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Review Article

Advances in Maize-Based Bioethanol Production and Its Prospects in Nepal

Kailash Pandey[1*], Bijay Shrestha[2], Samikshya Khanal[2], Debraj Adhikari[1], Chitra Bahadur Kunwar[3]

[1*]Prime Minister Agriculture Modernization Project (PMAMP), Junar Superzone, Sindhuli, Nepal
[2]Agriculture and Forestry University, Rampur, Chitwan, Nepal
[3] National Maize Research Program, Nepal Agricultural Research Council (NARC), Chitwan, Nepal

Abstract

The majority of energy demands of Nepal is met by petroleum imports. Massive use of gasoline and diesel has raised the annual CO_2 emission of the country. Thus, a search for the renewable, secure and sustainable source of energy is imperative. Maize (*Zea mays*) has been widely used across the world for bioethanol production. It is the second most important crop of Nepal in terms of both area and production. Productivity and attainable yield of maize in Nepal is 2.55 mt/ha and 5.70mt/ha respectively. The huge yield gap and scope of increasing productive area attest the possibility of increasing maize biomass production. Maize can be utilized in the bioethanol industry, generating ethanol for fuel and high-value co-products for feed. The starch fermentation process generates co-products like distillers dried grains with solubles, corn gluten meal, corn gluten feed and corn oil that can be used in the feed industry. Cellulosic ethanol is derived from lignocellulose, a substrate highly recalcitrant to enzymatic breakdown. Molecular and genomic tools have been used to study the cellulose assembly and its deposition pattern, biosynthesis of the lignocellulosic machinery and reducing lignin concentration. Transgenic maize with green-specific expression mechanism has been developed to produce cellulase within their biomass. Tropical temperate hybrid maize producing high biomass with low inputs have been developed. For a developing country like Nepal, maize-based bioethanol can meet energy demands, sustain agricultural production and create entrepreneurship opportunity.

Keywords: Bioethanol; energy; lignocellulose; maize; starch

Introduction

Nepal has been dependent on petroleum imports to meet energy demands. The country imported 486675 kiloliters of petrol and 1588869 kiloliters of diesel in 2017/18 (NOC, 2018). Coal, petroleum and fuelwood contribute to 87.27% of the total energy consumption of Nepal (WECS, 2014). Massive use of the fossil fuels has not only increased the economic burden but also raised annual CO_2 emission of this climate change vulnerable country. The territorial carbon dioxide emission of Nepal has increased to 9 mt in 2017 as compared to 2 mt in 1995 (GCA, 2019). Nepal is reported to be one of the top ten countries most likely to be impacted by the global climate change (WFP, 2009). In this context, a search for the renewable, secure and sustainable source of energy is imperative.

Bioethanol is a renewable source of energy produced from biomass. Ethanol is produced from the biological feedstock by the process of fermentation by bacteria or yeast. Maize, sugarcane, wheat, sugar beet, etc. are some of the carbohydrate containing feedstock that can yield starch for fermentation. Maize (*Zea mays*) has been widely used across the world for bioethanol production. Its use in the production of liquid transportation fuel has been recognized for more than 150 years. Globally, the number of ethanol production plants have been rapidly increasing and the

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[1*]Corresponding author
Kailash Pandey,
Prime Minister Agriculture Modernization Project (PMAMP), Junar Superzone, Sindhuli, Nepal
Email: kailashpandey748@gmail.com

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amount of ethanol produced is also increasing. In 2017, the U.S alone produced 15.80 billion gallons of ethanol, which is 58% of the total global production. U.S. and Brazil produce 85% of the world's ethanol (RFA, 2018). Agri-based biofuels can promote agricultural growth, meet energy demands, reduce the import of fossil fuels and minimize environmental impacts. In this paper, we propose a maize-based bioethanol production in Nepal, such that the demand for energy can be met without affecting the environment and the opportunity is created for entrepreneurship development and enhancing agricultural production.

Maize Production in Nepal

After rice, maize is the second most important crop of Nepal in terms of both the area and production. It is cultivated for food, feed and fodder. The area and production of maize in Nepal is 900288 ha and 1300121 mt respectively (MOALD, 2017). As shown in Fig. 1, the area, production and productivity of maize in Nepal has a variable trend. The area of maize increased from 870166 ha in 2007/08 to 906253 ha in 2010/11, decreased to 849635 ha in 2012/13 and gradually increased to 900288 ha in 2016/17. The area under maize cultivation is at increasing trend after 2014/15. Similarly, the production of maize is also at increasing trend from 2014/15 (2145291 mt) to 2016/17 (2300121 mt). Fig. 2 shows that the productivity of maize increased in all provinces of Nepal from 2014/15 to 2015/16. In 2016/17, the yield increased in all provinces except province no. 1 and 2. The province no. 3 had the largest increase in productivity. The yield in this province increased by 0.21 mt/ha in 2016/17.

