The Americas Journal of Plant Science and Biotechnology ©2011 Global Science Books

Jerusalem Artichoke: An Emerging Crop for Bioenergy and Bioproducts in North America

Jaswinder Singh[1*] • Michael Ngadi[2] • Anuradha Varier[1] • Suha Jabaji[1]

[1] Department of Plant Science, MacDonald Campus, McGill University, 21111 Lakeshore Road Ste. Anne-de Bellevue, Quebec, H9X 3V9 Canada
[2] Department of Bioresource Engineering, MacDonald Campus, McGill University, 21111 Lakeshore Road Ste. Anne-de Bellevue, Quebec, H9X 3V9 Canada

Corresponding author: * **jaswinder.singh@mcgill.ca**

ABSTRACT

Bioenergy has become an urgent topic worldwide. States, nations, and companies are investing heavily to enhance their energy security and reduce fossil-fuel carbon emissions and pollution. Ethanol from sugarcane and maize are arguably the first renewable fuels to supplement petroleum-based fuels for use in transport. However, major drawbacks include the limited availability of suitable agricultural lands to grow these crops as well as their nutrient and water demands. Moreover, use of these crops has a negative impact on the food supply, especially with the use of maize (grain) as a feedstock. Jerusalem artichoke (*Helianthus tuberosus* L.) with its low input requirement, high ability to sequester C producing high amounts of biomass and inulin promises to be a good alternative for producing bioethanol with the added advantage of obtaining profitable bioproducts. Jerusalem artichoke (JA) is a tuberous rooted perennial plant which is a close relative of sunflower, an important oil seed crop. The storage carbohydrate of JA tubers is inulin, a fructan which is sweeter than sugar and has both nutritional and health benefits. Additionally, fructan containing food is also known for increasing the bioavailability of minerals and for stimulating immune system. The inulin present in tubers and stem can be converted to bioethanol. JA can be grown on marginal lands and even on post industrial site. We will review the potential of this crop for bioenergy while highlighting genetics, genomics, biotechnological and bioconversion efforts to turn this native Canadian crop to a viable feedstock for sustainable production of bioproducts.

Keywords: ethanol, fructan, inulin, renewable energy, wild species
Abbreviations: DP, degrees of polymerization; **FEH**, fructan 1-exohydrolase; **FFT**, fructan,fructan 1-fructosyl transferase; **JA**, Jerusalem artichoke; **S/I**, sucrose/inulin; **SST**, sucrose:sucrose 1-fructosyl transferase

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INTRODUCTION

Overwhelming use of finite fossil fuels due to industrial revolution, increasing population and fears of global warming has dramatically accelerated our chase to capture, exploit and utilize renewable bio-resources. As a consequence, shift of food commodity agriculture towards bioenergy sector is inevitable. The future use of agriculture is set to play a new role to explore specialty crops and designing new strategies in current energy based world economies. Unpredictable oil prices and growing concerns over climate change are the driving force for the world over investments in the biofuels sector as countries and industry increasingly look towards infinite bioenergy to replace the non-renewable fossil fuels. This bright side of transition also has a dark side of possible effect on world food security which is of

Received: 28 November, 2009. Accepted: 10 December, 2010.

Invited Review

major concern for the world's food scientists and FAO. Despite the rapid development and deployment of high yielding varieties, the world food problems are far from over. The world has witnessed a steep rise in food prices due to diversion of food crops to produce bio-ethanol leading to depletion in world food stocks. Conquering the pressure of high energy demands without utilizing world's food supply certainly necessitates identification and domestication of specialty crops which require minimum inputs and can be grown in marginal lands.

North America is one of the world's most important regions for energy, producing about one-fourth of global energy supply and consuming about 30 percent of the world's commercial energy (http://www.pi.energy.gov/documents/NorthAmericaEnergyPictureII.pdf). Both the USA and Canada have become increasingly conscious of the need to reduce their dependence on fossil fuels and shift towards cleaner and renewable sources of energy for transportation such as biofuels. This is evident from their policies and the steps taken by them to reduce green house gas emissions providing for a cleaner environment. The United States has resisted endorsing the Kyoto Protocol, but its various internal policies implemented through organizations such as the US Department of Energy and the Environmental Protection Agency (EPA), provide for maintaining its own standards of reduction of environmental pollution, global warming and climate change. Legislations such as the Energy Policy Act of 2005, and Energy Independence and Security Act of 2007 have facilitated increased production of clean renewable fuels. The Renewable Fuel Standard Program of 2009 established under these acts requires that roughly 10% of gasoline be replaced with renewable fuels such as ethanol (EPA 2010).

The approach of the United Sates Government is market driven involving giving incentives to the industry in return for reduced CO_2 emissions. The recently passed "caps and trade bill" in the house of representatives provides tax incentives in the form of carbon credits to companies that meet the required "cap" in CO_2 emission thus encouraging industries such as those involved in production of bioethanol. At present, the United States is the largest producer of bioethanol, producing over 9 billion gallons in 2008 (http://www.ethanolrfa.org/industry/statistics/#E). Most of the ethanol produced in the US is used as an oxygenative additive to gasoline replacing methyl tertiary-butyl ether (MBTE) which was banned almost all over the country by 2006 (Goettemoeller and Goettemoeller 2007).

In Canada also, the use of clean renewable energy is an important part of the government's strategy to protect the environment and the health of its citizens. Canada is a signatory to the Kyoto protocol and is bound by its mandate for reducing GHG emissions and addressing issues related to climate change. The Canadian goal for the Kyoto Protocol is to reduce GHG emissions by 6% below their 1990 levels by 2012 (Environment Canada 2007). The Government of Canada is investing up to $1.5 billion to increase the supply and availability of bioethanol and biodiesel. Proposed regulations will require 5% renewable fuel content based on the national gasoline pool by 2010. To meet this goal, Canada will need nearly three billion liters of renewable fuels by 2012. The eco ENERGY for Biofuels initiative administered by Natural Resources Canada is to help reach this target by providing operating incentives to companies that produce renewable alternatives to gasoline and diesel based on production levels and other factors. It will make investment in production facilities more attractive by partially offsetting the risk associated with fluctuating feedstock and fuel prices.

At present there are only around 10 ethanol facilities either in operation or under construction in Canada (http://re.pembina.org/sources/bio-energy) and the bioethanol production was just around 0.25 billion gallons in 2008 which is less than that in many other countries (http://www.ethanolrfa.org/industry/statistics/#E). Thus, it is evident that the bioethanol industry in Canada is lagging behind and the Government needs to take more aggressive measures to step it up.

