Genetic Improvement of Biofuel Plants: Recent Progress and Patents

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Abstract: Due to depleting reserves of fossil fuels, political uncertainties, increase in demand of energy needs and growing concerns of environmental effects, bioenergy as an alternative source of energy needs had taken centre stage globally. In this report, we review the progress made in lignocellulose, cellulose and fermentation based biofuels in addition to tree borne oil seeds. Algae as a source of feedstock for the biofuel has also been reviewed. Recent efforts in genome sequencing of biofuel crops and molecular breeding approaches have increased our understanding towards crop improvement of major feedstocks. Besides, patenting trends in bioenergy sector were assessed by patent landscape analysis. The results showed an increasing trend in published patents during the last decade which is maximum during 2011. A conceptual framework of "transgenesis in biofuels to industrial application" was developed based on the patent analytics viz., International Patent Classification (IPC) analysis and Theme Maps. A detailed claim analysis based on the conceptual framework assessed the patenting trends that provided an exhaustive dimension of the technology. The study emphasizes the current thrust in bioenergy sector by various public and private institutions to expedite the process of biofuel production.

INTRODUCTION

Bioenergy refers to energy produced from biological materials, specifically photosynthetic organisms such as green plants, grasses and algae. Interest in the biofuel as an alternative to fossil fuel triggered not only due to continuous rise in crude oil prices but also to ensure energy security by sustainable energy supply through biofuels and long term environmental care. Bioenergy is currently the only alternative energy source able to supply liquid transportation fuels. This can be achieved in many ways, i.e., plants can be used as a source of fermentable sugar for the production of ethanol which is called bio-ethanol. The fermentable sugars can be obtained from sweet sorghum, sugar cane, sugar beet or sweet potato generated via hydrolysis of starch or cellulose and hemi-cellulose present in the plant cell wall. Ethanol derived from such process is called cellulosic ethanol. Lignocellulosic biofuels use all of the plant material instead of just the starch or sugar. Lignocellulose and lignin, a phenolic polymer, is the most abundant biomass. Residue products from agriculture, such as straw, corn stover could be used as feedstocks. Certain plant species are grown solely for the production of lignocellulosic biofuels (e.g willow, poplar, switch grass and miscanthus). Globally, plants produce an estimated 200 billion tons of biomass per year [1] in the form of sugars, polysaccharides, oils and other biopolymers representing unprecedented resources for biofuel production.

Further, lipids derived from algae and oil crops (e.g., canola, soybean, castor, sunflower, oil palm and *Jatropha*) and processed spent oil can be used as feed stock for the production of biodiesel. The processing of oils and lipids to biodiesel involves trans-esterification using a base catalyst and alcohol (methanol or ethanol). In commercial processes, methoxide (CH_3O^-) generated by dissolving sodium hydroxide or potassium hydroxide in methanol is used as base catalyst. This process results in the formation of glycerol, and methyl esters of fatty acids that can be used as a substitute for or additive to petroleum-based diesel fuel.

An alternative method for the production of liquid fuels is through the use of syngas obtained from gasifying plant biomass, resulting in a mixture of Hydrogen, Carbon Monoxide (CO), Carbon Dioxide, water and light hydrocarbons. Various low-molecular weight compounds, including methanol and ethanol, can be produced from syngas with the use of chemical catalysts.

Direct Biomass Combustion

Among the renewable resources, biomass occupies prime position and provides @ 10-15% of the worlds primary energy. While the developing countries utilize biomass for heating, cooking and power generation, it is in the industrialized nations that the energy from biomass has replaced the fossil fuels. Biomass is described as all non-fossil organic materials which are the direct and indirect products of photo-

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synthetic materials that have an intrinsic chemical energy content. The feedstocks are broadly classified by source (plant or animal) and physical state (solid, liquid or gas) and include fuelwood, forestry and agricultural residues, animal manures, industrial wastes and municipal solid wastes (www.bera1.org). Biomass is converted to energy through the technologies such as gasification, pyrolysis, advanced combustion, anaerobic digestion, biodiesel, and cellulose to ethanol conversion. The challenges in commercialization of biofuel from biomass include maximization of crop yield, biofuel standards allowing marketing and quality control, efficient means of biomass harvest and densification, methods of long term biomass storage that maintains its fuel quality and business structure for biomass supply chain. Technological developments in biomass are rapidly advancing and new methods to convert biomass into energy, fuel or chemicals are developed. During the past decade there is a modest increase in biomass patents being issued and intellectual property protection primarily covers the processes, machines and composition of biobased compounds. About 70% of these technologies are related to processes for production of fuels and chemicals from biomass while the rest include products from biomass, the devices and the systems.

Biomethane

Biomethane (Biogas) is a product of anaerobic degradation of diverse organic substrates such as manure from farm animals, fat from slaughter waste, used frying oil, organic household and garden waste, food-processing wastes, industrial wastes and municipal refuse. In addition to these, energy crops like grass, poplar, maize and willow are also used as feedstocks for biogas production. Biomethane is generally produced in small-scale industrial units and the applications include production of electrical power or heat and use in combustion engines. The solid and liquid effluents are used as fertilizer for soil amendment. Despite the significant potential of biogas by utilization of organic wastes, production of biomethane has its own limitations. Production is technically feasible but is uneconomical without incentives. Commodity costs also vary widely depending on the feedstock and the technology employed. Intellectual property protection covers the types of anaerobic digesters, the methanization process involving breakdown of complex organic material in different stages (hydrolysis, acetogenesis, methanogenesis) to biogas, methods for collecting and distributing biomethane and the storage containers.

