TURNING FOOD INTO FUEL: GM DROUGHT TOLERANT SOYBEAN AND ITS USE IN THE PRODUCTION OF BIODIESEL

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FOOD FOR FUEL

DROUGHT TOLERANCE

Water plays a crucial role in the survival of all organisms. In plants in particular, aside from fulfilling the roles of solvent, transport medium and evaporative coolant, water provides the energy necessary to drive photosynthesis, the natural plant process which synthesizes organic food. [1] Photoautotrophs are organisms that posses their own chlorophyll and are thus able to harness the energy associated with sunlight, in a process called photosynthesis. Under drought conditions the loss of water in the plant protoplasm may result in the concentration of ions in the protoplasm to toxic levels resulting in possible protein denaturation and membrane fusion and negatively impacting plant metabolism.

Water deficiency is a severe limiting factor in several countries and impacts on both food production and the economies of these countries. Approximately four tenths' [2] of the world's agricultural land is in arid or semi-arid regions with transient droughts causing death of livestock, famine and social dislocation. Several agricultural regions are reliant on irrigation to maintain yields. Climate change has been implicated in the fact that drought areas have more than doubled in the last thirty years.

Those crop plants which can make the most efficient use of water and maintain acceptable yields will be at an advantage in these regions. Research into drought tolerance and mechanisms for improving drought resistance are underway internationally to provide solutions to the problems of water deficiency, to save water used in agriculture and to ensure the development of sustainable agriculture. This includes research into elucidating the mechanism of drought tolerance in plants – different plants have different genetic makeup and hence different abilities for drought tolerance.

PLANT DROUGHT AND STRESS TOLERANCE MECHANISMS

There are several mechanisms of drought and stress tolerance in plants and research is currently underway to elucidate the mechanisms by which plants can survive during periods of water deficit.

Several drought tolerant plants occur naturally. Molecular biology has yielded several tools such as molecular markers that can be used under traditional breeding methods assist in the screening and identification of new drought tolerant plants and varieties in different genebanks. [3] Maize variety ZM521 for example, yields up to 50 per cent more than traditional varieties under drought conditions.

In 1998, Peter McCourt of the University if Toronto, isolated a gene that controls drought tolerance in plants. [4] The paper published in Science [5] outlines howplants endure periods of drought without dying. The plant hormone abscisic acid (ABA) can impact on plant control and development by mediating control of stomatal aperture in leaves.[6] Abscisic acid is controlled by the ERA1 gene and by inhibiting the gene's action; a plant becomes super-sensitive to drought. Suppression of the gene, hence closure of the stomata, enables control of water loss so that plants can last longer despite the onset of adverse conditions.

This study spawned several research programs into drought stress and tolerance by genetic manipulation and had led to a collaboration between the University of Toronto (UT) where McCourt is based and Performance Plants Inc. Testing is underway to apply this drought tolerance mechanism to canola and technology is being developed in other major species including corn, soybean, cotton, ornamental plants and turf grasses with a commercially available drought tolerant corn expected to be available by 2010. [7]

Outside of the UT, the ABA mechanism of drought tolerance has attracted much research attention as a potentially useful trait in selecting for drought tolerance in crops. [8],[9],[10] Beside the ABA mechanism for drought tolerance, several other studies are being conducted to identify other mechanisms andgenes related to drought tolerance and their biological functions.

CURRENT PROGRAMS TO ENGINEER DROUGHT TOLERANCE

There are several programs underway, internationally to engineer drought tolerance. A variety of wheat containing a gene from barley which requires one eighth as much water as its conventional counterpart is undergoing bio-safety testing in Egypt in preparation for commercialization. The International Maize and Wheat Improvement Center (CIMMYT) is currently evaluating a drought tolerant transgenic wheat variety which may be ready for commercialization within five years. Switching on transgenes is also an active area of research. The CIMMYT transgenic drought tolerant wheat, for example, does not do as well under conditions of sufficient rainfall as under water-deficient conditions and research is underway to switch on the drought tolerance mechanism only under conditions of water stress to reduce or eliminate yield drag.