FEEDSTOCKS FOR BIOETHANOL PRODUCTION

Research efforts are being made to identify and develop diverse bioenergy sources worldwide to supplement the current energy demands. Brazil has been a leader in bioethanol production from sugarcane and has recently been superseded by the US corn ethanol initiative. While biodiesel is being produced in Europe using rapeseed and sunflower seeds, it also focuses on ethanol production using sugar beets and wheat. Large-scale oil palm plantations in Malaysia for producing oil for biodiesel and using of cassava for ethanol production in other parts of Asia is also being explored. Feedstock for first generation bioethanol (produced from food crops) comes mainly from starchy grains crops such as corn and wheat and sugar crops such as sugar-cane and sugar-beet (Sims *et al*. 2008). This greater push for bioenergy has resulted in the diversion of food crops for energy, a trend that can be devastating from world's food security point of view. In 2007, 40 million metric tons of corn was used to produce ethanol in the USA and this quantity is estimated to increase in 2008 reaching the mark of 25% of total corn produced in the USA (Aho 2007; USDA 2007). This massive shifting of food and feed from human and livestock use to energy use is sending shock waves throughout the world. An immediate effect of this is reflected in the 34% increase in food prices (Aho 2007). The use of grains for biofuel production in Canadian province, Québec is discouraged and priority is given to non-grain agricultural biomass, due to the fact that such a production scheme is environmentally and economically more beneficial for the province. Second generation bioethanol (from non-food biomass) is mostly produced from ligno-cellulosic materials including cereal straw like corn stover, bagasse, forest residues and purpose-grown energy crops such as vegetative grasses and short rotation forests (Sims *et al.* 2008). There is a lot of interest in the present times to shift from first generation to second generation biofuels (fuels from non-food parts of crops or crops which are not primarily grown for food). Non-food crops such as switchgrass (*Panicum virgatum* L., Poaceae) and *Miscanthus x giganteus* are gaining importance as dedicated bioenergy crops (Sims *et al*. 2008). Jerusalem artichoke (JA) uniquely fits into an efficient bioenergy crop niche as it can be grown on marginal lands and will not compete with important food growing crops. It has been estimated that biofuels produced on marginal cropland could replace a fifth of the US transportation fuel by around 2020 if given the right level of support (Roberts 2005). Thus, JA is an important biomass for renewable energy as well as an interesting source of nutraceuticals such as inulin and fructose. The renewed interest in biofuel has placed JA on the forefront of bioenergy crops due to its unique chemical composition. Only a small number of plants like chicory (*Cichorium intybus* L.), agave and JA accumulate inulin in amounts sufficient for cost effective extractions. Because of the high carbohydrate content in JA tubers (up to 27.7% in some varieties) it can serve as a good source of fermentable sugars for production of ethanol at 80 to 90% conversion efficiency (Chubey and Dorrell 1974) and at levels either comparable to or above sugar beet, corn and wheat. JA has a unique blend of features to become a new bio-energy crop as it has high ability for carbon sequestration producing more dry matter than other crops. In addition, it is a perennial crop and does not require planting every year.

SYNTHESIS AND HYDROLYSIS OF INULIN

Inulin is the major storage carbohydrate in JA, chicory and dahlia. JA has a high inulin content of >15% on a fresh weight basis and >75% on a dry weight basis. Pure inulin contains about 3% glucose and about 97% fructose (Coussement 1999). Inulin is composed of β-(1-2)-linked fructose

chains with a terminal glycopyranose unit at the reducing end and functionally diverse due to number of fructose units in the fructan chain generally expressed as degrees of polymerization (DP). Most of the inulin (52%) present in JA has DP of less than 9% making it suitable for fermentation products like ethanol. There is also some amount of inulin with medium DP (42% with 10-40% DP) and a small amount of high DP inulins (6% with DP>40%) which make it suitable for fat replacement, as a prebiotic and for high-fructose syrups. Inulin extracts can be fractionated on a commercial scale into discrete chain length classes allowing tailoring of the product for different market uses (Kays and Nottingham 2008). Inulin is synthesized by the concerted action of two fructosyl transferases (Van Laere and Van den Ende 2002). The enzyme sucrose:sucrose 1-fructosyl transferase (SST: EC 2.4.1.99) catalyzes the initial step of the synthesis of 1-kestose from two molecules of sucrose. The other enzyme, fructan:fructan 1-fructosyl transferase (1-FFT; EC 2.4.1.100) is responsible for chain elongation transferring fructose from fructans of low DP to those of higher DP. Inulin degradation is catalysed by both 1- FFT and fructan 1-exohydrolase (1-FEH) which utilizes a multi-chain attack and hydrolyses fructan molecules at the terminal non reducing fructosyl residue releasing fructose. These three enzymes 1-SST, 1-FFT and 1-FEH appear to control fructan polymerization and depolymerization in JA. All these enzymes function inside the vacuole and the biosynthesis and degradation of inulin occur inside the vacuole (Frehner *et al.* 1984). There is a distinct temporal control over the gene expression of these enzymes (Edelman and Jefford 1968; van der Meer *et al*. 1998; Ritsema and Smeekens 2003). Control of fructosyltransferase enzymes concentration and that of sucrose could account for the variability of fructans in various tissues or species which awaits investigation using modern molecular and proteomics tools. Fructan polymer distribution in JA tubers studied using HPAEC-PAD chromatograms indicate that the range in polymer lengths varies depending upon the developmental stage of the organ in which they are present. In the fall as the tubers mature, long chain length polymers predominate, however during dormancy and with sprouting, fructans start breaking down and the mean chain length shortens dramatically (Ritsema and Smeekens 2003; Saengthongpinit and Sajjaanantakul 2005) A different role for fructans may be involved in the protection of plants during drought, salt or cold stress (Ritsema and Smeekens 2003).

Fructose can be obtained from inulin by either chemical or enzymatic hydrolysis (Kays and Nottingham 2008). Chemical hydrolysis can result in high yields of fructose, however, the combined effects of heat and pH causes unfavourable secondary reactions resulting in the appearance of foreign taste and smell (Yamazaki and Matsumoto 1986). Enzymatic hydrolysis of inulin is achieved by the enzymes fructan hydrolases and inulinases (Khanamukherjee and Sengupta 1989; Pandey *et al.* 1999; Singh and Gill 2006). Hydrolysis can be achieved either by using inulinase isolated from microorganisms or by using these inulinase producing organisms directly in bioreactors. Enzymatic hydrolysis has the advantages of using less energy and mild pH and not producing coloring or secondary products. Inulin hydrolysis yields many economically useful products such as high fructose syrups and inulin oligomers. The sugars released by inulin hydrolysis can then be fermented using microorganisms to produce ethanol and reagents such as acetone-butanol (Barthomeuf *et al*. 1991a), 2.3-butanediol (Fages *et al*. 1986), lactic acid (Shamtsyan *et al*. 2002) and succinic acid (Drent *et al.* 1993).