Extremophiles

Extremophiles are a group of organisms/crops that do not compete with traditional crops for arable land and other resources. Extremophile energy crops (EECs) are wild species adapted to harsh environments of drought, soil salinity, extreme temperatures, nutritionally poor habitats and produce stable enzymes that are often capable of tolerating extreme environmental conditions distinguishing them from traditional crops. EECs are less susceptible to stresses in the changing global environment and provide higher yields than existing crops [2]. Some of the potential EECs currently promoted as biofuel raw materials include poplar, miscanthus, switch grass, agaves, elephant grass, etc. Research on

the "extreme enzymes" isolated from extremophiles is gaining attention as they function under extreme conditions of pH and temperature. This property of the enzymes from extremophiles is harnessed for bioprocessing of lignocellulosic biomass into ethanol. Genetic engineering, enzyme engineering, computational techniques and genome sequencing of the potential extremophiles and EECs are underway for effective use of these class of organisms for biofuel production.

PLANT OIL METABOLISM

Triacylglycerols (TAG) serve as an excellent source for generation of biodiesel due to their similarity to fossil fuels. Current biofuel crops, such as soybean, canola, *Jatropha*, *Pongamia* have either low or unpredictable oil yields. Thus, increasing oil content in plants is needed to achieve optimum biodiesel production.

Increase in oil content in seeds has been achieved by manipulation of the expression levels of enzymes involved in synthesis of TAG. Overexpression of fungal DGAT2 (diacyl glycerol acyl transferase) in soybean seeds resulted in 1.5% increase in oil content [3]. Similar results were observed by expression of DGAT in *Arabidopsis* [4]. Overexpression of two soybean transcription factors in *Arabidopsis Dof4* and *Dof1* increased total fatty acid and lipid content [5].

Altering the carbon flux to TAG biosynthesis by affecting the supply of glycerol-3-phosphate (*gpd*), which is another TAG substrate in addition to fatty acids in the endoplasmic reticulum, is an alternate approach for increasing the oil content. Over expression of yeast *gpd-1* gene in canola seeds increased the lipid content by 40% [6].

Another approach for reducing the levels of saturated fatty acid palmitate is by down regulation of *FATB*, an acyl-ACP thioesterase, causing accumulation of oleic acid up to 85% from 18% in the wild type [7]. Jatropha cDNA encoding a putative *FATB* homolog, *JcFATB1*, has been cloned [8]. Down regulation of *JcFATB1* in transgenic Jatropha can be attempted to increase levels of oleic acid in seeds.

LIGNOCELLULOSIC BIOMASS

One of the major limitations to efficient lignocellulosic biofuels is the recalcitrant nature of complex polysaccharides and lignins. Recalcitrant nature is mainly due to heterogeneity and molecular structure of lignocellulose, where cellulose is arranged into a network of tight, intra-chain hydrogen bonds that form a crystalline core of microfibrils, embedded in a matrix of hemicellulose polysaccharides that are covalently linked to lignin. Therefore, high complex nature of lignocellulose requires prohibitively expensive and harsh pre-treatments to gain access to monosaccharides. Heterologous expression of cell wall deconstructing enzymes such as cellulases, hemicellulases and ligninases is an important research area in exploitation of plants for biofuel purpose.

Extensive studies on lignocellulose quality in the forage and pulping industry have identified lignin to possess a major effect in cell wall recalcitrance [9-10]. A recent study in transgenic alfalfa achieved a significant improvement in fermentable sugar release from lignocellulose by down regulating monolignol biosynthetic enzymes. Alfalfa lines silenced for cinnamate-4-hydroxylase (C4H), hydroxyl

cinnamoyl CoA shikimate hydroxycinnamoyl transferase (HCT) and coumaroyl shikimate 3-hydroxylase (C3H) had lower lignin amounts [11]. Another approach is to target the down regulation of enzymes at later stages of monolignol biosynthesis, such as cinnamoyl CoA reductase (CCR) and cinnamoyl alcohol dehydrogenase (CAD). Targeting CCR and CAD enzymes led to generation of alfalfa lines with improvements in enzymatic saccharification efficiency up to 60% and 40%, respectively. However, extensive metabolomic and transcriptomic analyses have shown that silencing CCR and CAD effected several other biochemical pathways in tobacco and poplar than just lignin biosynthesis [12,13] suggesting that additional genes can be targeted for more efficient pathway engineering. A candidate CCR gene has been recently identified in switch grass [14] that could serve as target for pathway engineering.

Lignin engineering approaches are currently being pursued at the Joint Bioenergy Institute, USA to replace monolignols with compounds containing easily cleavable chemical linkages such as ester and amide bonds [15]. Monolignol replacement strategies could avoid the undesirable developmental and structural phenotypes associated with the down regulation of lignin biosynthetic enzymes in transgenic plants.

The contribution of hemicellulose to plant cell wall recalcitrance is a much less well understood phenomenon. Reduction of xyloglucan in poplar expressing an *Aspergillus* xyloglucanase improved the saccharification of wood, probably by making cellulose more accessible to enzymatic hydrolysis [16]. In poplar, silencing of *PoGT47C*, a glycosyltransferase homologous to *Arabidopsis* FRA8 and involved in glucuronoxylan synthesis, caused an increase in glucose yield following enzymatic hydrolysis, indicating that reducing xylan content leads to improved saccharification efficiency [17].