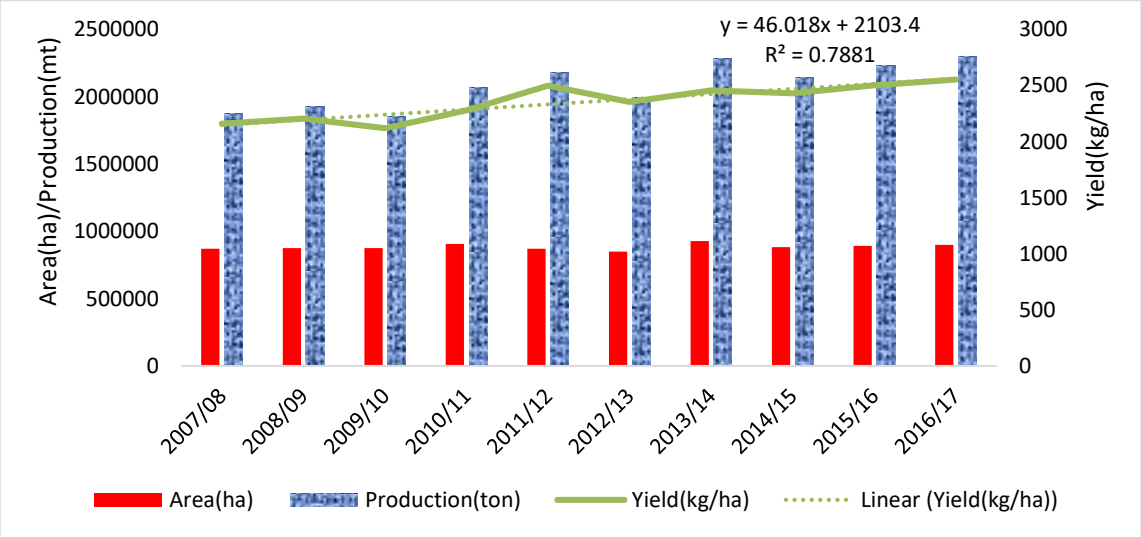

Fig 1: Area, Production and Productivity of Maize in Nepal *(Source: MOALD, 2017)*

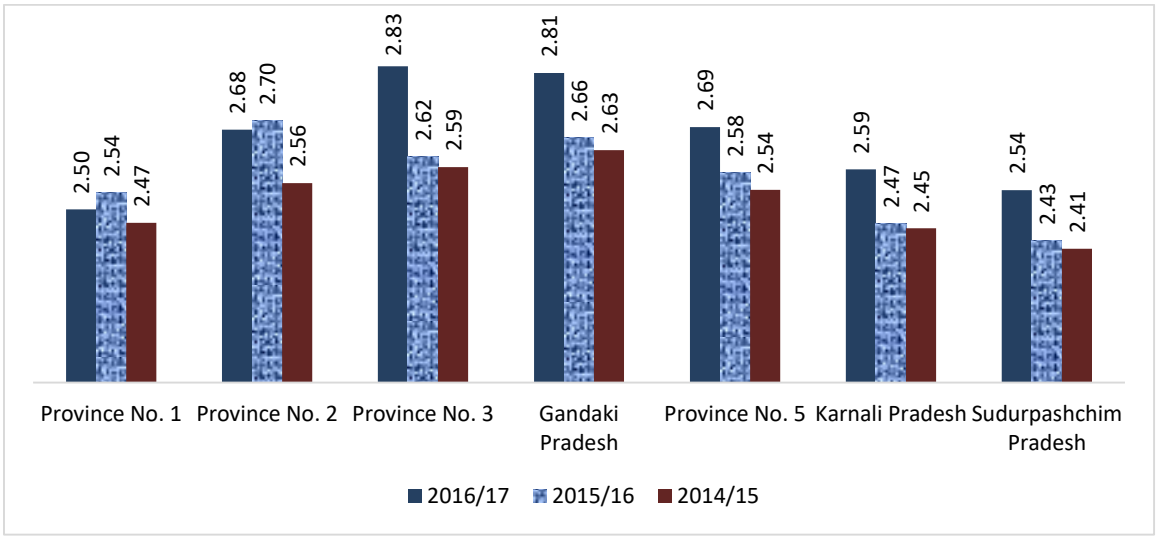

Fig. 2: Productivity of Maize (mt/ha) in different provinces of Nepal *(Source: MOALD, 2017)*

Relevance of Maize in Ethanol Production

Maize is a C4 crop and has a higher efficiency of converting solar energy into biomass. In C3 type of photosynthesis, CO_2 is fixated by ribulose bisphosphate carboxylase oxygenase (Rubisco). At low CO_2 concentration and higher temperature, Rubisco binds with O_2 instead of CO_2 by the process of photorespiration. In C4 crops, Rubisco activity is reduced by CO_2 concentrating mechanism that involves phosphoenolpyruvate (PEP) carboxylase. Thus, maize can accumulate more biomass as compared to C3 crops. In addition, the production cycle of maize is of three months, its grains can be stored, there is the possibility of off-season production, it consumes less water in the cycle to harvest and its harvesting cost is also lower. In the presence of climatic variability, maize can be produced for more than one cycle in the same year. Maize is cultivated for grain purpose and the stover i.e. leaf and stem fractions are available as the lignocellulosic residue. It is assumed that stover to the grain ratio is 1:1 in maize **(**Kim and Dale, 2004**).** This gives the instance of the biomass quantity that can be produced from maize. As a natural outcrosser, maize has a remarkable diversity and most of its desirable traits are yet to be utilized. This can be utilized in breeding activities for improving biomass characteristics. All these characteristics make maize a suitable feedstock for bioethanol industry.

Ethanol Production from Maize

Maize grain contains endosperm, germ, and hull. Principally, stover contains cellulose and hemicellulose. Cob contains more amount of cellulose and hemicellulose than in stalks and leaves. It is difficult to convert cellulosic biomass into fermentable sugars because of its crystalline structure and close association with the lignin and hemicellulose. Cellulose is recalcitrant to hydrolysis and a large portion of hemicellulose cannot be fermented by natural yeast. Pretreatment and hydrolysis are required to make the use of lignocellulosic material in ethanol production. It incurs extra input of energy and cost. Thus, the majority of the biofuels are produced from starch, as they are easily processed than the cell wall polysaccharides (Naik *et al*., 2010).