THE ENZYME INULINASE

Hydrolysis of inulin is an important step for obtaining various bioproducts from JA. Inulin hydrolyzing enzymes are classified into two major types: endo inulinases (2,1-β-D-fructan fructanohydrolase, EC 3.2.1.7) and exo-inulinases (β-D-fructan fructanohydrolase, EC 3.2.1.80) (Singh and Gill 2006; Ricca *et al*. 2007;Vijayaraghavan *et al*. 2009). The endo-inulinases cleave linkages within the chain, yielding fructans with reduced degrees of polymerization while exo-inulinases cleave single-D fructose molecules from the terminal end. Inulinases from plants and fungi belong to the glycoside hydrolase family 32 (GH32) while those from bacteria belong to GH68 (Coutino and Henrissat 1999; Goosen *et al*. 2008). Genes for inulinases have been cloned, sequenced and characterized from various yeasts, fungi and bacteria (Moriyama *et al.* 2002; Tsujimoto *et al*. 2003; Wen *et al.* 2003; Singh and Gill 2006; Goosen *et al*. 2008). The ORFs of the inulinase genes range from 1296 bp in *Thermatoga martima* to 2436 bp in *Arthobacter* sp. (Singh and Gill 2006). There are several conserved motifs among inulinases from different microoraganisms. Within these conserved motifs Asp and Glu residues exist which are involved in the catalytic activity of inulinases (Reddy and Maley 1996). These residues, particularly the Asp residue have been implicated in substrate recognition by providing hydrogen bonds (Nagem *et al.* 2004; Kim *et al*. 2008). Most inulinases from microorganisms are able to hydrolyse and transfructosylate with sucrose and are induced by it (Yuan *et al*. 2006; Goosen *et al.* 2008) unlike the inulinase (1-FEH) from higher plants such as that in JA which is inhibited by sucrose (Incoll and Neales 1970; Wiemken *et al.* 1986). However, the S/I ratio (relative activities with sucrose and inulin) in inulinases is lower than that of invertases (Ohta *et al.* 2002; Goosen *et al.* 2008). Some inulinases are induced by fructose while certain others are inhibited by it through catabolic repression (Yuan *et al*. 2006). Glucose also exerts a strong catabolic repression of inulinase genes (Moriyama *et al*. 2006). The pH and temperature of the medium are the most significant variables for achieving optimum activity of inulinase. The optimum pH for inulinase activity ranges between 3.5 and 6.5 (Kalil *et al.* 2001). The optimum temperatures for inulinase activity range from 45 to 70°C in the different microorganisms. There is also a wide range for the thermostability of the enzymes between species (Singh and Gill 2006). Temperatures at or over 60°C are conducive for solubility of inulinases which brings down the cost of production at industrial level. High temperatures also reduce microbial contamination (Vandamme and Derycke 1983). Inulinases from some strains of the yeast *Aureobasidium pullulans* possess thermostability above 60°C (Lima *et al*. 2009) and those from *Streptomyces* sp. and *Geobacillus stearothermophilus* are active at temperatures above 70°C (Tsujimoto *et al*. 2003; Sharma and Gill 2006). Among all the microorganisms that synthesize inulinase, *Kluyveromyces* spp. and *Aspergillus* spp. have proved to be the most versatile source of inulinases (Singh and Gill 2006). Among the different *Aspergillus* species, thermostability varies; exoinulinase isoform II of *A fumigatus* has a higher thermostability than *A. niger* and *A ficum* (Gill *et al*. 2006a, 2006b, 2006c). The thermostability of inulinases can be enhanced by immobilization on-to a support which provides a rigid backbone and reduces the susceptibility of the enzyme molecules to breakdown by heat (Ettalibi and Baratti 2001; Gill *et al*. 2006a, 2006b, 2006c). Also thermal stabilizing polyols such as PEG, ethylene glycol, isopropanol, dextran, sorbitol, glycerol and mannitol protect inulinases from high temperature degradation (Viswanathan and Kulkarni 1995; Gill *et al.* 2006a; Sharma and Gill 2006) and can be used commercially.

BIOCONVERSION AND ETHANOL PRODUCTION IN JERUSALEM ARTICHOKE

Raw material

Both the stalks and tubers of JA can be used for ethanol production (**Fig. 1**). Although tubers are used predominantly, the fructans in the stalks can be used in the late summer prior to flowering and the final stage of tuberization for ethanol production (Bajpai and Margaritis 1986b; Caserta and Cervigni 1991; Curt *et al.* 2005; Negro *et al.* 2006;

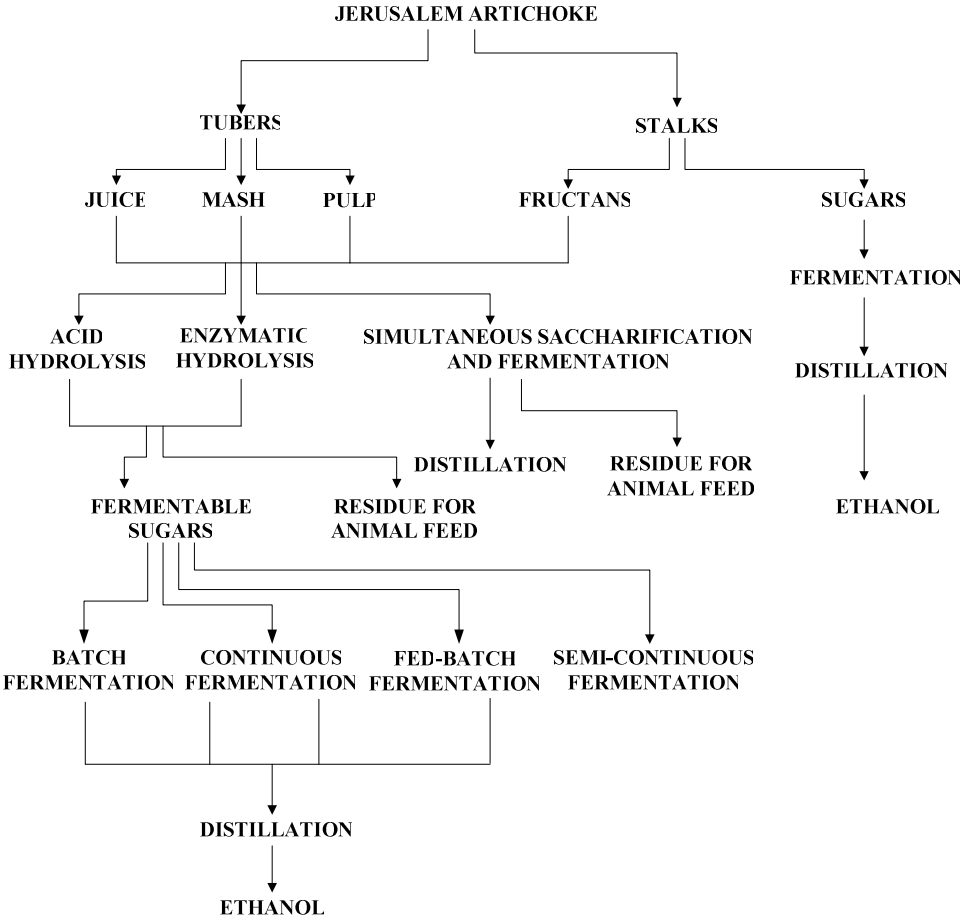

Fig. 1 Process of bioethanol production from Jerusalem artichoke.