Targeting cellulose biosynthesis is an alternative strategy for lignocellulose improvement. Cellulose synthase complexes and other proteins assisting in the process have been identified in *Arabidopsis* and other higher plants [18]. The discovery of a transcriptional regulation mechanism via small RNAs (SiRNAs) showed that cellulose synthase and several hemicellulosic biosynthetic genes are co-ordinately down regulated during leaf development [19].

Methods for genetic transformation using *Agrobacterium* have been developed for switch grass, *Jatropha*, *Poplar* and *Brachypodium* [20-24] paving the way for genetic engineering approaches to crop improvement. Successful engineering of a functional metabolic pathway for the production of polyhydroxy butyrate (PHB) in transgenic switch grass has been reported. A protocol for generating switch grass protoplasts for use in transient gene expression experiments has been recently published [25]. Tissue culture techniques for the propagation of *Miscanthus*, *Jatropha* and castor have been developed [22, 26-28]. The draft genome sequences of *Brachypodium*, poplar, sorghum, *Jatropha*, *Ricinus* and maize have been published [29-34]. Like wise chloroplast genome of date palm and *J. curcas* are sequenced [35, 36]. These resources will be instrumental in developing the tools for functional genomic and proteomic assays and will allow comparative genomic approaches between model species and biofuel crops to become a reality.

ALGAE BASED BIOFUELS

Micro algae are a large and diverse group of photosynthetic eukaryotes with a simple cellular structure ranging from unicellular to multicellular forms. Microalgae require only water, atmospheric CO_2 and sunlight that are available freely in nature. As to have a promising partnership between microalgae and biofuels requiring a multidisciplinary approach, Malcata [37] outlined such an approach encompassing fundamental biology, systems biology, metabolic modelling, strain development, bioprocess engineering, scale-up, biorefineries, integrated production chain and whole system design. Among the different groups of fresh water algae, *Chlamydomonas, Chlorella, Scenedesmus, Dunaliella* (green algae), *Spirulina* (Blue-green alga), *Porphyridium* (Red alga) and marine water algae like *Isochyrsis, Nannochloris, Nannochlopsis, Neochloris, Nitzschia, Phaeodactylum* are the commonly used microalgal species as source for biofuel production. Micro algae yield more oil per hectare than some traditional biofuel feedstocks. For example, microalgae containing 30% oil by weight of dry biomass could yield almost 587,000 litres per hectare, far in excess of what can be generated from rape seed or soybean grown in the same area [38, 39]. In species such as *Botryococcus braunii* and *Schizochytrium* spp. oil content can go up to 70% [40]. Biodiesel productivity could reach 121,104 Kg/ha per year using algal species with 70% oil content as feed stock. According to a recent estimate, oil from algae on 20-30 million acres of marginal land would replace the entire US supply of imported oil [41] leaving 450 million acres of fertile soil in the country entirely for food production.

Microalgal oils are mostly composed of four unsaturated fatty acids, namely palmitoleic acid (16:1), oleic (18:1), linoleic (18:2) and linolenic acid (18:3). Saturated fatty acids such as palmitic (16:0) and stearic (18:0) acids are also present in a small proportion [42]. Composition of fatty acid influences the quality of biodiesel produced. To obtain the best microalgae strains that possess high oil content, high growth rate and broad environmental tolerance for biodiesel production, much of industry's previous work has focused on species selection and cultivation techniques. Unexpectedly, genetic modification of microalgae has received little attention. However, with the development of molecular biology methods, now it is possible to increase microalgal oil yield by genetic or metabolic engineering.

Algae production requires favourable climate, available water depending on the algae type, a ready and essentially aseptic source of CO_2 and plastic liners. Currently commercial algae production is confined to small scale production which is inefficient. The issues concerning large scale cultivation systems include the prohibitively expensive infrastructure (bioreactors) for algal culture, its processing, waste treatment, water supply, other support systems required and operating costs. The limitations with regard to microalgae based biofuel production include the relatively low levels of productivity, low oil content, low photosynthetic efficiency in mass culture, stability, easy harvesting, oil extraction, susceptibility to algal contaminations and viral infections,

evaporation in open ponds, vulnerability to temperature and other environmental factors. Biodiesel produced from algae has many unsaturates and hence, is unstable and compares poorly to its alternatives.

Genetic engineering can be used to increase photosynthetic efficiency and thus higher microalgal biomass yield. Since photosynthesis drives the first stage of biodiesel production, any increase in photosynthetic efficiency will benefit the downstream production [43]. In the interest of engineering microalgae strain to effectively capture light energy, research attempts are focused on reducing the number of light-harvesting antenna complexes (LHC) which capture sunlight and transfer the derived energy to photosystem (PS) and PSα to drive the photosynthetic reaction. For example, Mussung *et al* [44] reported the successful down regulation of LHCs in *Chlamydomonas reinhardtii* for improved photosynthetic efficiency and light penetration. The resulting LHC mutant offers more efficient conversion of solar energy to biomass.

Genetic engineering can be used to increase microalgal biosynthesis of oil by regulating the activity of the enzymes involved in lipid production. The committed step in fatty acid synthesis is the conversion of acetyl CoA to malonyl CoA catalyszd by ACCase. Further, TAG biosynthesis in microalgae has been proposed to occur via direct glycerol pathway. Fatty acids produced in the chloroplast are sequentially attached to glycerol backbone, which are catalyszd by cytosolic glycerol-3-phosphate acyl transferase, lysophosphatidic acid acyl transfease (LPAT), phosphatidic acid phosphatase and DGAT, respectively [45]. Researchers have amply demonstrated that over-expression of LPAT and DGAT gene led to increased seed oils in higher plants [46]. However, very few successful results have been reported in enhancing the oil content from microalgae.

