

# MONSANTO'S GENETICALLY MODIFIED DROUGHT TOLERANT MAIZE IN SOUTH AFRICA

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## INTRODUCTION

During March 2007, the South African GMO authorities gave Monsanto permission to conduct experiments involving GM drought tolerant maize in open field trials in South Africa. As a result of the extremely limited opportunities for civil society to intervene in GMO permit applications in South Africa the African Centre for Biosafety (ACB) was prevented from objecting to the application in a timely manner. Nevertheless, we offer this paper as a contribution to the biosafety discourse and our commitment to monitoring the GM industry.

Our research has revealed that transgenic drought tolerance is at least 8 to ten years away from approaching commercialisation,<sup>1</sup> and involves a large set of genes in the expression of a complex trait like drought tolerance.

Nevertheless GM drought tolerant crops are being used as powerful PR tools by the biotech machinery and strategic philanthropy such as the Rockefeller Foundation to promote acceptance of GM crops, expand existing markets and develop new markets.<sup>2</sup> The field trials in South Africa is designed to win Monsanto credibility in Africa since it can now claim that it is developing GM crops adapted to the needs of poor African farmers. Already Monsanto is claiming that drought-tolerant technology would lead to yield insurance, yield enhancement and cost-savings on irrigated land<sup>3</sup> and is reported as stating that trials conducted during 2006 showed an increase in yield of 23.2% compared to non-GM production.<sup>4</sup>

Recently, Europabio's Simon Barber revealed that GM drought tolerant crops would go a long way towards changing European perceptions towards GMOs, particularly in Eastern and Central Europe where their use is limited due to moral and health concerns.<sup>5</sup>

## ABIOTIC STRESS: DROUGHT

### DROUGHT TOLERANCE

Water plays a crucial role in the survival of plants by fulfilling the roles of solvent; transport medium and evaporative coolant as well as providing the energy necessary to drive photosynthesis, the natural plant process that synthesizes organic food.<sup>6</sup> 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<sup>6</sup> 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<sup>7</sup> 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. 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.

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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 tools can assist in the screening and identification of new drought tolerant plants and varieties.<sup>8</sup> Maize variety ZM521 for example, yields up to 50 per cent more food than traditional varieties under drought conditions.

A 1998 paper published in *Science*<sup>9,10</sup> outlines how plants 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.<sup>11</sup> 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.<sup>11</sup>

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.<sup>12</sup>

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.<sup>13,14,15</sup> Beside the ABA mechanism for drought tolerance, several other studies are being conducted to identify other mechanisms and genes related to drought tolerance and their biological functions.<sup>16</sup>

### **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.

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The University of Connecticut (UC) has engineered a drought resistant tomato by enabling transgenic tomato plants to produce more of the enzyme H<sup>+</sup>-pyrophosphatase (H<sup>+</sup>-PPase) which was shown in *Arabidopsis* plants to confer resistance to drought. The UC is currently studying this effect in rice, poplar trees and legumes.<sup>16</sup> 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.<sup>17</sup>

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.<sup>18</sup> Additionally, Bayer,<sup>19</sup> Syngenta,<sup>20</sup> Dow, BASF<sup>21</sup> and Dupont<sup>22</sup> all have extensive research programs in the area of drought tolerance.<sup>23</sup>

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 to 72 hours, depending on the species.<sup>24</sup> 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.<sup>24</sup> Another plant under study in South Africa is the *Xerophyta humilis*<sup>25</sup>

#### **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.<sup>26</sup> 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<sup>27</sup> 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.<sup>28</sup> By 2002, these transgenic were in their fourth year of testing by ARC.<sup>28</sup> In May 2005, ARC identified four high yield sense transgenic lines that are currently being tested in field trials.<sup>29</sup> Seedstock from these trials is being stockpiled by ARC and if

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the transgenic lines prove more drought tolerant than the parent line breeding trials might be conducted.<sup>28</sup>

## **DROUGHT TOLERANT GM MAIZE**

### **IMPORTATION AND FIELD TRIALS OF DROUGHT TOLERANT GM MAIZE INTO SOUTH AFRICA**

Monsanto have been granted permits (Permit No. 17/3(2/07/014)) by the South African Department of Agriculture, firstly to import 100kg of drought tolerant GM Maize and secondly to conduct a trial release of this maize (Permit No. 17/3(4/07/015)). The maize events in question, designated ZM\_M39872, ZM\_M38714, ZM\_M38721 and ZM\_M38835, have been produced by *Agrobacterium* mediated transformation with the plasmid vector PV-ZMAP595.<sup>30</sup> The stated aim of these trials is to test the efficacy of these events to increase yields. Further suggestion is that this assessment will be to assess suitability of the events for Feed and Food (page 2 of the application).<sup>30</sup>