Stolzenburg 2006). It has been suggested that, at 80-90% conversion efficiency, ethanol yields of 3900-4500 L/ha could be attained from JA's carbohydrate, the content of which is as high as up to 27.7% in the tubers of some varieties. JA could yield relatively more alcohol, approximately, 1.7, 2.0 and 3.7 times more compared to sugar beet, corn and wheat respectively (Duvnjak *et al.* 2004). It is also possible to ferment ethanol directly from sugars present in the stalks avoiding the additional step of inulin hydrolysis (Harris and Bautista 1983). Stalks are easier to harvest than tubers because the irregular size and shape of the tubers make them difficult to harvest (Amato 1993).They have to be harvested by the slow labour-intensive process of hand-digging. Also, storing the tubers needs expensive infrastructure for maintaining the required high humidity and low temperature (0°C) conditions. Another disadvantage with using tubers is that their thick, irregular, fibrous skins make drying and distilling exceptionally difficult.

Grinding and milling

The raw material (stalks or tubers) is subjected to washing and grinding. Stalks are ground in a hammermill to release sugars or fructans from the central cylinder, pith, ligneous cells and to a small amount from the bark (Harris and Bautista 1983). The cellulose from the stalks can be broken down using sulfurous acids, exogenous enzymes or enzymes from cellulolytic strains of fungi or bacteria (Kays and Nottingham 2008). Ethanol from tubers can be produced either from mash, pulp or juice obtained by crushing and pressing the tubers (Rosa *et al.* 1986; Barthomeuf *et al.* 1991a; Szambelan and Chrapkowska 2003; Szambelan *et al.* 2004a; Ge and Zhang 2005; Beckers *et al*. 2008). Higher ethanol yields are obtained with juice than mash or pulp (Szambelan *et al*. 2004a). Kosaric *et al.* (1982) developed a method for extracting juice from JA containing high levels of fructans by a 2-step expression process with maceration.

Acid and enzyme hydrolysis

Acid hydrolysis was the original method of obtaining fermentable sugars from plant feedstocks using either high acid concentration at low temperatures or low acid concentration at high temperatures (Lampe 1932; Underkoffler *et al*. 1937; Boinot 1942; for comprehensive review see Kays and Nottingham 2008). During acid hydrolysis, the most important factors to be considered are the pH, temperature and time of incubation. Maximum hydrolysis of inulin is obtained with sulphuric acid at a pH of 2 or less and incubation temperatures above 90°C for 1 h (Szambelan and Nowak 2006; Nasab *et al.* 2009). Although both acid and enzymatic hydrolysis can be used, acid hydrolysis has certain disadvantages, for example it leads to degradation of fructose and formation of difructose anhydrides (Barthomeuf *et al.* 1991b; Singh and Gill 2006). Enzymatic hydrolysis of inulin can be performed using industrial enzymes or by inulinases extracted and purified from different microorganisms (Zubr 1988; Ohta *et al*. 2004; Gill *et al.* 2006a, 2006b, 2006c). The industrial enzymes such as "Novozyme" are thermostable inulinases derived from *Aspergillus* spp. The most commonly used Novozyme is "Novo 230" (Zubr 1988; Baumgartner and Praznik 1995). In order to improve the efficiency and stability of enzymes, it has been suggested (Singh and Gill 2006) that the enzymes can be mobilized on-to various supports including chitin with gluteraldehyde (Kim and Rhee 1989), amino-cellulofine (Nakamura *et al.* 1995), glass beads with silane reagent and glutaraldehyde (Ettalibi and Baratti 2001); calcium alginate (Catana *et al*. 2005); amberlite (Catana *et al*. 2007), gelatin-water after glutaraldehyde treatment (Paula *et al.* 2007); and other substances. Immobilization increases the activity of inulinase several folds (Elnashar *et al.* 2009).The optimum pH for the activity of inulinases ranges from 4.5-6.5 and the optimum temperature ranges from 30-60 (Ricca *et al.* 2007).

Fermentation systems

The sugars released after inulin hydrolysis can be fermented using several microorganisms. The most commonly used among these are *Saccharomyces cerevisiae*, *Zymomonas mobilis* and *Kluyveromyces marxianus*. There are mainly four types of fermentation systems used by industry including batch, fed-batch, continuous and semicontinuous processes (Caylak and Sukan 1998; Kosaric and Vardar-Sukan 2001). The batch process is the classical method of producing alcoholic beverages and is the method by which most of the ethanol is produced today. In this process substrate and separately grown cell slurry are charged into the bioreactor together with nutrients and enzymes required. The time required to completely utilize the substrate is 36-48 h. After fermentation the material is pumped to distillation supply tank**.** This system has advantages and disadvantages. The advantages include low investment costs, use of unskilled labor, well defined cultivation periods and higher conversion levels, less risk of infection and cell mutation, and a greater flexibility because a bioreactor with various product specifications can be used. In a continuous process patented by MacLennan and MacLennan (1998), ethanol is produced in the gaseous phase after multi- stage fermentation in a series of fermentation vessels with step wise reduction in pressure. Continuous processes have little downtime, can produce more ethanol of a more uniform quality than batch processes and are better suited to large-scale production. However, they are less flexible, have higher investment and operating costs and have a higher risk of adverse microbial mutation due to longer culturing periods. Fed-batch processing combines batch and continuous cultures, with substrate and microbial cultures added at regular intervals and effluent removed discontinuously Semi-continuous production is effectively repeated fed-batch processing with culture withdrawn at intervals and new microbes and fresh medium added. Fermentation of JA extracts in experimental trials has been mostly carried out by batch, continuous and semi-continuous processes (Thuesombat *et al*. 2007; Kays and Nottingham 2008) and occasionally by fed batch process (Ge and Zang 2005). Fermentation by all processes can be conducted with either free (Margaritis and Bajpai 1982a; Margaritis *et al.* 1983; Bajpai and Margaritis 1986a; Bajpai and Bajpai 1989) or immobilized (Margaritis and Bajpai 1982b; Margaritis *et al.* 1983; Margaritis and Merchant 1984; Bajpai and Margaritis 1986b) cells. Free cells are suspended in the culture medium and may aggregate through flocculation while immobilized cells are held on support systems. The same substances that are used for immobilizing the enzyme inulinase can be used for immobilizing cells. In addition, agro-industrial wastes such as sugarcane bagasse and soyabean bran can be explored for their use as alternatives to synthetic materials as immobilization supports. Immobilization increases the cell concentration thus avoiding the need for cell recycling systems to replace washed out cells. Optimum rates of fermentation occur at acidic pH, 3.5-6.0 (Bajpai and Margaritis 1982c; Margaritis *et al.* 1983; Bajpai and Margaritis 1987). In non-acidic conditions more contaminants occur which reduce the efficiency of fermentation. The optimum temperature for fermentation ranges from 25-36°C. Immobilization of cells could extend the higher temperature range up to 45°C.