Omics technologies such as genomics, transcriptomics, proteomics and metabolomics are unraveling metabolic pathway regulation and are providing targets to optimize biofuel production [43, 47]. The *C. reinhardtii* genome sequence [48] revealed several unexpected pathways that are involved in fundamental metabolic processes such as inorganic carbon fixation, fermentation, selenoprotein expression and vitamin biosynthesis [49]. Application of 454 pyrosequencing platform [50] resulted in the first discovery of non-coding RNAs in *C. reinhardtii* and led to first report from any single-celled eukaryote of miRNAs [51]. Since few algal genomes have been sequenced, a concerted effort from the research community must be initiated to sequence potential strains for biofuel applications. The intellectual property protection in algae production is for the biorefineries, process of lipid extraction from microalgae, methods of harvesting, dewatering and drying of algae and the genes for enhancing the photosysnthetic efficiency.

PATENT LANDSCAPE

Against the set background of different types of feedstocks and the technological innovations for their potential for efficient conversion to biofuels, emerging technologies like 'transgenic for biofuels' generally have a potential for immediate commercial application. In an earlier study done in 2008 [52] as many as 2,796 biofuel related patents have been known to be published in 6 year period with 150 per cent increase in last two years of the study. Hence using standarised tools of patent analytics, a conceptualized framework was prepared with emphasis on the genetic engineering aspects. Patent analysis provides a platform to assess the current status and trends in technology development. Data mining from a set of relevant patent documents can give the entire dimension of the technology. In the present analysis, patents related to transgenic technology for biofuel production were retrieved from Thomson Innovation Database [53] using a set of subject specific keywords and standardized search strings. One thousand two hundred fifty one (1251) patent records were retrieved and after following the usual methods of filtering out the irrelevant patents and duplicates, a final set of 1243 patents were used for analysis.

Basic bibliographic analysis through patent timelines and geographical distribution; technology trend analysis through Patent analytics viz., IPC category composition, Theme Maps and claim analysis were used to get the technology landscape of relevant set of patents.

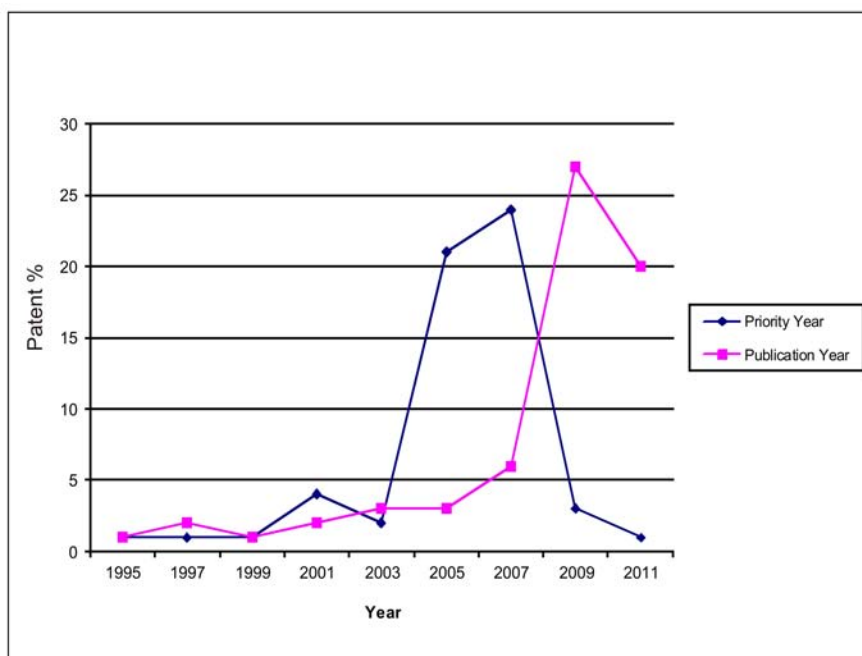## BASIC BIBLIOGRAPHIC ANALYSIS

Patent Timeline Analysis

Trends in inventive activities over a span of 15 years were gauged from reference dates viz., priority date[1] and publication year[2]. Reference dates reflect timing of invention, process and strategy of the applicant [54]. Beginning of the transgenic era did not show much patenting activity, however, there was a drastic increase in filing for a patent from 2006 and publication of the same from 2007 onwards with a maximum number of published patents in 2011 (Fig. **1**). Though the trend is more or less similar for both the reference dates, patent publishing dates always followed priority dates. This is similar to the Transgenic Bt cotton patenting trends [54] which inferred that the difference may be due to the fact that patent offices need to examine filed applications in conjunction with relevant national laws and thus the time taken for publication differs in each country.
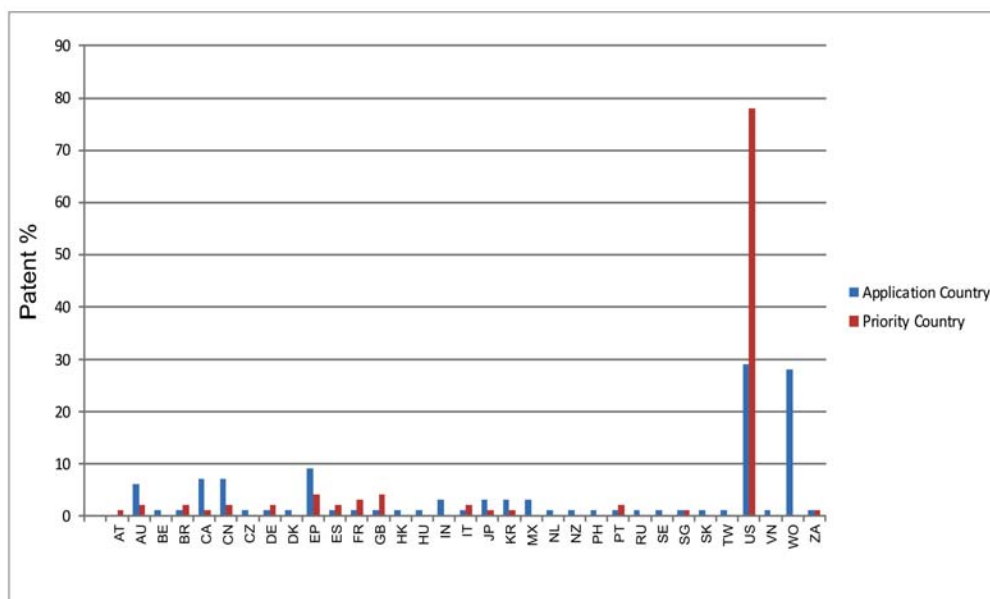
Geographical Scope of Patent Protection