Maize contains 15% water, 59-70% nitrogen-free extract (starch, pectin, and sucrose), 5-15% proteins, 1.5-8.5% lignocellulose and 1.3-4% ash (Johnston and Moreau, 2017). Starch is composed of amylose (15-25%) and amylopectin (75-85%). Amylose is linear with D-glucose molecules of α-1,4-glucosidic bonds and amylopectin is highly branched consisting D-glucose with α-1,4-glucosidic bonds and α-1,6- glucosidic branching points. Corn starch contains 25-28% amylose (Cai *et al*., 2014). α -amylase is responsible for the liquefaction of starch. It is optimized at 90°C and pH 7 (Warren *et al*., 2011). It cleaves α -1,4- glucosidic bonds and cannot hydrolyze α-1,6-glucosidic bonds. The glucoamylase (amyloglucosidase) cleaves the glucose at α-1,4- glucosidic bonds and α-1,6-glucosidic bonds and is mainly used for the saccharification of the hydrolyzed material from liquefied processes. Glucoamylase acts at an average temperature of 60°C and pH 5 (Warren *et al*., 2011). Thus, the industrial processing using α-amylase for liquefaction requires cooling of the material. *Saccharomyces cerevisiae*, a facultative aerobe, transforms the glucose into ethanol and CO_2 under anaerobic condition.

Ethanol can be produced from maize by the two major commercial processes; dry grinding processing and wet milling. The dry grinding process is used for the production of ethanol only, whereas the wet milling is used for the production of corn oil and corn starch also. The yield of ethanol is greater in the dry grinding process as compared to the wet milling process. Thus, this paper will mainly focus on the dry grinding process.

Step 1: Milling

In this process, the grains are screened to remove any debris. The kernels are separated from the cob and ground to produce corn flour. The cobs are also ground so that they can be mixed with dry distillers grains (DDGs) after the co-products are generated.

Step 2: Cooking and liquefaction

The corn flour is mixed with water and enzyme α-amylase. The process involving the cooking of this slurry is called liquefaction. The jet-cookers inject steam into flour slurry to cook at a temperature above 100°C. This process breaks the starch present in the endosperm. The cooked mash is then cooled at 80-90°C and additional α-amylase is added.

Step 4: Saccharification

The slurry after liquefaction is called corn mash. During saccharification, the mash is cooled to approximately 30°C and glucoamylase is added to breakdown starch into fermentable sugars.

Step 5: Fermentation

Yeast is added after the mash is transferred to the fermenter. Saccharomyces cerevisiae is a commonly used species. The fermentation products are carbon dioxide and ethanol. The carbon dioxide is released to the atmosphere or is captured and used in carbonated soft drink, dry ice or beverages industry. The fermentation process is completed in about 48-50 hours. The fermented corn mash is now called beer.

Step 6: Distillation

The boiling point of ethanol is lower than water, so it can be separated by distillation. The beer is pumped into a multi-column distillation system and ethanol at 92-95% purity is obtained. The residual solids with water remaining after distillation is called stillage. It is processed to produce wet distiller's grains or distiller's dried grains with solubles.

Step 7: Dehydration

The ethanol produced from distillation contains 5% water. It is passed through the molecular sieves, where the desiccants absorb water to produce the anhydrous ethanol.

Step 8: Denaturing and Storage

To make the ethanol inconsumable, it is denatured with 2-5% petrol. Thereafter, it is sent to the tanks for storage.

The wet milling process differs from the dry grinding process in the initial treatment of the grain. The grain is soaked in water for its fractionation into various components of the kernel. The starch, fibre and germ are processed separately to produce different products. The economic byproducts of the wet milling process are corn gluten meal (high protein, 40%) and corn gluten feed (low protein, 28%). These can be used for animal feed (Mosier and IIeleji, 2006). The corn germ is processed to produce corn oil.

In the cellulosic production process, pretreatment is required, it softens the cellulosic material so that it can be broken down. Lignocellulosic material has a network of hemicellulose and cellulose in close association with lignin. It provides strength and complexity. During pretreatment, the lignin seal is broken, cellulose structure is disrupted and the hemicellulose is partially removed. The area exposed to the enzymes is increased. Pretreatment includes the use of a combination of heat and chemicals (water, caustics, acid and/or ammonia) to partially break down cellulose (Mosier *et al*., 2005). The pretreatment is followed by hydrolysis and fermentation. The yeast used in the grain-based ethanol production can be used to ferment glucose obtained from the cellulosic materials. 50-60% of the sugar obtained from the cellulose rich material is glucose but the remaining 40-50% is mostly xylose. The natural yeast can't ferment it to ethanol. Biotechnology has been used for the genetic modification of yeast and some bacteria that can produce ethanol from both the glucose and xylose.

The dry grinding process produces ethanol, distillers grains and carbon dioxide. In this process, the distillers dried grains with solubles (DDGS) are produced by drying the distillers grain to 10% moisture content or even less. This increases the storability and reduces the shipment cost. Distillers wet grains (DWG) have moisture content generally greater than 50-60% and has a limited shelf life. DDGS is the most prevalent distillers grains in the market. The common rule of thumb is that one bushel of corn (~25.4kg) produces up to 11 litres ethanol, approximately 8.2 kg distillers grain and nearly 8.2 kg of carbon dioxide (Rosentrater, 2011). DDGS from most of the modern fuel ethanol plants contains about 30% protein, 10% fat, at least 40% neutral detergent fibre, and up to 12% starch (Rosentrater and Muthukumarappan, 2006)**.** However, this may vary with the quality of raw material and production practices. As it has a high level of nutrients and is digestible, it can be used in the animal feeds. Moreover, its value addition can be done by pelleting/densification. If the lipids are removed from the DDGS, it can be converted into biodiesel (Rosentrater, 2011). Fig. 3 shows dry milling process of butanol production.