Fermentation with the bacterium *Zymomonas mobilis* has some advantages over that with the yeast *Saccharomyces cerevisiae*. *Z. mobilis* has a natural tendency to flocculate raising the efficiency, grows both in high sugar and ethanol concentration without inhibition has more favourable fermentation kinetics than *S. cerevisiae*, produces fewer contaminants and consistently outperforms *Saccharomyces* strains in terms of ethanol yield (Kays and Nottingham 2008). The disadvantage with *Z. mobilis* is that there is a need to sterilize the culture medium making it economically less viable than yeast strains. Use of *Z. mobilis* in mixtures with the yeasts *S. cerevisiae* and *Kluyveromyces fragilis* results in higher theoretical yields of ethanol than those obtained from using these organisms individually (Szambelan *et al*. 2004b). JA mash, pulp, and juice provide a complete medium containing carbohydrates, minerals and vitamins necessary for the growth of these microorganisms (Duvnjak *et al*. 1981; Toran-Diaz *et al.* 1985). Other factors such as temperature, pH, inulin degrees of polymerization (DP), sugar concentration and method of fermentation should be standardized to optimize the efficiency of conversion. It has been noted that the ethanol accumulation during fermentation can cause damage to the microorganisms decreasing their efficiency. This can be overcome by using microorganisms with high ethanol tolerance such as *Saccharomyces* and *Zymomonas* (Singh and Mishra 1993) and strains of other microorganisms such as *K. fragilis* selected for improved ethanol tolerance (Rosa *et al*. 1988). In addition, yeasts can be genetically engineered to improve ethanol tolerance (Alper *et al.* 2006). Expression of FPS1, a gene encoding a plasma membrane aquaglyceroporin contributes to decreased ethanol accumulation in yeast cells and improves ethanol tolerance during fermentation (Teixeira *et al.* 2009). Other strategies to improve ethanol tolerance of microorganisms include addition of Ca ions to the medium (Nabais *et al.* 1988) and immobilization of cells (Krisch and Szajani 1997; Jirku 1999; Desimone *et al*. 2002; Zhou *et al*. 2008).

Simultaneous saccharification and fermentation using microorganisms

Traditional fermentation yeasts such as *S. cerevisiae* are not adapted to utilize inulin. However, a number of yeast strains have been discovered with inulinase activity which can both hydrolyse inulin and ferment the resulting sugars. These include strains of *Kluyveromyces marxianus*, *K. fragilis*, *Candida pseudotropicalis*, *C. kefyr*, *C. macedoniensis*, *Saccharomyces fermantati*, *S. diasticus*, *Schwanniomyces castelli* and *Torulopsis colliculosa* (Duvnjak *et al*. 1981; Guiraud *et al.* 1981; Echeverrigaray and Tavares 1985; Guiraud *et al.* 1986; Rosa *et al*. 1986; Padukone 1996; Ge and Zhang 2005). It is possible to produce ethanol from JA extracts using these yeasts in a single vessel without prior hydrolysis or saccharification in a one-step process called simultaneous saccharification and fermentation (Kays and Nottingham 2008). Of all these species, *Kluyveromyces* spp. is the most versatile and has received maximum attention (Fonseca *et al.* 2008; Yuan *et al.* 2008). Aeration is not essential for ethanol fermentation with *K. marxianus* and the optimum temperature is 35°C for fermentation (Yuan *et al*. 2008). The inulinase gene from *K. marxianus* can be transformed into other species of yeast that are not able to hydrolyse inulin such as *S. cerevisiae* and these transformed strains then be used for simultaneous saccharification and fermentation of inulin (Kim *et al*. 1998). Alternatively, *K. marxianus* can also be mixed with other microorganisms like *Z. mobilis* and *S. cerevisiae* for optimizing ethanol yields (Szambelan *et al*. 2004b). Simultaneous saccharification and fermentation can also be done by co-immobilizing inulinase and *Z. mobilis* cells (Kim and Rhee 1990). A schematic summary of ethanol production from JA is shown in **Fig. 1**. Though JA is not an oil producing crop the possibility of producing biodiesel from its tubers by the heterotrophic microalga *Chlorella protothecoides* has been explored recently (Chen *et al.* 2008). The alga utilizes the hydrolysate of JA as carbon source and accumulates high amounts of lipid under *in vivo* conditions. The lipids are extracted and converted to biodiesel by transesterification. Methyl esters of cetane acid, linoleic acid and oleic acid are the dominating components of the biodiesel produced through this process. Unsaturated methyl ester constitutes over 82% of the biodiesel content.

Commercial production of bioethanol in North America

Bioethanol processing plants in North America are either bio-chemical biorefineries or thermochemical bio-refineries. The comprehensive list of these companies has been compiled recently by Sims *et al.* (2008). Some of them are already in operation and others are under construction. Examples of biochemical-biorefineries are Abengoa Bioenergy, Bluefire Ethanol, Iogen Biorefinery, Poet, Ecofin LLC, Mascoma and ICM and those of thermo-chemical refineries are ALICO, Range Fuels and New Page. Companies such as Iogen have plants in both US and Canada. Some companies such as Abengoa Bioenergy and Ecofin LLC only produce first generation bioethanol from corn. Others also involved second generation bioethanol from raw materials like corn stover, wheat straw, wood chips and wood wastes, forest residues, energy crops and others. Although, JA is not currently used as a feedstock for bioethanol production at commercial scale it holds great promise for future use in biorefineries due to its unique features. Thus, there is a pressing need to promote this versatile crop for bioethanol production on a commercial level.

Other uses of Jerusalem artichoke's inulin and its products

The tubers of JA can be processed in different ways to yield various products. These include flour, fructose syrup, short chain fructooligosaccharides, inulin with high DP, native inulin and inulin concentrate. A schematic procedure for the conventional extraction and fractionation of JA inulin into various products has been recently documented by Kays and Nottingham (2008). This scheme combines information generated by various researchers including Vogel (1993), Barta (1993), Aravina *et al*. (2001) and Ji *et al*. (2002). Other methods for extraction include direct and indirect sonication (Wei *et al.* 2007).

