Chapter

Nature of Importance of Various Parameters for Ideal Biofuel Crops: Special Reference to Rapeseed Mustard

Vanya Bawa and Sunil Kumar Rai

Abstract

To increase the performance of diesel engine and environment, the utilization of biofuel as a major source of renewable energy is justified. It is well understood that agri-based biofuel is always also the choice in case of utilization as biofuel because of requirement of processing and threat to food security. Therefore, scope of improvement increases as it is yet to efficiently exploit as major full in the world. There are numerous factors that influence the efficiency of a fuel and its combustion. The physiochemical properties, namely viscosity, surface tension, flash point, latent heat of vaporization, oxidation, etc., allow the fuel to work efficiently during combustion. Thus, interests in biofuels have been increased, and various experimental studies have been developed for diesel engines consisting of methanol and methyl ester of rapeseed oil. In a relevant study, to achieve ideal biofuel, various biotechnological advances at the frontiers of plant science to dissect the underlying traits for identification of fatty acid profile useful for oil production and quality are essential, thereby ensuring food security. The plant-based fuel and its efficient utilization depend upon its oil quality and quantity, which thereby can be evaluated and enhanced by various conventional and nonconventional approaches of engineering and plant sciences.

Keywords: biofuel, combustion, fatty acid profile, physiochemical, biotechnological

1. Introduction

The development of efficient fuel and its sustainability is the major topic of concern in the time of climate change and food insecurity. The generation of efficient sources of fuel and fiber delineates the ways for the development of sustainable sources of fuel. Biodiesel is an alternative to petroleum-based fuels derived from a variety of feedstocks; including vegetable oils, animal fats, and waste cooking oil [1]. At present, biodiesel is mainly produced from conventionally grown edible oils such as soybean, rapeseed, sunflower, and palm. The cost of biodiesel is the main obstacle to commercialization of the product [2, 3]. Biodiesel produced from edible oils is currently not economically feasible. On the other hand, extensive use of edible oils for biodiesel production may lead to food crisis. The complexity is diverse in case of food and fuel crops, and raw material is derived from plant seed oils and animal fats and is a mixture of the alkyl esters of long-chain fatty acids, mainly produced by trans-esterification methodology [4]. The concentration of useful fatty acid is the basis of selection, and the utility of biodiesel can be increased by focusing on the traits related to direct production of oil and its quality.

Before focusing on the mitigation practices, other ways of dissection of traits can be done to improvise the genetic architecture of crop plants. The dissection of various properties relevant to biofuel development can be targeted. The physicochemical properties of biodiesel are very similar to those of petroleum diesel and, therefore, could be used as an alternative to diesel in conventional diesel engines without the need for any modifications [5]. In contrast to conventional fuels, other advantages of biodiesel include higher cetane number, flash point, and lubricity, absence of sulfur, and lower aromatics content compared with the petroleum diesel [6, 7]. The density, viscosity, cetane number, linolenic acid, methyl ester, polyunsaturated methyl esters, acid value, glycerides content come under the major parameters of physiochemical properties and are considered before making suitable mixture with commercial diesel [8, 9]. These parameters are of prime importance to study before subjecting oilseed crops to get biofuel. The quality and content of oil are also of basic and utmost important feature to observe and determination for fatty acid profile and important physiochemical properties [10]. Currently, the majority of the commercial production of biodiesel is dependent on oils derived from palm, soybean, and rapeseed by conventional methods. Changing the direction of biodiesel production from food crops to nonedible plants requires significant improvements in oil yield and quality of these plants as well as in their tolerance to biotic and abiotic stresses. As discussed throughout the present article, biotechnological interventions and genetic engineering approaches have shown great potentials to achieve these goals [11]. However, the application of such technologies in oil plants is at their starting points, and there is no commercial oil plant with enhanced oil content or composition yet; there are laboratory and pilot-scale samples though. Moreover, it is also necessary to thoroughly evaluate the potentials of genetic engineering technology in improving several other attributes of oil plants, i.e., environment adaptation, production cost, and economic feasibility in field scale [12]. Lack of the presence of superior genotypes as a base for genetic engineering especially for nonedible plants such as *Jatropha,* which is known as one of the most important to study but not implemented and proved as one of the drawbacks in pursuing this path [13]. Developing high-throughput tissue culture and transformation protocols for oil plants is also very important to produce a large number of primary GM lines. This is very critical for especially woody perennial trees. Most of the GM oil crops have been evaluated at laboratory or greenhouse levels, and it is not clear that their responses under field conditions are yet to be looked into [14]. It should be noted that genetic engineering methods might sometimes seem technically successful but would lead to commercial failures at large-scale production [15]. Identification and characterization of major genes involved in TAG biosynthesis pathway as well as in adaptation to biotic and abiotic stresses are also of grave importance [16]. In addition, it is necessary to carry out gene pyramiding programs to collect different agronomical traits in single superior genotypes of oil plants. This would help breeders to accelerate achieving superior genotypes suitable for commercial biodiesel production. Another challenge would be the biosafety issues related to GM oil plants, necessitating performing environmental and health risk assessments for such plants before commercial release. This would include evaluation of GM plants risks related to gene flow and potential negative effects on non-target organisms as well as the risks of potential negative effects on human and animal health.