The University of Connecticut (UC) has engineered adrought resistant tomato by enabling transgenic tomato plants to produce more of the enzyme H+-pyrophosphatase (H+-PPase) which was shown in *Arabadopsis* plants to confer resistance to drought. The UC is currently studying this effect in rice, poplar trees and legumes.[11] Cornell University reported a new strategy for genetically engineering rice and other crops to make them more tolerant of drought, salt and temperature stresses, whilst improving yields. [12]

Monsanto’s drought tolerant corn may be ready for commercialization as early as 2010 and studies on drought tolerant soybean and cotton are in the pipeline. [13]

Additionally, Bayer, [14] Syngenta, [15] Dow, BASF [16] and Dupont [17] all have extensive research programs in the area of drought tolerance. [18]

In South Africa alone, several institutions and organizations are involved in or may have units especially dedicated to the study of stress tolerance, albeit not only by genetic manipulation. These include, amongst others, The Agricultural Research Council (ARC) Institute for Tropical and Subtropical Crops (ITSC), the Foundation for Research Development (FRD) Arid Zone Ecology Forum (AZEF), The Grootfontein Agricultural Development Institute, the National Botanical Institute (NBI) Stress Ecology Research Unit, the University of Fort Hare Agricultural and Rural Development Research Institute (ARDRI), the University of Cape Town (UCT) Plant Stress Research Unit and the University of the Witwatersrand School of Molecular and Cell Biology.

The South African plant*Xerophyta viscose*(known as isiphemba or isiqumama in Zulu) has many medicinal applications including treatment for asthma, nose bleeds, general aches and as an anti-inflammatory. *X. viscose,* a so-called resurrection plant, is able to survive long periods without water and has the remarkable property of being able to rehydrate completely and resume full metabolic functions within 24 to72 hours, depending on the species. [19] Scientists at the Plant stress Research Unit at UCT are studying *X. viscosa* genes that code for proteins responsible for the resurrection phenomenon. Several of the genes implicated in this drought tolerance have been identified and are being cloned into drought sensitive species of plants such as the monocot grass *Digitaria sanguinalis* and the weed *Arbidopsis thaliana*. Future plans include engineering tolerance in agronomically important crops such as wheat and maize. Another plant under study in South Africa is the *Xerophyta humilis*.[20]

THE DEVELOPMENT OF GM DROUGHT TOLERANT SOYBEAN IN SOUTH AFRICA

Soybean production in South Africa is affected by frequent periods of drought. The Agricultural Research Council (ARC) embarked on a program of producing genetically modified soybean to withstand drought conditions by the application of sense and antisense gene technology. [21] More specifically, biosynthesis of the amino acid proline under conditions of drought stress was explored. Proline levels had been observed to increase far in excess of protein synthesis requirements during periods of drought [22] and it was thought that this was an adaptive mechanism by the plant to counter the accumulation of NADPH that would occur under conditions of reduced photosynthesis.

The Soybean cultigen *Glycine max* (L.) Merr cultivar Ibis were transformed using a patented non-tissue culture*Agrobacterium*-mediated vacuum infiltration transformation system for soybean (patent SA 2001/3076 in the name of ARC/PRF). [23] The *Arabidopsis* L-D1-Pyrroline-5-carboxylate reductase (P5CR) gene was cloned in the sense and antisense orientation into a heat shock cassette (hsE2019) containing an inducible heat shock promoter (IHSP)·[24] which allowed proline gene expression and production to be manipulated under conditions of drought stress. Further, the transgenic line has kanamycin resistance marker gene under the control of the nopaline synthase promoter. ARC analyses found that transgenic plants contained at least 5 copies of the P5CR gene with at least three integrations.

By 2002, these transgenic plants had already been tested in the ARC greenhouse for three years and were in their fourth generation. The 2002 application by ARC to the South African Department of Agriculture (permit 17/3(4/02/233)) was granted and six genotypes that indicated improved drought performance under greenhouse conditions, one antisense line and three wild type cultivars were planted in a rainout shelter trial. ARC reported that all six sense transgenic lines were more heat tolerant than the three wild type cultivars and that four of these sense lines produced very high yields under drought conditions.21 In May 2005, ARC requested permission under the Department of Agriculture's fast-track application system, to extend the field trial period for two more growing seasons. [25] The four identified high yield sense transgenic lines would be further tested at 4 different locations. This permission was granted (permit 17/3(4/05/080)), and these trials are currently underway.

Seedstock from these trials is being stockpiled by ARC and the original application states that if the transgenic lines proved more drought tolerant than the parent line that breeding trials might be conducted (page 3 of application). These field trials appear to have attracted a great deal of attention from several players involved in plant biotechnology (University of North West, University of Johannesburg, and Protein Research Foundation) as well as CSIR (Council for Scientific and Industrial Research) Bio/Chemtek which is the business arm of the CSIR. The current trials are being conducted by all of these bodies in consortium.