JA flour is a low calorie, fat-free source of energy and fiber rich in nutrients including calcium, potassium and iron. Addition of flour or inulin to bread confers positive attributes including improved softness, prolonged preservation and improved bread volume (DeMan and Weegels 2005). The flour is also used to supplement animal feed. Seiler (1988) found that whole plants of wild and cultivated JA populations had a crude protein content of 60 to 90 g/kg. This is adequate for maintenance of ruminant animals.

The medicinal values and health benefits of JA has been firmly established in decreasing cholesterol, maintaining healthy intestinal microflora, suppressing intestinal infection, combating obesity, maintaining blood sugar levels in diabetic patients, improving blood lipid composition and stimulation of immune system (Hidaka *et al*. 1986; Coundray *et al*. 1997; Milner and Roberfroid 1998; Bonaparte and Kneifel 1999; Roberfroid 1999; Frank 2000). Inulins with high DP have applications in medicine. They help maintain the health of the cardiovascular system by lowering cholesterol and homocysteine levels (Hidaka *et al.* 2001; Tungland 2003). Inulin and fructooligosaccharide supplements boost the immune system and help preventing a wide range of diseases such as ulcerative colitis, inflammatory bowel diseases and colorectal cancer (Hidaka *et al*. 2001; Pool-Zobel *et al*. 2002; Kanauchi *et al*. 2003). Research in experimental animal models has revealed that inulin type fructans have anti cancer properties (Reddy *et al.* 1997; Milner and Roberfroid 1998; Rowland *et al.* 1998; Roberfroid 1999).

Inulin is a prebiotic, and its effect on human and animal health has been widely acknowledged (Rocha *et al.* 2006). Because inulins resist hydrolysis by small intestinal digestive enzymes, they are sometimes referred to as non-digestible carbohydrates and may be useful in the management of diabeties. Fermentation of inulin in the large intestine is known to improve gut health by stimulating the growth of health-promoting bacteria particularly bifidobacteria. Inulin extracts are sold widely as food supplements in health food stores. Inulin is a good bulking agent and thickener in ice-cream, sandwich spreads ,chocolate products and mayonnaise (Berghofer *et al.*1993; Frippiat and Smits 1993) and an additive for improving textural and sensorial characteristics of yogurt (Yi *et al.* 2009). It can particularly substitute probiotics in yogurts which may not survive beyond the stomach to contribute to colon microflora (Graham-Rowe 2006) and improve the retention of beneficial bacteria in yoghurts during storage (Paseephol and Sherkat 2009). Inulin-containing functional foods are useful for increasing mineral absorption (Hidaka *et al.* 2001). Inulin is a recognized source for the production of fructose syrups. Fructose that has GRAS (Generally Recognized As Safe) status as a sweetener, is up to 1.5 times sweeter than sucrose and enhances flavour, color and product stability in many foods and beverages. Further, fructose metabolism bypasses the known metabolic pathway of glucose and therefore does not require insulin (Millo and Werman 2000). Fructose syrups produced by the hydrolysis of inulin are widely utilized in the food industry because they are sweeter than sucrose thus allowing less sugar to be used to achieve a given level of sweetness. Methods for producing high fructose syrups from JA have been standardized (Duvnjak and Koren 1987; Bajpai and Bajpai 1991; Koren and Duvnjak 1991; Leenheer and Booten 1998). To achieve high-purity fructose syrup, either low-DP inulin is removed before hydrolysis or glucose is removed after hydrolysis. Low-DP inulin is removed by chromatography, enzymatic removal or precipitation of higher molecular weight fractions using ethanol, low temperatures or ultrafiltration (Chabbert *et al*. 1985; Kamada *et al*. 2002).

GENETICS AND GENOMICS OF JERUSALEM ARTICHOKE

JA possesses huge untapped variability which provides ample opportunity to use plant breeding and molecular boiology techniques for its improvement (**Fig. 2**). It is highly suited to temperate climates of Canada, is widely distributed and often labeled as a weed. Though its nutritional value as feed is greatly established, JA never emerged as a major feed crop due to lack of efforts to develop suitable cultivars.

Breeding

Breeding improved varieties of JA is crucial for the future of the crop. The existing commercial cultivars are closer in appearance to their wild ancestors than in most crop species because it has not been subjected to the same degree of genetic manipulation as other crops. However there is lot of genetic diversity in JA particularly with respect to tuber characteristics. Recently, Terzic and Atlagic (2009) reported that total sugar content in JA varied from 14-23%. JA is mostly bred by public institutions. In Canada, JA breeding is being carried out mainly in the Canada Research Station in Morden, Manitoba. Research is aimed at increasing tuber yields, and the fructose and inulin content of tubers, in accessions adapted to the conditions of Western Canada. A number of Morden accessions have been bred and selected in experimental trials and few of these have gone on to become commercially grown cultivars like 'Columbia' (Chubey and Dorrell 1974, 1982; Amato 1993). Recently, development of improved lines of JA has been initiated in the Alberta Research Council at Vegreville (Slaski and Anyia 2008; Anyia *et al*. 2009). In the US, a number of small-scale breeding programs have aimed to improve JA for industrial applications. For example research in USDA-ARS, Northern Crop Science Laboratory, Fargo, ND has focused on enhancing the crop's value for forage and silage (Seiler and Campbell 2004). Several accessions of JA including wild and weedy accessions, landraces or traditional and obsolete cultivars and advanced and improved cultivars are available in germplasm collections worldwide. These