2. Insight to physiochemical traits

The engine manufacturers are primarily focused on the efficiency of utilization of fuel. But while efficient biofuel is one priority area of research, the development of biofuel-efficient engines is another. Understanding the characteristics of biofuel leads to a vast field of study based on utilization, engine requirements, and efficiency. The criteria used to identify modifications in biofuel properties that either directly or indirectly affect fuel atomization and combustion define the biofuel's quality [17]. Key properties of efficient biofuel are its well to mix nature with air and ability to combust. The volatile nature of fuel is the major aspect of physical property. The volatile nature determines the Reid Vapor Pressure (RVP), measure of propensity of fuel. The ability of SI engine to startup when cold is another important requirement, at that time fuel vaporization signifies the utility and efficiency, as it determines the ease at which engine will start and blending percentage for good ignition. In the context of biofuels, ethanol has more latent heat of vaporization than gasoline fuel, requires more heat to get vaporized [18]. The higher electrical conductivity of biofuels restricts its 100% usage; hence, blending for good ignition is required, which opens up the scope of improvement in terms of ignition properties.

Commercial incorporation of biofuels as a major fuel is still critically overlooked as in case of diesel engines, fuel atomization process is having little time in combustion chamber when fuel is injected; the crucial step is determined by its important physical properties, namely fuel density, viscosity, fuel surface tension, and obviously the quality of biofuel occupies the position and creates huge scope of processing. The quality of the fatty acid profiles of biofuel or biodiesel fuels, with biodiesel having all higher values, significantly influences these physical properties. A sensor in the exhaust of modern engines can also detect changes in mixture, whereas biofuels with higher blends require more engine modifications. The chemical properties of biofuels are strongly affected by their different molecular structures; presence of oxygen is one of them. Whereas, resistance to self-ignition determines its higher octane number, shows it has resistance to knock [19].

The chemical properties of biofuel mixture are simply the sum of chemistries of each of the constituents, When consideration is taken in the context of modeling of biofuels in terms of molecular weight, the example can be used as methyl-butanoate, the approaches can be envisioned for answering the question of whether utilizing methyl-butanoate a model to study the kinetics of qualitative characteristics of long-chain fatty acids combustion. May be this can be used as a prerequisite to address the issues in modeling of ideal biofuel in order to mitigate the negative temperature coefficient behavior that causes ignition delay of biofuels [20]. The oxidative stability of biofuel is another important concern, with reference to canola/rapeseed derived biofuels, which contain unsaturated components in the oil. Presence of small amount of unsaturated fatty acids creates instability and is prone to photo oxidation, etc. [21]; hence, additives for oxidation stability are preferred in plant-derived fuels. Likely the concern is lowered when looking forward toward the oxygenated diesel fuels rather than the conventional ones; the presence of oxygen in biofuels is helpful in reducing the harmful emissions. Therefore, the oxidation of every individual part is critical in