In October 2006, the CSIR announced the receipt of funding from the Department of Science and Technology to conduct research into the potential uses for biodiesel waste with soybeans and sunflower seeds being the two main crops used for biodiesel production.[26] This, according to the CSIR, "links well with related investigations" by the CSIR and ARC. Any full-scale biofuel or biodiesel initiative would necessitate a steady and reliable source of feedstock as might be satisfied by a drought tolerant crop. It is assumed that the development of these drought resistant soybean studies form part of these "related investigations".

BIOFUEL & BIODIESEL

Biofuels is any fuel that derives from biomass i.e. recently living organisms or their metabolic by-products, such as corn grain or manure from cows. Unlike natural resources such as petroleum, coal and nuclear fuels, biofuels are a renewable energy source. [27] Several agricultural crops are grown specifically for the production of biofuels including corn and soybeans (United States), flaxseed and rapeseed (Europe), sugarcane (Brazil) and palm oil (South East Asia). Biodiesel is a biodegradable transportation fuel for use in diesel engines that is produced through transesterification of organically derived oils or fats. [28] Biodiesel can be made from soybean oil, canola oils, animal fats, waste vegetable oils, or microalgae oils. [29]

TURNING FOOD INTO FUEL

Both the White Paper on Energy Policy as well as the Integrated Rural Development Strategy with regard to the supply and consumption of energy in South Africa, highlight the greater need for the development and implementation of renewable energy applications and their application to rural development to develop internal capacity for integrated and sustainable development. One of the identified sources of alternative energy is the development of biofuels. There is a great deal of interest in biodiesel worldwide because it is made from renewable resources and reduces air-polluting emissions by diesel engines.

Typically, many commercially available biodiesel fuels are made from soy oil and then added to diesel to levels of twenty percent (v/v). Several technologies are being applied to the production of biodiesel from lower value less pure lipids such as soapstock and not only from highly refined oils as has been done thus far.[30] Soybean oil soapstock is a plentiful and relatively inexpensive resource and is seen as the ideal candidate for the application of this technology. How this will impact on the energy balance calculation is not clear.

The value of biofuels as part of an alternative energy strategy is hotly debated worldwide. No consensus has been reached on the net energy value of biofuels and publications on both sides of the debate highlight the inadequacy of the currently applied calculations of arriving at a consensus figure. [31],[32],[33] It is recognized however that the costs of renewable energy technologies, compared to conventional energy supplies for bulk energy supply require significant initial investment and potential long term support before reaching profitability. The energy balance calculations are fraught with difficulty because of the choice of inputs of the various researchers. Some researchers ignore inputs such as natural gas, electrical inputs and labour, whilst over-emphasising the value of by-products. The results of a comparative study of different approaches to calculating inputs were reported earlier this year. This study took all the identified inputs from several papers and fed them into the same model. [34] There was no significant shift in the movement of the figures from the negative to the positive.

Soybean is viewed as a very attractive crop for the production of biodiesel. Pimentel and Patzek conducted a net energy analysis for deriving biodiesel from soybeans. [35] Soybean yield is as high as 2,668 kilograms/hectare translating into 480 kg of oil per hectare, as compared to say sunflower seeds which will produce only 390 kg of oil. Soybeans can fix atmospheric nitrogen and require minimal nitrogen fertilizer inputs, which often can account for the single largest energy input in agriculture.

The high energy cost however, lies in processing with about 11.9 million kcal required to produce 1000kg of soy oil which will have an energy content of 9 million kcal. This translates to a net energy loss of 32%. The contribution of the soy meal produced as a useful by-product, when added to the energy calculation, reduces this net energy loss to 8%. Still, at 2002 figures soy oil was found to be 2.8 times more expensive than petroleum diesel to produce. The use of a genetically modified crop such as the soybean discussed above for the production of biodiesel would only add to the cost because of the economics associated with the growth of transgenic plants. Patent protection of GE crops ensures that there is a fixed cost associated with their planting which would have to be taken into consideration in the energy calculation.

Ultimately, biofuels are not a green option. It is estimated that the replacement of all our fossil fuels consumption with biofuels would require at a minimum, 22% of the net primary productivity (NPP) of the Earth’s current biota. A figure, that would be substantially higher if biofuel production remains less energy efficient than the generation of energy from fossil fuels. All of the prime productive land has already been given up to agriculture. How much more would be required or have to be appropriated to serve the needs of producing crops for energy.

It would be more energy efficient to lay a field of photovoltaic panels and get 200 times the amount of energy than it would be to plant maize on a hectare of land.[36] However, the reason for planting maize should be for food and feed and not for producing food crops for the production of energy. Producing crops for the production of biodiesel is not only costly, but subverts valued human food and animal feed from direct use.[37] Further, it would require economic inputs from government in order to be viable, inputs that could be more appropriately allocated within the current South African socio-economic context.

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