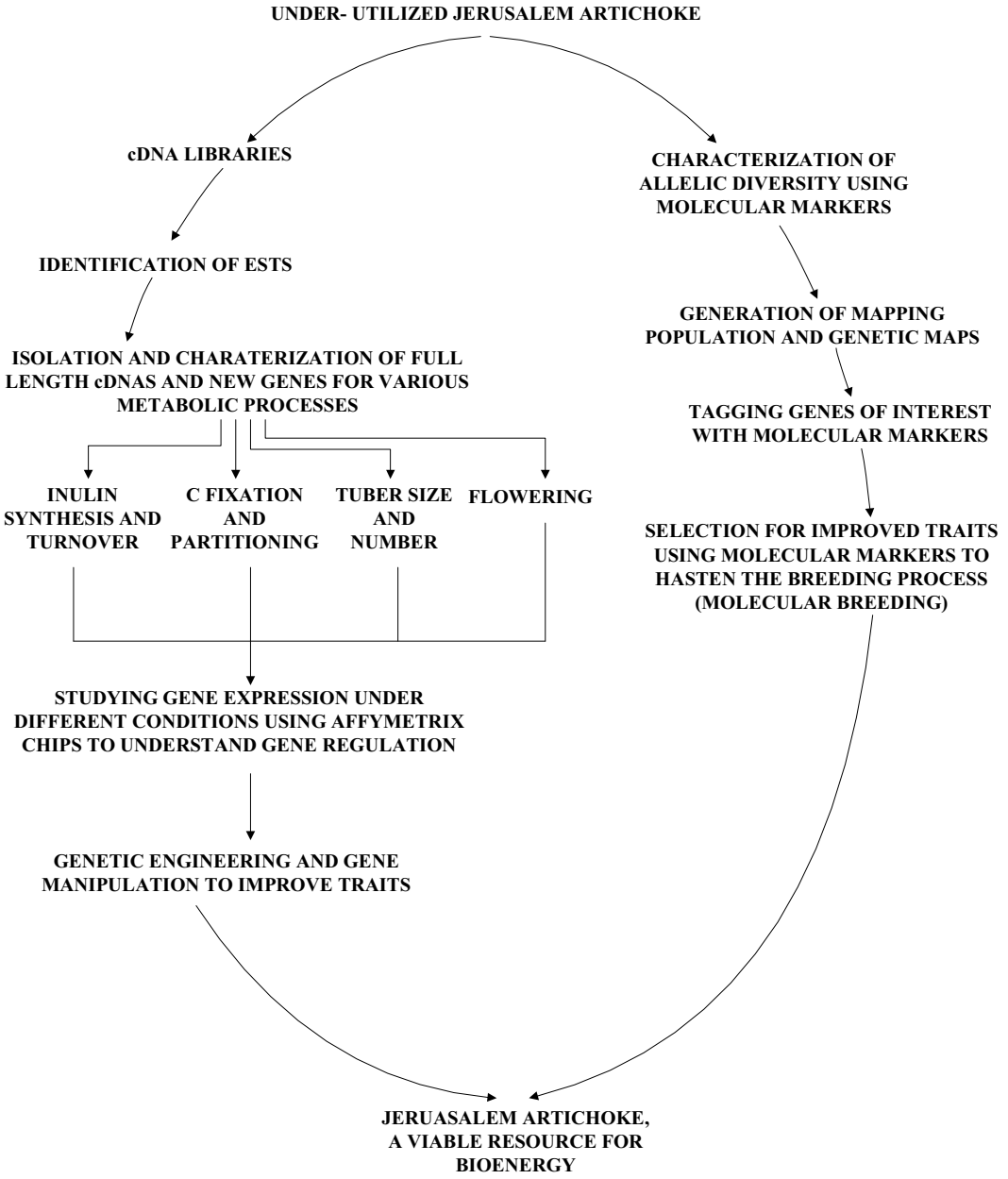

Fig. 2 Upgrading Jerusalem artichoke through geneomics tools.

accessions are maintained as tubers, seed in seed banks or in tissue culture. Propagation of JA is mainly through tubers as seed setting is difficult. Different explants from JA are amenable to regeneration under tissue culture conditions so it becomes an important means of preserving JA (Volk and Richards 2006). In Canada, the main repository for JA germplasm is at Plant Gene Resources of Canada, Saskatoon. Research Centre which maintains around 175 accessions (Volk and Richards 2006). In the United States most of the collections are held at the North Central Regional Plant Introduction Station (NCRPIS) at Iowa State University, Ames that holds around 112 collections (Kays and Nottingham 2008). Selection criteria for breeding include increased tuber yield, tuber size, and smoothness of tuber surface, inulin quantity and quality, capacity for photosynthesis, resistance to diseases such as such as sclerotinia wilt/rot, southern wilt and powdery mildew. Interspecific hybridization between JA, *H. tuberosus* and other *Helianthus* species has been attempted for introgression of disease resistance, stress tolerance and other advantageous genes. In most instances, the objective has been to move critical genes into *H. annus* the dominant crop of the oil seed family. However such crosses have also provided improved traits for cultivated JA (Kalloo 1993; Encheva *et al.* 2003, 2004). Low fertility is a main problem in these crosses because meiosis in JA is irregular. Efforts have been made to overcome through direct organogenesis and some hybrids between *H. annuus* and *H. tuberosus* have been developed which show resistance to the parasite broomrape, and the fungal pathogens *Phoma macdonaldii* and *Plasmopara halstedii*, the causal agents of sunflower black stem and downy mildew, respectively (Encheva *et al.* 2004).

Jerusalem artichoke as a source of genes

JA served as an excellent source of genetic material for transformation of other crop species. Genes for fructan biosynthesis including 1-sucrose:sucrose fructosyltransferase (1-SST) and 1-fructan:fructan fructosyltransferase(1-FFT) have been isolated, purified and characterized from JA and introduced into plants like *Petunia* where their expression has led to fructan biosyntheis in tissues (Van der Meer *et al.* 1998; Sevenier *et al*. 2002). These genes have also been used to transform sugarbeet which is an industrial crop with an established processing infrastructure for the production of sucrose (Sevenier 2002). Sugarbeet was transformed using *35s-1-sst* and *35s-1-fft* constructs and the resulting transformants show accumulation of fructans. The effici-

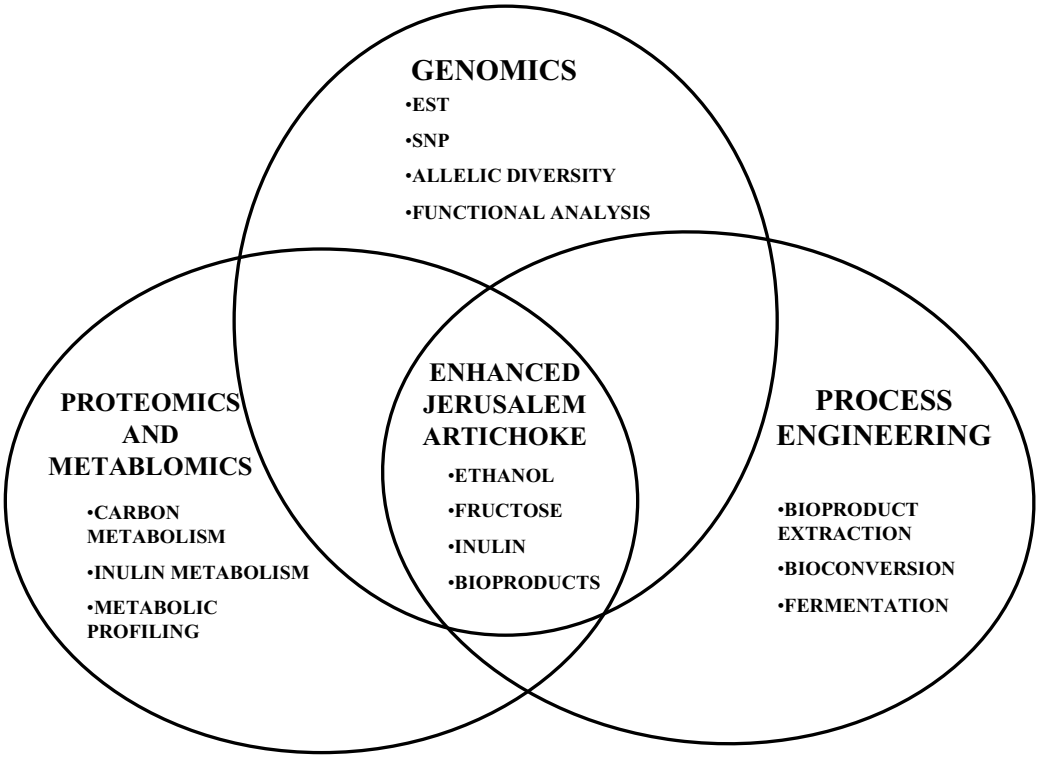

Fig. 3 Integrated approach to explore bioenergy capability of Jerusalem artichoke.