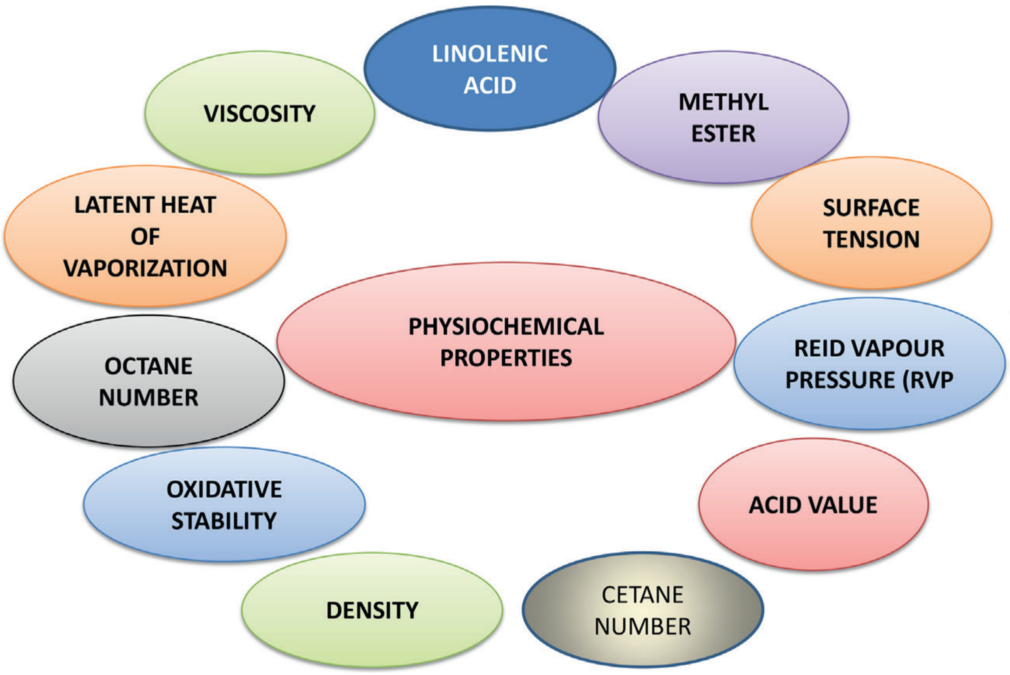

Figure 1.
Physiochemical parameters/factors affecting quality of biofuel.

the context of influencing the oxidation of other components, this result in the scope of determination of detailed chemical and kinetics study. The all physiochemical factors occupy the equivalent position when it comes to the production of efficient biofuel; the summary is shown in **Figure 1.**

3. Demand and area to address

The extent of biofuel production and its testing at various levels has increased in past few years as countries of the world are achieving in higher share in context of "green energy." The world is going toward sustainable options to minimize the global pollution and energy scarcity problem. The need of proper manipulation and effective utilization is required to for achievement of self-sufficiency, thereby working under national and international policies. To expedite the process of identification of diversity in fuels and the pattern in demand based upon quality of biofuel can describe the greater way to exploit specified crop plants for fulfilling global energy demands. The pattern of various results showed that consumption of plant-derived fuel has been increased since 2000 [22].

The history suggests that initially production of first generation biofuels was from agricultural raw material. The renewable source of biofuels includes vegetable oil, which is utilized for its production by transesterification method. Whereas the major input material for biodiesel production in EU is rapeseed mustard oil and accounts for 57–70% of biodiesel production [22]. The utilization of plant-based fuel brings the idea of HEAR oil and to utilize it as biodiesel for its characteristic properties with high number of carbon atoms (C22:1) and potentially to produce good amount of energy [23]. Studies reported that rapeseed mustard utilization for biofuel production has become more independent and is gradually converting into significant in terms of industrial use [24], claiming as per EU biofuel directive (2003/30/EC) implemented targets for biofuel. The total biofuel product using rapeseed as primary input for biofuel production for industrial purpose shares total production until 15–40%., whereas the rest of the produce was utilized as primary purpose, which includes food feed and postharvest processing. Zentková and Cvengrošová (2013) reported that the ever-increasing demand of biodiesel leads to an increase in production and acreage of rapeseed as an input for biodiesel production. This need to render the demands of global fuel can open the significant opportunities for farmers and allied sectors, the placement of their production to mitigate the farmers paradox of too high production and low demand. The production followed by better utilization will accelerate the biodiesel production via identified efficient methods.

It is to be noted that global food demand will change with the bifurcation of utilization of rapeseed mustard especially for the countries where it is major edible oil seed crop. Hence, methods to develop quality fatty acid profile with special reference to biodiesel production will develop the case of its production and consumption security. To achieve this by balancing the food demand and oil production for fuel manipulation and efficient usage of conventional and nonconventional approaches are required. However, to offer the better choice of healthy and acceptable food to the consumer a coordination of plant breeding, food processing and nutrition science is required, which can exponentially increase the specific traits in relation to oil quality suitable for biodiesel without altering the yield.

The focused quality production by rendering the modification of fatty acid composition of rapeseed oil is required and recommended. The production of rapeseed lines with good amount of lauric acid and myrestic acid identifies the one quality toward energy source. To address the check points in case of quality parameters plant pathways and various linked genes can be identified and triggered leading to the efficient carbon fixation and its conversion to lipids. Therefore, identification and understanding lipid biosynthesis pathways can delineate the ways to induce the relevant system in development of mutation breeding programs in plant breeding for the development of long-chain fatty acid rapeseed genotypes beyond current level [23, 25].

