accumulates in storage tissues without any reduction in the amount of stored sucrose, caused a doubling of the total sugar content in fruit juice [15].

The solution to increase the digestibility of lignocellulosic materials is to modify the materials to have modified lignin and cellulose content. Plants with less lignin and more cellulose content are more digestible by carbohydrates in bioreactors [16]. Increasing cellulase by 20% makes biofuel production economical. This can be done transgenically, by creating transformants with increased cellulosity with a more open of biodegradable nature using the CBD gene and using RNAi techniques to modulate lignin content. Silencing of lignin-causing phenylpropanoid pathway

enzymes encoded by all gene families, anti-detection using siRNAs matching the consensus sequence of the gene family, or other RNAi technologies can be applied. Decreased function of a single gene resulted in decreased expression of lignin, increased digestibility in sorghum, millet, maize, pine and poplar [13].

#### 4. Concluding remarks

The importance of renewable energy sources is increasing due to the depletion of fossil fuels and the damage they cause to the environment. Among the renewable energy sources, the use of bio-containing raw materials used in biomass-based energy production has been increasing recently. Plants, plant wastes and algae to be used in biofuel production are an alternative sources and nature friendly that do not harm human nutrition and the environment. Today, the use of these resources should be increased and they should be more sensitive to the environment. The process of removing lignin in bioethanol production from plants is long and problematic, but this problem can be solved by making various modifications on lignin genes or by changing external factors. In order to increase the oil content of oilseed plants used in biodiesel production, different types of genes should be studied. With the studies carried out, more biofuels can be obtained by using different methods. The use of plant wastes in biofuel production should be increased and the use of energy from waste should be expanded. Production of bioethanol and biodiesel using algae should be expanded because it is the best and latest technology. Therefore, they are systems that need to be worked on. We must use renewable energy sources to protect nature and provide the necessary energy. The limited availability of existing energy resources (fossil fuels) and the use of sustainable resources as an alternative to these resources sheds light on the future and nature.

#### Conflict of Interest

The authors declare no conflict of interest.

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