As geographical scope of patent protection reflects market coverage of an invention, patents were analyzed for application country and priority country (Fig. **2**). Application country is the country from where an application was made and where patent protection has to be sought first forms the priority country. US topped both for application and priority country and is also the most preferred country even by applicants from non-US countries indicating presence of market for industrial application of biofuels. Analysis based on location of assignees and inventors indicates comparative strengths of countries. US, Australia, Brazil and Mexico are among the top countries by assignee. The presence of 3% patents from a developing country like India indicates initiation of research interests in this area.

Fig. (1). Patent Timeline Analysis (n=1243). Based on application date and priority date. Priority date/year is the first date of filing of application anywhere in the world and considered closest to the invention date. Publication date/year reflects the time of information disclosure to the public from statutory offices.

Fig. (2). Geographical scope of patent protection (n=1243). Based on application country and priority country.

TECHNOLOGY TREND ANALYSIS: PATENT ANALYTICS

IPC Analysis

As it is known that International Patent Classification (IPC) Codes for each patent categorize the patents concerned into technology fields, a technology assessment was made based on the distribution pattern of patents in IPC classes. Distribution of patents in top IPC classes presented in Table **1** clearly indicates the relevant technology used in transgenic crops for biofuel production. Transgenic technology is basically a recombinant DNA technology (C12N 15/09) involving introduction of foreign genetic material (A01H 1/00) for alteration of fatty acid or oil content (C12N 15/82) in crop plants (C12N 15/82).

Patent Landscape Visualization: Aureka Themescape Maps

Aureka Themescape IP analysis platform helps in visualizing trends and relationships within the patent landscape. Language of the title, abstract and claims of the patents were used to generate a ThemeMap with a systematic removal of

Table 1.   Distribution of Patents in top 10 IPC Classes.

IPC Code	Description	Patents (%)
A01H 5/00	Flowering plants, i.e. angiosperms	31
C12N 15/82	For plant cells	27
C12P 7/64	Fats' Fatty oils; Ester-type waxes; Higher fatty acids, i.e. having at least seven carbon atoms in an unbroken chain bound to a carboxyl group; Oxidized oils or fats	13
C12N 1/21	Modified by introduction of foreign genetic material	11
A01H 1/00	Processes for modifying genotypes (AO1H 4/00 takes precedence)	8
C12N 5/10	Cells modified by introduction of foreign genetic material, e.g. Virus-transformed cells	8
A01H 5/10	Seeds	8
C12N 15/09	Recombinant DNA technology	7
C12N 5/04	Plant cells or tissues	7
C10L 1/02	Essentially based on components consisting of Carbon, Hydrogen and Oxygen only	6

generic "stop words", e.g., "reacting", "designing", "providing" etc. and progressive refinement (8 cycles of analysis). Eliminating stop-words in generating ThemeMap can reduce noise and improve precision [55].

The most patented concepts (as indicated by the contour peaks) are related to the cultivar (cultivar, cultivar lines, plants produced by crossing), genes, nucleic acids and enzymes (phospholipase, lipase, lignocellulase) and fatty acid derivatives reflecting the most active areas of innovation (Fig. **3a**). Further the ThemeMaps were analyzed in specific areas for enzymatic methods (Fig. **3b**), biomass utilization (increasing plant yield, biomass, biomass utilization) (Fig. **3c**), and biofuel (Fig. **3d,e**). Red dots in the ThemeMap indicate active areas of innovation in respective themes. Specific keywords were identified based on the initial clustering in ThemeMap and used for manual claim analysis (Tables **2** and **3**).

As claims are the heart of the patent document, language in the claims determine the hold on the technology and thus Themscape Map based on claims would provide an initial overview of patent rights and possible overlaps (Fig. **3**). However, further a detailed analysis of the patent claims was conducted to determine breadth of patent rights (Fig. **4**; Tables **2** and **3**).

Transgenesis and Biofuels: Transgene Source to Industrial Application

Unlike published research articles, in a patent document title and abstract do not give a crisp understanding of the technology involved and often mislead researchers. Aureka Themescape Maps only provided an overview of the technology involved and a detailed assessment of whole text patent documents with a specific focus on technology description, background of the invention and claims was made to get an exhaustive view of the transgenic technology involved in biofuel production keeping in view the general method of transgenic development.