During the last decades, different plant breeding strategies have been used to improve oil yield and quality, and work has been done to improve tolerance in terms of biotic and abiotic stresses in edible and nonedible oil plants. New biotechnological tools such as marker-aided selection, next-generation sequencing, “omics” technologies, and genetic engineering have accelerated the breeding process for such traits in these kinds of plants. Identification and isolation of major genes involved in the lipid biosynthesis pathways using omics technologies and their transfer to edible and nonedible oil plants are expected to result in economical oil production in such plants as feedstock for biodiesel production [26]. Different genetic engineering strategies aimed at changing the structure of the enzymes as well as overexpression or silencing of the genes involved in the oil production pathway have been used to enhance oil yield and quality in nonedible oil plants. In this article, recent advances in the field of plant genetic engineering for improving oil production in biofuel crops and in particular in tow of the most promising nonedible plants, i.e., *Jatropha* and *Camelina* are reviewed and discussed [27].

4. Conventional strategies to enhance oil production in biofuel crops

To achieve commercial biodiesel production from nonedible oil plants, development of new varieties/hybrids of oil plants with high oil contents, tolerance to biotic and abiotic stresses, and no toxic proteins are a critical step. During the last decade, different breeding strategies have been used to improve these traits in crop plants of interest. The main activities pursued in plant breeding include developing variation, selection, evaluation for target traits, multiplication, and finally, release and distribution of new varieties [28]. Commonly, creation of genetic variation is performed through domestication, germplasm collection, introduction, intra- and inter-species hybridization, mutation, polyploidy, somaclonal variation, germaclonal variation, and genetic engineering [29, 30]. During the last decades, using conventional breeding strategies, different edible and nonedible oil plants have been improved to enhance oil quality, oil yield, and tolerance to biotic and abiotic stresses [31, 32]. In spite of such successful experiences, these methods face some disadvantages, such as being laborious and time-consuming, low accuracy in achieving desired traits, impossibility of inter-species hybridizations and wild crosses. The long methodologies and less precision created gap between identification of lines with potential to be the source of biofuels and the application for production of biodiesel.

5. Nonconventional tools and techniques

Recent advances in next-generation sequencing technologies (NGS) have encouraged rapid development of bioenergy crops. Different new "omics" technologies (genomics, transcriptomics, metabolomics, and metagenomics), marker-aided selection (MAS), and genetic engineering approaches have been considered and are widely used as the most promising solutions to improve agronomic traits in bioenergy oil plants. "Omics" technologies can accelerate and be used for the identification of novel and useful genes responsible for oil production, to discover oil production pathways including mapping of significant QTLs in the family [33] and also to improve oil content and composition [34]. The tremendous efforts in terms of genome analysis have been done in Crucifereae family [35]. To facilitate such efforts, whole genomes of some oil plants including soybean [36], rapeseed [37] sunflower [38], castor [39], Jatropha [40], oil palm [41, 42], Camelina [43] have been already sequenced. Such huge deal of data has opened a new high-throughput way to discover

Germplasm	Marker system	QTL identified	References
520	60 K SNP array		[44]
177	Sequence by genotyping	12	[45]
370	60 K SNP arrays	11	[46]
	37,721 filtered SNPs	37SNP	[46]
300 inbred lines	201,817 SNPs (SLAF sequencing by an Illumina HiseqTM 2500)	30 SNP	[47]
RILs(GH06 X P174)		40 QTLs	[48]
segregation population BC_1F_1	251 markers of RAPD, SSR, and SRAP.	19 QTLs	[49]
94 $F_{7–8}$ recombinant inbred lines (RILs)	143 (SRAPs) and 38 (SSRs)	4QTLs	[50]
DHs (Polo × Topas)		131 QTL	[51]

Table 1.
Nonconventional/biotechnological efforts.