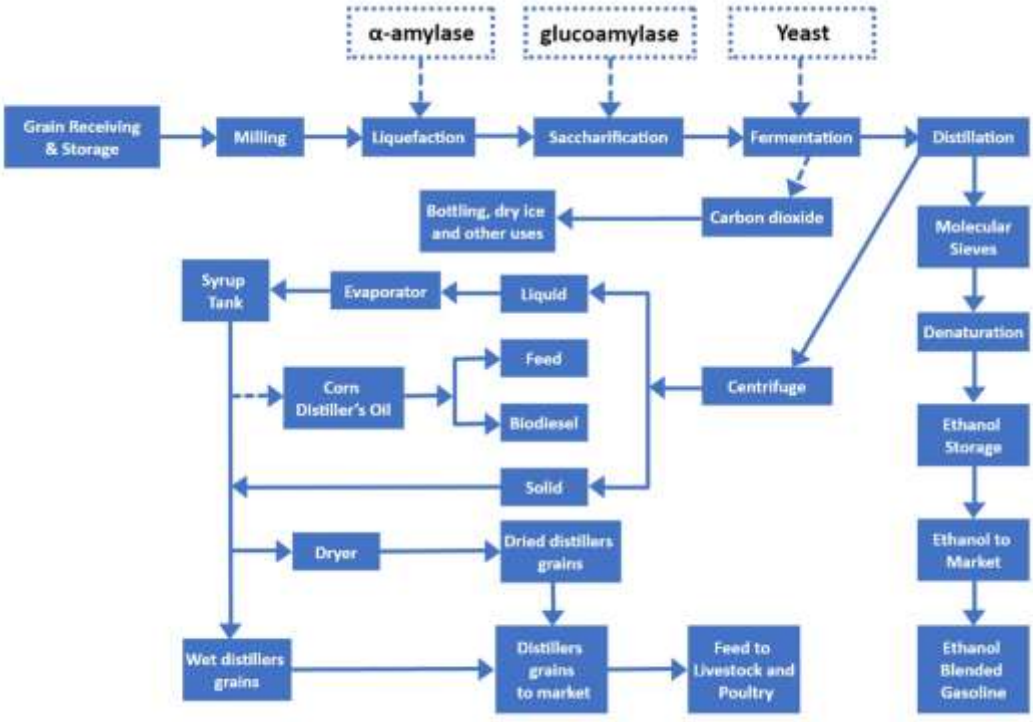

Fig. 3: Dry Milling Process (Adapted from Renewable Fuel Association, www.ethanolrfa.org)

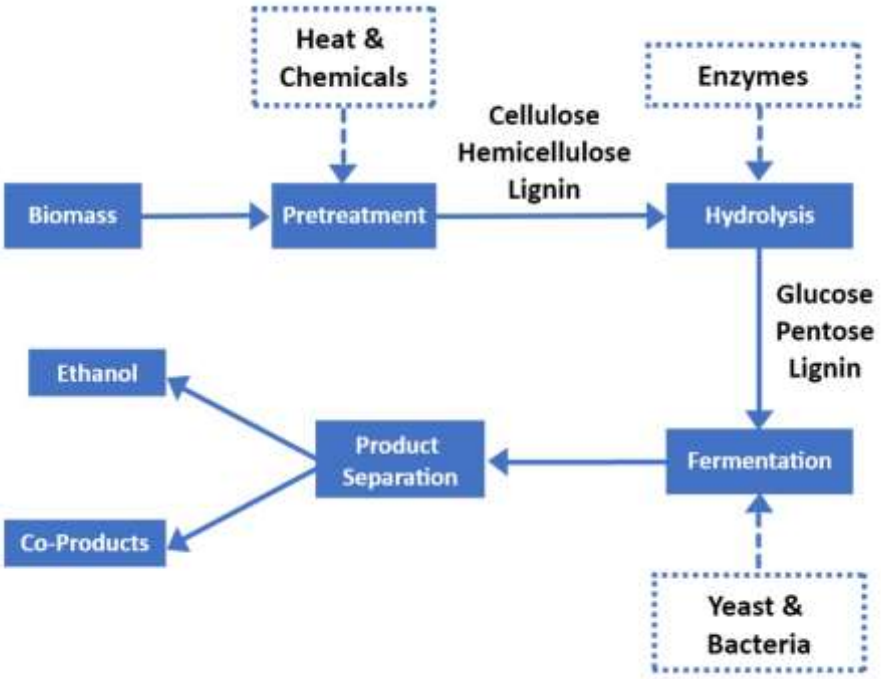

Fig. 4: Ethanol Production from Lignocellulose (Adapted from Mosier N., 2006)

Recent Advances

Bioethanol produced from the plant cell wall fragments is called lignocellulosic bioethanol. Lignocellulosic biomass are the most abundant polymers with limited use in food and feed and can be utilized for large scale biofuel production (Wyman, 2008). Difficult physical degradation makes the lignocellulosic biofuel production comparatively expensive. The major challenge with respect to the cost competitiveness of lignocellulosic conversion technology is the higher input requirement of energy and chemicals to extract and hydrolyze the cell wall carbohydrates (Wyman, 2007). The pretreatment procedure may account for up to 25% of the total processing expenses (Gnansounon and Daurlat).