The table of genetic elements of the PV-ZMAP595 construct include an octopine left border sequence, a nopaline right border sequence, a Lox site derived from Bacteriophage P1, a neomycin phosphotransferase II gene, a Nos terminator, a second Lox site and several other elements, including the gene expression cassette which have been designated Confidential Business Information and not included in the information we have received. Also information on the “probable consequences (positive and negative), of the release of such an organism, including impacts on human, animal or plant health” have also been designated as Confidential Business Information (page 15 of the application).<sup>30</sup>

## **ENGINEERING STRESS TOLERANCE**

### **IMPRECISION OF PLANT MODIFICATION TECHNIQUES AND POSSIBLE CONSEQUENCES**

The lack of complete sequence information makes an assessment of the gene expression cassette nigh impossible. *Agrobacterium*-mediated transformation is characterized by multiple fragments and gene rearrangements.<sup>31,32</sup> Inserted gene sequences may interrupt native gene sequences and/or their promoters and additional code fragments are not necessarily non-functional and may be transcribed. Extra gene fragments in Monsanto's Roundup Ready Soya were also claimed to be non-functional and not-transcribed,<sup>33</sup> but were later found to be transcribed to produce RNA.<sup>34,35</sup> Unintended effects that are not detected in the lab and that may only become apparent in the long term cannot be ruled out.

Further, it is not clear if the insert or fragments thereof lie on any transposons and what the impact of the DNA insert is on flanking sequences. The lack of sophisticated methods for targeted insertion,<sup>32</sup> especially in higher organisms necessitates more rigorous research into possible position effects prior to the granting of any release of

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transgenic organisms into the environment. Further, if transgenes behave just like naturally occurring genes, then they have the potential to be inherited in the same way and persist indefinitely in cultivated or free-living populations. Any mixing of native and transgenic plants whether by dispersal, improper handling etc., can result in the spread of transgenes. The consequences, both ecological and evolutionary of crop-to-crop gene flow are only now beginning to be investigated in any meaningful way and the possible exposure of non-target organisms, including humans to novel proteins cannot be discounted.

### **ANTIBIOTIC RESISTANCE MARKER GENES**

Antibiotic resistance marker genes are used often in the development of transgenic crops as selectable markers. Selectable markers allow the modified form to be selectively amplified while unmodified forms are eliminated. The use of antibiotic resistance markers has application in development of the transgenic line allowing for selection of modified plants in the laboratory. The transgenic crop line however, will retain the marker gene for its lifetime in each of its cells. The *nptII* gene from *Escherichia coli* expresses the enzyme neomycin phosphotransferase II (NPTII), which inactivates principally kanamycin, geneticin and neomycin by phosphorylation, that is used to select transformed cells.

### **HORIZONTAL GENE TRANSFER (HGT)**

Horizontal gene transfer (HGT) is the transfer of genetic material between organisms, outside the context of parent to offspring reproduction<sup>36,37</sup>. It is most commonly recognized as infectious transfer<sup>38</sup>. HGT frequencies are now known to be much higher than originally thought. The evolution of antibiotic resistance, for example, is an indicator of the frequency of gene transfer, given that antibiotics have been used in medicine only for about 50 years<sup>38</sup>. The intentional modification of plants could through horizontal gene transfer result in the unintentional modification of other organisms. What the possible impacts of such gene transfer might be is not known.

### **POTENTIAL FOR HGT OF ANTIBIOTIC RESISTANCE MARKER GENES (ARMG)**

The significance of any potential gene transfer is dependent on the marker being transferred and what its existing or future therapeutic application is or might be. Kanamycin, contrary to popular belief, is still used in medical applications, e.g. prior to endoscopy of the colon and rectum<sup>39</sup> and to treat ocular infections<sup>40</sup>. It is well known that there is cross resistance between antibiotics of a particular type<sup>37</sup>. Neomycin was found to cross react with kanamycin B in inhibiting RNase P ribozyme 16s ribosomal RNA and tRNA maturation<sup>41</sup>. Other aminoglycoside antibiotics including streptomycin, gentamycin and tobramycin, which are used to treat human disease, have exhibited cross resistance<sup>37</sup>. The possibility of transfer of the marker by HGT, and subsequent adverse effects on human and animal health, cannot be ruled out in those cases where these antibiotics are still being used.

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## RESISTANCE OF DNA TO DIGESTION

There are however several reported cases in the literature of both the persistence and transfer of gene sequences after ingestion of GM products. Polymerase chain reaction (PCR) has been used to demonstrate the presence of large fragments of M13 phage DNA, which had been fed to mice, in the faeces and bloodstream and in white blood cells<sup>42</sup>. Research published by the UK government in 2002 has shown that bacteria in human intestines had in fact taken up a novel gene from processed food containing GM Soya<sup>43</sup>. It has been reported that people with ileostomies (i.e. who make use of a colostomy bag) are capable of acquiring and harbouring DNA sequences from GM plants in the small intestine<sup>44</sup>. Recombinant DNA fragments and Cry1Ab protein was also found in the gastrointestinal contents of pigs fed genetically modified corn<sup>45</sup>.