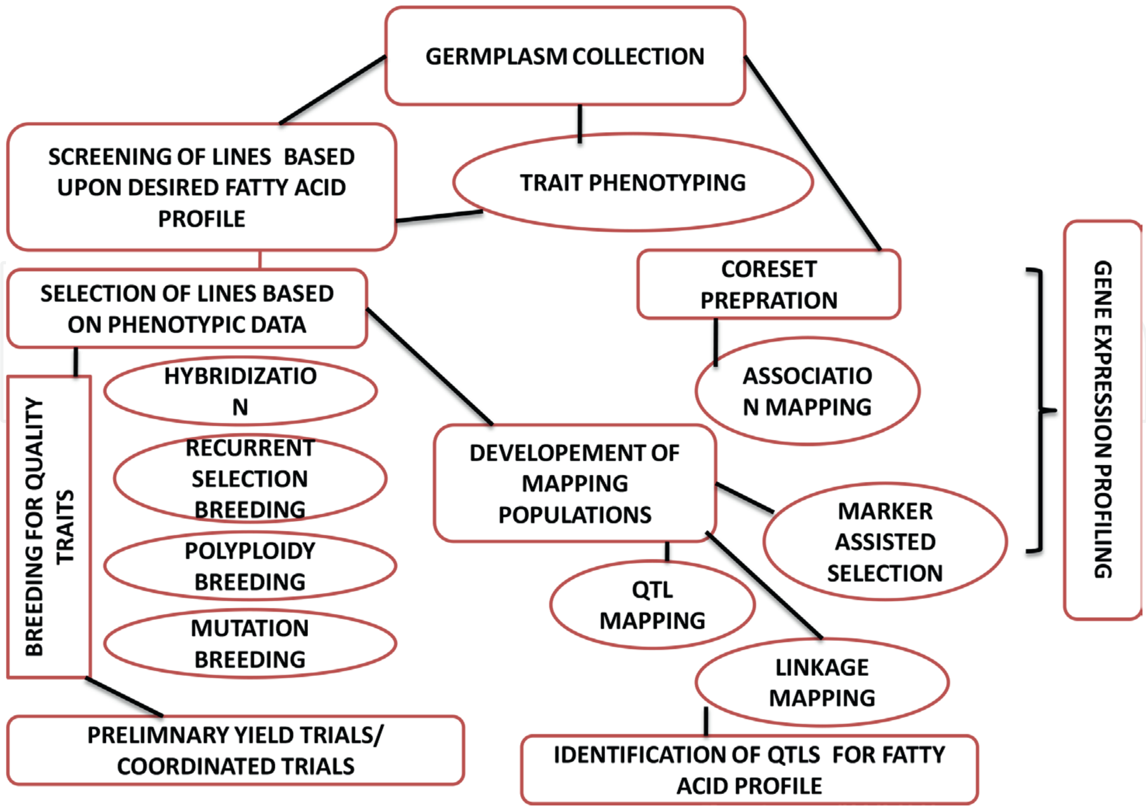

Figure 2.
Conventional and nonconventional approaches to improve fatty acid profile in oilseeds.

genes and pathways involved in oil production as well as the genes affecting oil quality in these plants. Moreover, by using omics technologies, such as quantitative trait locus (QTL) mapping, single-nucleotide polymorphism (SNP), and expressed sequence tag (EST)-based molecular markers, cDNA libraries, and RNA-seq analysis, researchers could identify and characterize genes involved in lipid synthesis pathways, tolerance to biotic and abiotic stresses, and the other important traits (Tang et al., 2017) [27], and similar few nonconventional efforts are summarized in **Table 1**.

This chapter creates the two paths that cross each other while selection and development of ideal biofuel crops in conventional and nonconventional ways. The strategies are not only based upon the extraction and utilization of oil but first going into the system of plants using genetic dissection methods that can delineate the mechanisms of gene responsible for the development of useful fatty acid profile. The triggers can be developed to identify useful genes, thereby understanding their expression profiling in **Figure 2**.

6. Conclusion

Utilization of biofuels has gained popularity as population is growing and need for the more diverse sources for fuel production. The plant-derived fuel still requires lot of efforts in terms of its modification to fit into the system of efficient utilization. The efficiency and usage can be increased by intensive study into the characteristic features of these plant-derived fuels with minimizing the threat to food security. The first aspect is to understand and evaluate the physiochemical nature taking petroleum based fuel as standard check. Other approach can be through understanding the mechanism of fatty acid formation and identification of potent gene sources to increase the fatty acid profile. Thereby molecular dissection is another suggested approach as it is believed to be successful in many crops. The omics technologies can accelerate the process to dissect the genetic features for required traits. Hence, comprehensive approach is required to mitigate the problem of underutilization of biofuels. The barrier can be reduced by focusing on evaluation of all properties of the oil and its maximum efficiency.

Author details

Vanya Bawa[1*] and Sunil Kumar Rai[2]

1 Central University Jammu, India

2 Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, India

*Address all correspondence to: vanyaadarsh@gmail.com

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