Ethanol Production from Lignocellulose is shown in Fig. 4. The major requirements to enhance the lignocellulosic bioethanol production are;

- Increase the supply of lignocellulose in a sustainable and cost-effective way.
- Improvement of the conversion efficiency of the lignocellulosic biomass into ethanol.

The recent studies are directed towards understanding the effect of lignin content on recalcitrance in lignocellulosic biomass. In a study with brown midrib mutants in maize and sorghum, reduced cell wall lignin content often led to the improved enzymatic digestibility. Similar results were obtained in transgenes that down-regulate monolignol biosynthesis genes in maize, sugarcane and switch grass (Sattler *et al*., 2012; Vernerris, 2008; Wu *et al*., 2011)**.** Pui Ying Lam *et al*. (2017) reported the substantially reduced lignin content in *FNSII* mutant rice. In *Oryza sativa*, the flavone synthase II is crucial for biosynthesis of soluble tricin-derived metabolises. Majority of grasses (including cereals) utilize a member of flavonoids, tricin as a natural comonomer with the monolignols for cell wall lignification. The chemical analysis of *OsFNSII*-Knockout mutant rice cell wall showed 34%-58% reduction in lignin content and 30-37% enhancement of the saccharification.

The characteristics of cell wall other than lignin also determine biomass recalcitrance. The cellulose crystallinity, degree of cell wall porosity, polysaccharide accessible surface area and protective sheathing of cellulose by hemicellulose also cause resistance to enzymatic degradation (Gross and Chu, 2010; Himmel *et al*., 2007; Mosier *et al*., 2005; Zhao *et al*., 2012). Studies have been made on making alterations in cellulose synthesis machinery that may lead to modifications in the structure of cellulose microfibrils and help in the simplification of its enzymatic depolymerization. The cellulose recalcitrance is related to its high degree of polymerization and crystallinity index (Mansfield *et al*., 1999)**.** Vandenbrink *et al*. (2012) demonstrated that genotypes with lower cellulose crystallinity have higher enzymatic hydrolysis. The understanding of complex biosynthetic machinery is necessary for the modification of cellulose assembly and deposition pattern in maize. 12 members of the maize cellulose synthetase (*CesA*) gene family have been annotated and characterized. These genes encode the

catalytic subunits of maize cellulose synthetase complex (CSC) (Holland *et al*., 2000). Hemicellulose binds to the microfibrils of cellulose and threads them via cross-links with lignin. It is important in structural integrity and recalcitrance of cell wall (Carpita, 1996). It appears to be synthesized by members of the *Cellulose Synthase Like (Csl)* gene family. It is a multigene complex that is highly homologous to the *CesA* family (Richmond and Somerville, 2000; Richmond and Somerville, 2001). Despite of the significant advances, much of it remains to be explored for the manipulation of the properties of cell wall and efficient conversion of lignocellulosic biomass.

The cell wall recalcitrance also depends on types of hemicelluloses and the ratio of monomers of lignin (Girio *et al*., 2010). Grasses have two major types of hemicellulose; MLG (β-1,3- β-1,4-glucan) and GAX (β-1,4-linked xylose backbone with single arabinose and glucuronic acid side chains) (Doblin *et al*., 2009; Reiter, 2002; Vogel, 2008). GAX other than MLG links tightly to lignin. It can be replaced by MLG by the expression of *CsIF* and *CsIH* genes that are known to catalyze MLG biosynthesis (Fry *et al*., 2008). The three monolignol polymers acting as the precursors are *p*-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol. These are incorporated into lignin in the form of phenylpropanoids, guaiacyl (G), *p*-hydroxyphenyl (H), and syringyl (S). The ratio of coniferyl lignin to syringyl lignin is a crucial factor that determines the biomass recalcitrance (Chen and Dixon, 2007). It is also demonstrated that lower S/G ratio can reduce the biomass recalcitrance.

At least three groups of cellulase enzymes are required for the enzymatic hydrolysis of cellulosic materials. The cellulases are endo-cellulase or 1,4-β-endoglucanase (E1), exocellulase or cellobiohydrolase and cellobiase or β-D-glucosidase. Attempts have been made to express the microbial cellulase genes in plants and determine hydrolysis activity in the transgenic plants. It had no side effect on the plant growth and biomass yield (Himmel and Bayer, 2009). The heterologous production of cellulase in a plant cell can reduce the microbial production cost. Expression of 1,4-β-endoglucanase (E1) of *Acidothermus cellulalyticus* in maize shows the possibility of production of cellulose degrading enzymes in maize (Biswas *et al*., 2006). It is also important that the cell wall degrading enzymes are expressed at the appropriate developmental stage. Senescence-induced promoter can be used to drive the expression of cellulase in senescing maize. The gene expression should occur towards the end of growth or during the post-harvest operations (Torney *et al*., 2007). Mei *et al*. (2009) successfully produced *A. cellulalyticus* endo-cellulase E1 in transgenic maize plants. The heterologous enzyme was specifically targeted for accumulation in endoplasmic reticulum or mitochondria of plant leaves and stalks. Further, the heterologous cellulase was successfully used to convert cellulose into fermentable sugars for the biofuels. Green-specific expression mechanism of cellulase in maize can avoid controversies associated with the production of the transgenic products in the maize seeds and/or pollen. The endoplasmic reticulum contains molecular chaperones that enhance protein folding, reduced protein degradation, increased stability and higher biological activity. So, the sub-cellular targeting of the cellulase may result in the better expression of the transgenic products.