Several European countries including Austria, Luxembourg, France, Norway and the United Kingdom have expressed grave concerns about the presence of antibiotic genes in GM products and the EU has as a result, decided to prohibit GMOs with antibiotic resistance genes after the 31<sup>st</sup> December 2004 (directive 2001/18EC and Revising Directive 90/220/CEE)<sup>46</sup>

## POLLINATION

The Monsanto application to the South African government states that current agronomic processes will control any maize volunteers. It is not expected that the GE maize will become a persistent or invasive weed, should a seed spill or inadvertent planting occur. However, maize plants have been shown to survive over a growing season, under comparatively colder conditions<sup>47</sup> than found in South Africa. Should any volunteers arise, the resulting pollen could cross-pollinate with maize in fields, producing genetic contamination. Recently conducted research by the University of Exeter applied a new method for predicting the potential for cross-pollination, which takes account of wind speed and direction. The findings showed huge variation in the degree of cross-pollination between GM and non-GM crops of maize, oilseed rape, rice and sugar beet.<sup>48</sup> The levels vary depending on whether the GM field is upwind or downwind of the non-GM field. Current guidelines relating to field-to-field distances do not take into account this variation. If the GM field in a trial is downwind of the non-GM field, the trial will underestimate the potential for cross-pollination.<sup>48</sup>

## CONCERNS WITH DROUGHT TOLERANT PLANTS

Promoting the idea of a gene tolerant plant is very attractive, especially in semi-arid South Africa and more so in the light of the current debates relating to climate change and the potential difficulties the region might face. The reality is that drought tolerant crops are a long way off. Improved stress tolerance in plants by genetic methods requires a better understanding of the underlying physiological, biochemical and molecular events.<sup>49</sup> However, the fundamental mechanisms of stress tolerance in plants are not well understood.<sup>50</sup> The coding for drought tolerance in particular, is incredibly complex with up to as many as 60 genes implicated, all interacting in a subtle and complex way. The

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successful manipulation and transfer of many complex genes, which can respond to a variety of conditions, and not produce unwanted toxins and allergens, is a long way off for current scientific knowledge with some geneticists admitting that even hoping for drought tolerance in the next 10 or 20 years may be too ambitious.<sup>51</sup>

Plants modified to withstand abiotic stress are much more susceptible to external influences than say plants modified for insect resistance. Environmental stresses such as drought are unpredictable in various respects such as timing and intensity. Other variables include the presence of disease, day length, temperature, and soil characteristics.

### **MARKER ASSISTED SELECTION (MAS)**

We are aware of several marker-assisted selection (MAS) techniques being developed for the improvement of polygenic traits. The advances in the development of molecular tools has allowed for improved identification, mapping and isolation of genes in a wide range of crop species.<sup>52</sup> Initially, markers called restriction fragment length polymorphisms (RFLPs), were used to construct linkage maps for several crop species, including maize, tomato, and rice. Later the polymerase chain reaction (PCR) revolutionized molecular marker assays because of the easy and suitable for automation.<sup>52</sup>

MAS has been touted as a promising non-GM modern biotechnology technology by industry players such as Syngenta who has been conducting research on drought tolerance in sugarbeet using MAS.

### **TRADITIONAL BREEDING**

Traditional breeding and selection methods have served farmers well in identifying drought tolerant plants. It is well documented that approaches to improving crop quality by enhancing soil quality greatly improves water retention, and generally improves crop growth, at much less cost. The US Rodale institute has carried out long-term comparisons between organic and conventional crops and found that during the drought years the organic yielded better because the soil holds more water.

## **CONCLUSIONS**

Of particular concern in the Monsanto application is the omission of the human health impacts from consuming the transgenic events under consideration from the publicly available dossier. This combined with the lack of sequence information make an assessment of the available information extremely difficult if not impossible. This hampers the public's ability to contribute or engage meaningfully in any discussions regarding GE foods or be able to make informed choices about matters that so closely impact on them.

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The ability of ecosystems to develop gradually, the ability to anticipate environmental health effects and very importantly, the establishment of regulatory mechanisms that can effectively, efficiently and credibly manage risks associated with the use of GMOs has not kept pace with the rapid introduction of GMOs. Traditional breeding practices have an established history of safe use dating back several years as opposed to the application of recombinant DNA technology for human use, which is as young as 22 years when genetically modified bacteria-produced insulin was first introduced and even younger for genetically modified plants at ten years.

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