ency of fructan accumulation in sugar beet is higher with cDNA from JA than that from any other plant or microorganism (Smeekens 1998; Sevenier *et al.* 1998, 2002). Other genes which have been cloned, characterized and used for genetic transformation include cytochrome P450 (Didierjean *et al*. 2002), lectin (Chang *et al*. 2003) and ATP/ADP transporter protein (Meng *et al*. 2005). No attempt has been made yet to improve JA as a crop via genetic transformation. Genetic modification through genetic engineering of JA should not be difficult since regeneration by tissue culture was easily accomplished. The experience gained from the numerous sunflower transformations can also be extrapolated to JA.

Jerusalem artichoke genome

Jerusalem artichoke belongs to the compositae family and is a close relative of sunflower, an important cultivated oil seed crop. The genus is native to temperate regions of North America and contains 12 annual and 36 perennial species (Schilling and Heiser 1981). Cultivated sunflower (*H. annuus* L.) is an annual diploid (n = 17), whereas the JA is a perennial hexaploid (n = 51). Very little is known about the JA genome. The haploid DNA sequence length of JA was estimated as 0.23×10^{12} Da (Nze-Ekekang *et al*. 1974). The c value of JA DNA has been estimated to be 12.55 pg (Ingle and Sinclair 1972) and the genome size has been roughly estimated to be 10 Gb (Kays and Nottingham 2008).The task of sequencing a genome of this size is daunting so the lack of information is not surprising. As of 2009, Genbank has listed only 207 entries with DNA sequence information and 178 entries with protein sequences. In addition, cDNA library of around 6000 clones has been established in Canada to search for gene promoters that enhance the crop's value as a bioreactor (Elridge *et al*. 2005). Therefore, partial or complete gene sequences encoding for a relatively small cross section of polypeptides are available. Recently, Compositae Genome Project included *H. tuberosus* as one of the 21 species to develop resources for functional, comparative and evolutionary genomics of the genus. The main objective of this project is to identify ESTs (Expressed Sequence Tags) and using these as tags to identify complete gene sequences for as many proteins as possible. A total of 40,362 ESTs have been identified for *H. tuberosus* (http://compgenomics.ucdavis.edu/).

CONCERNS AND SOLUTIONS

Although JA possesses several unique properties including its capability to grow in marginal lands, it has the disadvantage of being invasive. Concerns have been raised that cultivation of JA could result in a serious weed problem as elimination of the plant is difficult because of the extensive underground tuber production. Frequent mowing of the top growth over a two to three-year period has been suggested to reduce heavy infestation. It has been observed that cereal crops compete effectively with JA by weakening JA plants for reduced tuber and rhizome formation. Plant breeding and agronomic remedies should also be taken into account while domesticating this weedy plant towards commercialization; these include identification of appropriate germplasm with large and approximately constant number of tubers. Breeding of such lines will be valuable to reduce its invasiveness. Introduction of such traits with JA's capacity to grow in marginal lands can, indeed enhance the value to the land and bio-energy sector.

CONCLUSIONS AND FUTURE AREAS OF RESEARCH

Regardless of our dependency on foreign oil, current levels of reliance on a petroleum-based industrial economy is not sustainable; crude oil reserves are finite and at current global consumption levels, the mathematical end of oil is predicted to occur in less than 70 years (McLaren *et al.* 2005). Consequently, development in the near term of viable alternatives is imperative. Further, the search for renewable energy sources must also address environmental concerns, like greenhouse gas emissions. A domestic, biomass source offers an alternative to conventional sources and provides more energy security, economic growth and environmental benefits. Plant-based systems are key to such approaches because they capitalize on renewable solar energy sources. Although biofuels offer a diverse range of promising alternatives to petroleum-based products, at present ethanol constitutes the majority (99%) of all biofuels in the US.

JA is an ideal crop for bioenergy production because of the unique properties of its tuber, stems and leaves and its high biomass production – even under extreme environmental conditions. Although the idea of bio-based resources has received much attention, little research has been conducted to optimize crop plants for energy capture or for the harvest of their structural materials. The modern tools of biotechnology can be used in a number of ways to develop optimized JA phenotypes for energy production, for example, by improving the plant's physical structure to enhance extraction of its energy components, thereby providing a more robust energy source. To provide a more optimal energy source, the first step is to develop a detailed understanding of the function of the genes involved in the traits related to energy production, *e.g.*, development of biomass and tubers, inulin enhancement and accumulation in tubers and stem, in order to increase the total biomass harvest for biofuel production. Through a more in-depth understanding of these processes it should be possible to create JA varieties with specifically designed phenotypes for the bioindustry through molecular breeding and process engineering approaches (**Fig. 3**). Being a native Canadian crop, it will be in the interest of Canadian agriculture and bio-resource development plans to tap this valuable resource and develop it into a commercial bio-energy and bio-product crop. To achieve this purpose research should be intensified on both the development of JA as a crop and optimizing the technologies for efficient bioconversion and production of biofuel from this crop species. Possible research areas include developing tools for functional, comparative and evolutionary genomics to improve traits of economic importance in JA (**Fig. 2**). For determining the extent of variability in the gene pool of JA allelic diversity should be determined using advanced molecular techniques. Since translocation of sugars to the tubers starts after flowering, it is important to identify genes that regulate these processes. Modification in genes for flowering would prolong the growing season and increase the amount of sugar that can be eventually translocated to the tubers. To optimize bioconversion, it is important to improve the efficiency of both the hydrolysis and fermentation of inulin. This includes research for improving inulinase production by microorganisms and the efficiency of its action, identifying more strains that can perform simultaneous saccharification and fermentation and designing industrial reactors for SSF. Research on metabolomics of JA involving metabolic profiling of the various plant parts for biproduct enhancement and identification of novel biproducts is also an area of important consideration. With consistent research efforts JA can be converted from an underutilized and neglected plant to a viable resource for production of bio-ethanol and other innumerable bi-products.

ACKNOWLEDGEMENTS

Thanks for Han Qi Tan for critically reading the manuscript and providing help to refine figures.

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