The production of lignocellulosic ethanol not only requires fermentation of glucose but also the fermentation of pentose sugars. The wild type of *Saccharomyces* can't ferment pentose sugar. So, the genetically modified yeasts, capable of co-fermenting xylose and glucose is needed (Ho *et al*., 2000). The surface-engineered yeast strains based on α-agglutinin codisplaying amylolytic enzymes have been successfully used to produce ethanol directly from corn starch. The yeast strains codisplayed the expression of glucoamylase from *Rhizopus oryzae* and α-amylase from *Streptococcus bovis* (Shigechi *et al*., 2004).

Tropical maize is a hybrid bred produced by crossing the inbred lines of tropical and temperate-adapted cultivars (White *et al*., 2011). These are also popularly known as temperate × tropical maize (TTM) hybrids. It accumulates a large amount of fermentable sugar, produces a large amount of cell wall biomass and produces little or no grain. It obtains the ability of higher stress tolerance and lower disease and pest susceptibility from its temperate parent. The TTM hybrids are 40% taller, exhibit late maturity, remain green for a longer time, require lesser input and are highly responsive to the nitrogen application (White *et al*., 2012). TTH can be used for the production of lignocellulosic ethanol.

The complete genome of maize has been released (Schnable *et al*., 2009). Also, there are many genomic tools available for maize (genetic markers, genomic annotations, quantitative trait loci (QTL's), extensive expressed sequence tag (EST) libraries, well-mapped populations, a large collection of mutants, etc.). It is important to improve the ability to introduce transgenes into inbred or elite genetic backgrounds for bioethanol production. The recent studies and technologies aim to improve the quality of transgene expression. It includes tissue or developmental stage-specific expression (Potenza *et al*., 2004), site-specific integration of transgene (Srivastava and Ow, 2002), multiple transgene expression and gene stacking (Halpin, 2005). In addition, it is also necessary to enhance the biomass yield by improved stress tolerance, reduced pest damage, enhanced biomass accumulation and increased yield.

Prospects in Nepal

The productivity and attainable yield of maize in Nepal is 2.55 mt/ha and 5.70 mt/ha respectively (MOALD, 2017;

KC *et al*., 2015). The huge yield gap and opportunity for increasing productive area reflect the possibility of increasing maize biomass production. More than 86% of maize production in the hills is used for human consumption and 80% maize production in the Terai region is used for poultry and animal feed (Gurung *et al*., 2011). The feed demand is increasing at a rate of 11% per annum (CDD, 2011). KC *et al*. (2015) reported that the demand of maize is shifting from food to livestock and poultry feed. This scenario shows that maize can be utilized for bioethanol production and the co-products of the ethanol industry can be used as feed for livestock and poultry. The presence of different agro-ecological zones in Nepal can provide the opportunity for multiple production cycles of maize. This can be helpful to increase biomass production. The cellulosic ethanol has 94% lesser greenhouse gas emission as compared to gasoline (Schmer *et al*.). Bioethanol comes from a renewable source. The crops absorb carbon dioxide during the growth. Thus, a balance is obtained for the carbon dioxide emission during the combustion of biofuel. Bioethanol is less toxic than fossil fuels and is biodegradable. Thus, bioethanol is environment friendly and can help to combat the negative impacts of climate change. Ethanol can be blended with gasoline and used as transportation fuel. The E10 blend consists of 10% anhydrous ethanol and 90% gasoline. In the United States, E10 is the standard blend for ethanol. It is safe and effective for all engine platforms (RFA, 2011). E20, E30 and E50 are mid-level ethanol blends (MLEBs). To use the MLEBs and higher-level ethanol blends (like E85), the vehicle must be fitted with flexible fuel vehicle (FFV) technology (RFA, 2011). The transportation sector has a 7.12% share in energy consumption of Nepal (WECS, 2014). The use of bioethanol as a gasoline blend can reduce the dependence of Nepal in petroleum imports. The chemicals produced in the bioethanol industry can serve several industrial uses. Acetaldehyde, acetic acid, isopropyl alcohol, potassium sulphate, etc. can be produced depending upon the plant type (EBPIA, 2019). In contrast to the wind, water, nuclear and photovoltaic energy; bioethanol can be stored as a liquid energy carrier and can have practical feasibility of fuel transportation in Nepal. Research and development initiatives on diesel engine to use biodiesel and simultaneous Jatropha plantation were carried out in past but it couldn't be materialized. Government of Nepal decided for several times in the past to use sugarcane-based bioethanol blended with petrol but the plan couldn't gain implementation (Pokharel and Sharma, 2008). Moreover, the sustainability of sugarcane-based ethanol industry can be questioned for its competition with the sugar industry. In contrast to the former approach, the higher feasibility of maize-based bioethanol can be a milestone for sustainable energy production and usage system in Nepal. The initiation of maize-based bioethanol industry can create entrepreneurship opportunity and employment generation in maize production, industrial processing, ethanol storage and transportation facilities and ethanol suited vehicle innovation and development.

Conclusion

The energy demand of Nepal is increasing and it is being met by the use of fossil fuels. It has resulted in dependence on imports and raised carbon dioxide emission. As the world is moving towards renewable energy, maize-based bioethanol has emerged as a strong alternative for transportation fuel. The co-products of the maize-based bioethanol industry can be used as a high value feed for livestock and poultry. In the scenario of shifting of maize demand from food to feed, the utilization of co-product creates no competition between the two industries. In the light of modern biotechnological development, many advances have been made in starch-based and lignocellulosic ethanol production. Thus, there may be a gradual simplification and economic optimization of the bioethanol industry. In Nepal, research to characterize and identify the maize varieties that are best suited for bioethanol production can be carried out. Study on the vehicles currently under use in Nepal and the possibility of using ethanol blended gasoline should be conducted. This technology can reduce petroleum imports, minimize greenhouse gas emissions, strengthen agricultural development and create entrepreneurship opportunities. Adoption of this technology in Nepal can not only help to sustain the economy and protect the environment but also to follow the global pace of technological advancement towards clean energy.