The information from all the methods of analytics employed were then conceptualized into a framework (Fig. **4**). This framework consists of 3 basic sections: Section A- or transgene and its source; Section, B- process of feedstock conversion; and Section C- the product (Fig. **4**).

Section A-Transgene(s) and Its Source (s)

The fundamental step in any transgenic development is the identification of transgene (s) hitherto a transgene may be a native gene modified or from any living organisms crossing the species barrier that will have a multiplier effect on the ultimate performance of a transgenic crop. From the set of patents analyzed, clear indications that the current research is being directed for locating transgene(s) in a variety of bioresources consisting of 3 major groups viz., microorganisms, grain crops and any other bioresources. FATB, acyl-ACP thioesterases and glucanases) are extracted from microorganisms like *Acetobacter sps.* (US20110162259A1) [56], *Psuedomonas aeruginos, Saccharomyces cervesiae* (US20100257778A1) [57], *Aspergillus niger* (US20090253169A1) [58]. Rice (*Oryza sativa*) and Maize (*Zea mays*) are the two grain crops identified as source of transgene(s). HSC71 gene was isolated from *Oryza sativa* (US20080254989A1) [59] and genes for Transcription factor Nuclear factor B (NBF2) gene, Transcription factor NST gene, Fructose 1, 6 biphosphate aldolase, Pyruvate orthophosphate dikinase and Ribulose biphophate carboxylase activase were obtained from *Zea mays* (US7629455B2) [60]. Genes for enzymes like lipase, phospholipase (US7977080B2) [61] and lipid transferase protein (LTP) were identified from other bio resources like *Pinus taeda* (Gymnosperm), mammalian cells, plant cells, yeast and fungi (US20090178163A1) [62], (US20070130644A1) [63]. Much emphasis seems to be focused on maize as a source of transgene(s). It has been well documented that the maize system serves as a potential source of gene(s) which can be further used in the development of transgenics. The results clearly indicate the application of such gene(s) for transgenics in the biofuels as well.
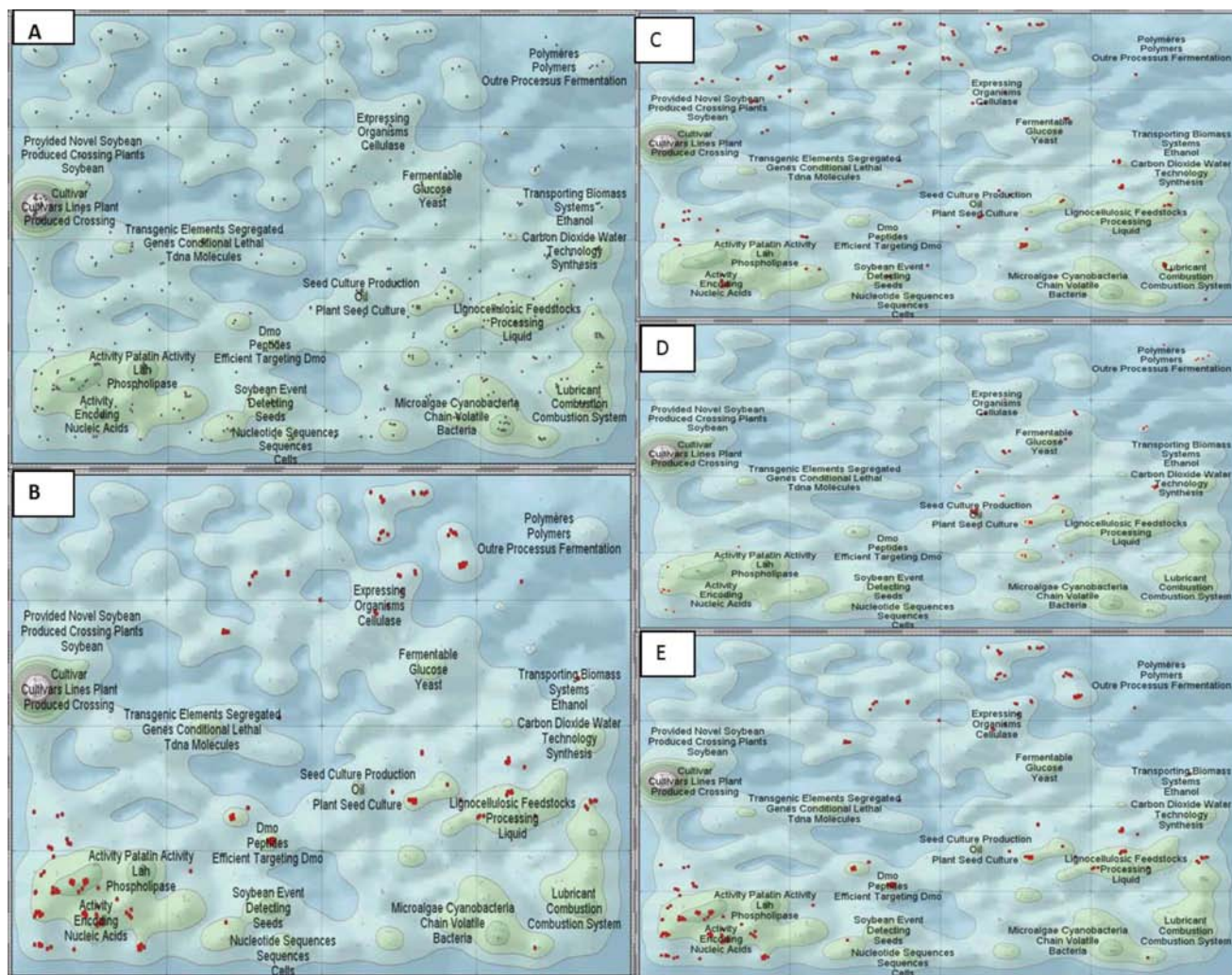
Fig. (3). Theme Maps for visualization of trends and relationships within the patent landscape (n=1243); **A**. General Theme Map; **B**. Enzymatic Methods; **C**. Biomass; **D**. Biodiesel and **E**. Bioethanol.