References

Biswas G, Ransom C and Sticklen M (2006) Expression of biologically active Acidothermus cellulolyticus endogluanase in transgenic maize plants. *Plant Science* **171**: 617-623.

Cai C, Zhao L, Hunag J, Chen Y and Wei C (2014) Morphology, structure and gelatinization properties of heterogeneous starch granules from high-amylose maize. *Carbohydrate Polymers* **102***:* 606-614.

Carpita N (1996) Structure and biogenesis of the cell walls of grasses. *Annual Review of Plant Biology* **47**: 445-476.

CDD (2011) *Impact of maize mission program.* Hariharbhawan, Lalitpur, Nepal: Crop Development Directorate.

Chen F and Dixon R (2007) Lignin modification improves fermentable sugar yields for biofuel production. *Nature Biotechnology* **25***:* 759-761.

Doblin M, Pettolino F, Wilson S, Campbell R, Burton R, Fincher G, Newbigin E and Bacic A (2009) A barley cellulose synthase-like CSLH gene mediates(1,3;1,4)-beta-D-glucan synthesis in transgenic Arabidopsis. *Proceedings of National Academy of Sciences of the United States of America* **106**: 5996-6001.

EBPIA (2019) *Creating Markets for Renewable Energy Technologies.* Brussels: European Biomass Industry Association.

Fry S, Mohler K, Nesselrode B and Frankova L (2008) Mixed-linkage beta-glucan: Xyloglucan endotransglucoxylase, a novel wall-remodelling enzyme from Equistem(horsetails) and charophytic algae. *The Plant Journal* **55**: 240-252.

GCA (2019) *CO_2 Territorial emissions.* Global Carbon Atlas, Global Carbon Project. Retrieved from http://emissions2018.globalcarbonatlas.org/exportGraphic

Girio F, Fonseca C, Carvalheiro F, Duarte L, Marques S and Bogelukasik R (2010) Hemicelluloses for fuel ethanol: A review. *Bioresources Technology* **101:** 4775-4800.

Gnansounon E and Daurlat A (n.d.) Techno-economic analysis of lignocellulosic ethanol: a review. *Bioresource Technology* **101:** 4980-4991.

Gross A and Chu J (2010) On the molecular origins of biomass recalcitrance: the interaction network and solvation structures of cellulose microfibrils. *Journal of Physical chemistry B* **114**: 13333-13341.

Gurung D, KC D, Ortiz Ferrara G, Gadal N, Pokhrel S, Bhandari D and Tripathi M (2011) Maize Value Chains in Nepal. *11th Asian Maize Conference.* China.

Halpin C (2005) Gene stacking in transgenic plants-the challenge for 21st-century plant biotechnology. *Plant Biotechnology Journal* **3**: 141-155.

Himmel M and Bayer E (2009) Lignocellulosic conversion to biofuels: Current challenges, global perspectives. *Current Opinions in Biotechnology* **20**: 316-317.

Himmel M, Ding S, Johnson D, Adney W, Nimlos M, Brady J and Foust T (2007) Biomass recalcitrane: engineering plants and enzymes for biofuels production. *Science* **315**: 804-807.

Ho N, Chez Z, Brainard A and Sedlak M. (2000) Genetically engineered Saccharomyces yeasts for conversion of cellulosic biomass to environmentally friendly transportation fuel ethanol. *American Chemical Society Symposium Series*.

Holland N, Holland D, Helentjaris T, Dhugga K, Xoconostle C and Delmer D (2000) A comparative analysis of the plant cellulose synthase(CesA) gene family. *Plant Physiology* **123**: 1313-1324.

Johnston D and Moreau D (2017) A comparison between corn and grain sorghum fermentation rates, distillers dried grains with solubles composition, and lipid profiles. *Bioresource Technology* **226**: 118-124.

KC G, Karki T, Shrestha J and Achhami B (2015) Status and prospects of maize research in Nepal. *Journal of Maize Research and Development* 1-9.

Kim S and Dale B (2004) Global potential bioethanol production from wasted crops and crop residues. *Biomass Bioenergy* **26**: 361-375.

Lam P, Tobimatsu Y, Takeda Y, Suzuki S, Yamamura M, Umezawa T and Lo C (2017) Disrupting Flavone Synthase II Alters Lignin and Improves Biomass Digestability. *Plant Physiology* 1-36.

Mansfield S, Mooney C and Saddler J (1999) Substrate and enzyme characteristics that limit cellulose hydrolysis. *Biotechnology Progress* **15**: 804-816.

Mei C, Park S, Sabzikar R, Qi C, Callista R and Sticklen M (2009) Green tissue-specific production of a microbial endo-cellulase in maize (Zea mays L.) endoplasmic-reticulum and mitochondria convert cellulose into fermentable sugars. *Journal of Chemical Technology and Biotechnology* **84**: 689-695. doi:10.1002/jctb.2100

MOALD (2017) *Statistical Information on Nepalese Agriculture.* Ministry of Agriculture and Livestock Development.

Mosier N (2006) Cellulosic Ethanol-Biofuel Beyond Corn. *BioEnergy*.

Mosier N and IIeleji K (2006) How Fuel Ethanol is Made from Corn. *BioEnergy*.