### Section B-Transgenic Substrate and Its Feedstock Components

The next level in the framework was defined as type of transgenics and their substrates that form the feedstock for biofuel conversion process wherein the transgene(s) identified from the potential donor species (Section A) are deployed in transgenic biofuel development. The analysis of the set of patents indicated primarily focus of research on transgenic development in 4 major groups ranging from transgenic microorganisms to transgenic perennials and their waste. The substrate emanating from such transgenic material provides a vast range of feedstock components for biofuel conversion. The feedstock component emerging from the identified transgenics and their substrates are classified into 3 major groups. These are Oils and their components, cellulosic material and biomass. Oil is obtained from transgenic oil crops like soybean, sunflower, palm, coconut and jatropha, cellulosic material from sugar or grain crops like sweet sorghum, sugar beet, sugar cane, potato, maize, barley, rice, wheat and perennials like alfalfa, plum, switch grass,

miscanthus, ryegrass, ginger and microorganisms like microalga, bacteria, cyanobacteria, fungi and yeast. Irrespective of the transgenic crop category, biomass is the universal feedstock component.

Section C-Process and Products

This section accentuates the processes leading to the various products through the use of transgene(s) in the transgenic substrate. The 4 processes viz., trans-esterification, fermentation, catalytic conversion and gasification were found to be the focus of current work that lead to the production of different types of bioenergy products like biodiesel, bioethanol, biobutanol, dimethyl furon, hydrocarbons and syngas.

Some of the possible applications found to be enumerated from this set of patents include use of bioethanol for reduction of harmful effects in the environment like biodiesel for sensor techniques and hydrocarbons for enhancing the life span of injectors [64]. While it is contended that this may not be a holistic picture, the analysis conveys an emerging
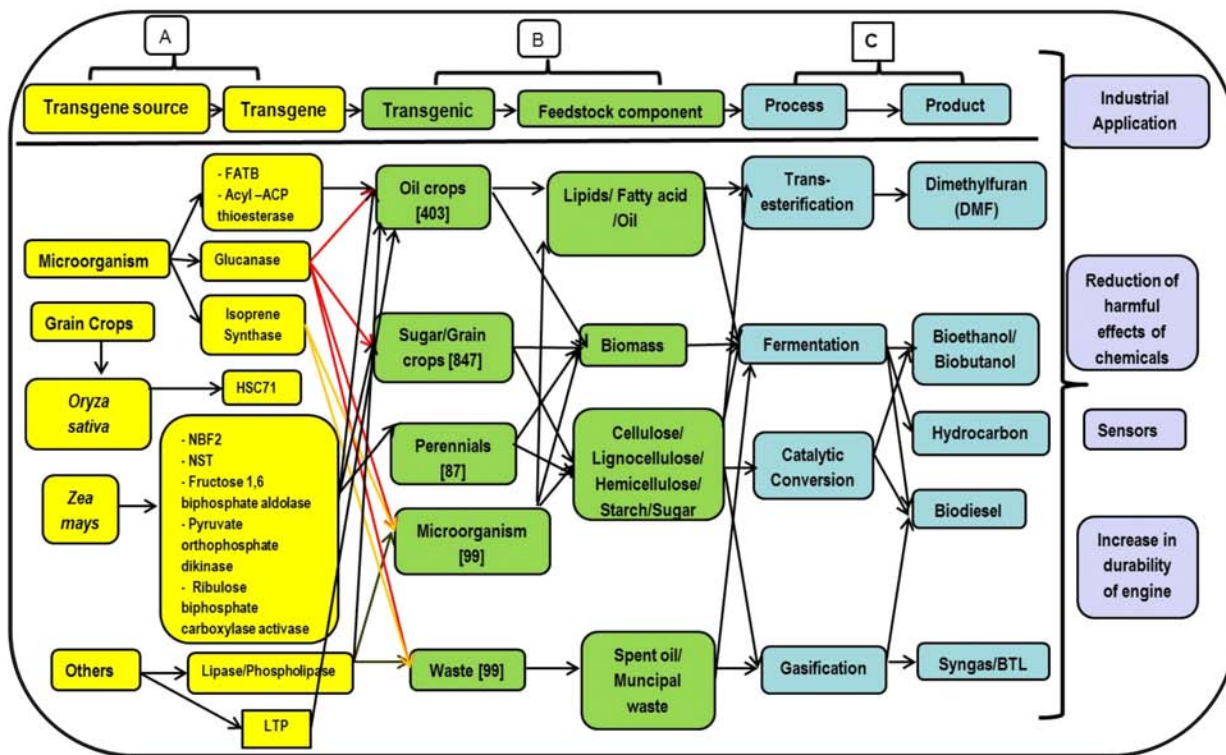
Fig. (4). Conceptual Framework for integration of Transgenesis and Biofuels. The figure is based on secondary data and information collected from a set of 1243 patents. Numbers in parenthesis indicate total number of applied/granted patents from the analyzed patent set.

indication on the type of transgenics, their substrates and processes being currently researched for possible application for biofuel production.