Mosier N, Wyman C, Dale B, Elander R, Lee Y, Holtzapple M and Ladisch M (2005) Features of promising technologies for pretreatment of lignocellulosic biomass. *Bioresource Technology* **96(6)**: 673-686.

Naik S, Goud V, Rout, P and Dalai A (2010) Production of first and second generation biofuels: a comprehensive review. *Renewable and Sustainable Energy Reviews* **114**: 578-597.

NOC (2018) *Import of Petroleum Products for 2074/75 (2017-18AD).* Nepal Oil Corporation Limited.

Pokharel G and Sharma S (2008) Biofuel: Potential and Prospects in Nepal as Alternative to Fossil Fuels. *International Energy Journal*(9) 19-24.

Potenza C, Aleman L and Sengupta G (2004) Invited review: Targeting transgene expression in research, agricultural, and environmental applications: promoters used in plant transformation. *In Vitro Cellular and Developmental Biology* **40:** 1-22.

Reiter W (2002) Biosynthesis and properties of the plant cell wall. *Current Opinion in Plant Biology* **5:** 536-542.

RFA (2011) *Building Bridges to a More Sustainable Future, 2011 Ethanol Industry Outlook.* Renewable Fuels Association.

RFA (2018) *World Fuel Ethanol Production.* Renewable Fuels Association.

Richard T (2010) Challenges in scaling up biofuels infrastructure. *Science* **329**: 793-796.

Richmond T and Somerville C (2000) The cellulose synthase superfamily. *Plant Physiology* **124**: 495-498.

Richmond T and Somerville C (2001) Integrative approaches to determining Csl function. *Plant Molecular Biology* **47:** 131-143.

Rosentrater K (2011) Overview of Corn-Based Fuel Ethanol Coproducts: Production and Use. In A. R. United States Department of Agriculture, *Biofuel's Engineering Process technology* (pp. 141-166). U.S.A: InTech.

Rosentrater K and Muthukumarappan K (2006) Corn ethanol coproducts: generation, properties, and future prospects. *International Sugar Journal* ***108*****(1295):** 648-657.

Sattler S, Palmer N, Saballos A, Greene A, Xin Z, Sarath G, Vermerris W and Pedersen J (2012) Identification and characterization of four missense mutations in brown midrib 12 (Bmr12), the Cafefeic-O-Methyltransferase (COMT) of sorghum. *Bioenergy Resources* **5:** 855-865.

Schmer M., Vogel K, Mitchell R and Perrin, R. (n.d.) Net energy of cellulosic ethanol from switchgrass. *Proceedings of the National Academy of Sciences of the United States of America* **105**: 464-469.

Schnable P, Ware D, Fulton R, Stein J, Wei F, Pasternak S and al e (2009) The B73 maize genome: complexity, diversity, and dynamics. *Science* **326**: 1112-1115.

Shigechi H, Koh J, Fujita Y, Matsumoto T, Bito Y, Ueda M, Satoh E, Fukuda H and Kondo A Author information (2004, August). Direct production of ethanol from raw corn starch via fermentation by use of a novel surface-engineered yeast strain codisplaying glucoamylase and α-Amylase. *Applied and Environmental Microbiology* **70**: 5037-5040.

Srivastava V and Ow D (2002) Biolistic mediated site-specific integration in rice. *Molecular Breeding* **8:** 345-350.

Torney F, Moeller L, Scarpa A and Wang K (2007) Genetic engineering approaches to improve bioethanol production from maize. *Current Opinion in Biotechnology* **18**: 193-199.

Vandenbrink J, Hilten R, Das K., Paterson A and Feltus F (2012) Analysis of crystallinity index and hydrolysis rates in the bioenergy crop Sorghum bicolor. *Bioenergy Resources* **5**: 387-397.

Vernerris W (2008) "Why bioenergy makes sense". *Genetic Improvement of Bioenergy Crops* 3-42.

Vogel J (2008) Unique aspects of the grass cell wall. *Current opinion in Plant Biology* **11**: 301-307.

Warren F, Royall P, Gaisford S and Butterworth P (2011) Binding interations of alpha-amylase with starch granules: the influence of supramolecular structure and surface area. *Carbohydate Polymers* **86**: 1038-1047.

WECS (2014) *Energy Data Sheet.* Water and Energy Commission Secretariat.

WFP (2009, December) The Future of Food: Creating sustainable communities through climate adaptation. *World Food Program- Food for Thought Series*(2).

White W, Moose S, Weil C, McCann M, Carpita N and Below F (2011) Tropical Maize: exploiting maize genetic diversity to develop a novel annual crop for lignocellulosic biomass and sugar production. *Springer* 167-179.

White W, Vincent M, Moose S and Below F (2012) The sugar, biomass and biofuel potential of temperate by tropical maize hybrids. *Bioenergy* 1-13. DOI:10.1111/j.1757-1707.2012.01158.x

Wu L, Arakane M, Ike M, Wada M, Takai T, Gau M. and Tokuyasu K (2011) Low temperature alkali pretreatment for improving enzymatic digestibility of sweet sorghum bagasse for ethanol production. *Bioresource Technology* **102**: 4793-4799.

Wyman C (2007) What is (and is not) vital to advancing cellulosic ethanol. *Trends in Biotechnology* **25** 153-157.

Wyman C (2008) Cellulosic ethanol: a unique sustainable liquid transportation fuel. *Material Research Bulletin* **33**: 381-383.

Zhao X, Zhanf L and Liu D (2012) Biomass recalcitrance. Part I: the chemical compositions and physical structures affecting the enzymati hydrolysis of lignocellulose. *Biofuels, Bioproducts and Biorefining* **6***:* 465-482.