Claim Analysis

A detailed analysis of claims of patents was done with an objective to categorize the entire set of patents on basis of the framework (Fig. **4**). Patents were grouped into 7 types with possible overlaps in claims (Table **2**). Maximum claims were for source of biofuel (Group 3) followed by feedstock component (Group 4). Focus was more on enzymatic compositions or fatty acids and their derivatives and their methods of preparation and processing. Among the patents on whole organism, oil crops and more specifically soybean hybrids or varieties or inbred lines or cultivars top the list (Table **3**). Even though transgenic research in biofuel plants is still in its infancy, several patents showed claims. The objective of the present study is to review the current developments in each of these areas and then discuss the research centring on genetic improvement of these resources. Using patent analytics as a tool, it then attempts to assess the current trends in the technology and commercial application of transgenic biofuel.

Out of set of 1243 patents, 27% patents were for whole organisms covering major biofuel crops including microorganisms (Fig. **4**; Table **3**). Both plant species and different microorganisms used as biofuel source were found to be part of patent documents. It is known fact that while most jurisdictions afford patents to microorganisms if found patentable, plant patents are allowed only by few countries.

CURRENT AND FUTURE DEVELOPMENTS

The paper brings forth current emphasis of research in area of alternate energy sources and use of emerging tools like biotechnology and molecular genetics in the biofuel crops. While the three major sources of biofuels viz., sugar or grain crops, oil crops and microorganisms are widely considered as first, second and third generation biofuels, respectively, the study indicates the gradual shift from sugar or grain crops to microorganisms along with intersections in oil sector as source of biofuel. Food security has been an issue of concern especially in the developing countries when there is a huge dependency on food crops and any use of these crops for non-food uses threatens the models for more food production. The results indicate a more clear research emphasis on exploring non-food bioresources for biofuel production. This shift from use of food crops to non-food organisms for alternative uses as biofuel provides a definite relief to the apprehension of diversification of food crops for non food uses. Research initiatives at plant genetic engineering for biofuel production is still in its infancy but the rise in the number of published patents during the past 2-3 years is indicative of the growing interest in biofuel research. This trend of accelerated rate of patenting on the research output in these areas sends new and alarming signals of ownership issues on a wide array of bio-resources. Such overly broad patents in this area can bring up issues of access to this large pool of bio-resources and biodiversity.

Table 2.   Claim Analysis.

Group	Claim		% Patents
1	Whole organism	Plant	13.19
		Microorganism	13.91
2	Methods and composition *	Enzymes	19.47
		Fatty acid derivatives	5.55
		Methods	26.71
		Processing	1.21
3	Biofuel source	Oil crops	40.3
		Sugar/Grain crops	84.7
		Perennial crops	8.7
		Microalgae	9.9
		Waste	9.9
4	Feedstock component	Biomass	16.3
		Oils/Fatty acid/Lipids	69.9
		Cellulose/ Lignocellulose/ Hemicellulose	20.4
		Sugar/Starch	39.3
5	Process of biofuel production	Trans-esterification	2.4
		Fermentation	11.1
		Catalysis	6.5
		Gasification	0.3
6	Type of biofuel	Biodiesel	11.9
		Bioethanol/Biobutanol/hydrocarbon	33.3
		DMF	0.3
		Syngas/BTL	0.8
		GTL	0.6
		Isoprene hydrocarbons	8.6
7	Industrial utility	Sensors	0.88
		Reducing the deleterious effects of manganese	4.67
		Enhancing the performance of injector	0.64

* Claims for methods and composition include claims for gene/part of the genome / nucleotide sequence / protein / enzyme preparation and their respective compositions.

Table 3.   Patents for Bio Resources.

Patented Organism		Category	Major Country	% Patents	
Soybean	Oil crop	Plant	US	26.71	32.64
Sunflower	Oil crop		US	3.56	
Canola	Oil crop		US	2.37	
Sorghum	Sugar/Grain crop		US	1.19	8.31

Patented Organism		Category	Major Country	% Patents	
Sugar beet	Sugar crop		US	4.75	
Rice	Grain crop		US	2.37	
Tobacco	Commercial crop		US	2.67	2.67
Switch grass	Grass	Tree	US	0.59	0.59
Perennials	Perennial		US	1.78	1.78
Any other plant/Tree	Plant/Tree	Plant/Tree	US	2.67	2.67
Yeast	Micro organism		US, EP, WO, CA, KRMX, AU	8.90	45.10
Bacteria	Micro organism	Micro organism	US, EP, WO, CN, CAAU, MX	23.74	
Microalga	Micro organism		US, EP, WO, CN, CA, AU, MX	18.69	

The upstreaming of these technologies also can be challenging and warrants more detailed studies before the commercial cycles can be put into operation. A sustainable biofuel industry would need to rely on food and non-food crops like cellulosic feedstocks for large scale production. The current low yields in this early-stage research may be obstacles for commercialization. Hence, if genetic engineering strategies for production of cellulases and hemicellulases, modification of lignin and increased polysaccharide content can reduce the need for pretreatment processes, this technology can become more sustainable for upstreaming by the industry. Likewise, metabolic engineering for altered fatty acid composition and increased oil content in candidate crops or their heterologous expression in suitable target crops may be areas worth exploring for meeting the fuel demands.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

PATIENT CONSENT

Declared none.

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