Advances in Life Science and Technology
ISSN 2224-7181 (Paper) ISSN 2225-062X (Online)
Vol.48, 2016 www.iiste.org

Genetic Improvement of African Maize towards Drought Tolerance: A Review

Joshua Kiilu Muli[1*] Nancy L. M. Budambula[2] Cecilia Mweu[3] Mary C. Imbo[1] Sylvester E. Anami[3]
1.School of Biological Sciences, Jomo Kenyatta University of Agriculture and Technology, P.O.BOX 62000-00200, Nairobi, Kenya
2.Department of Biological Sciences, Embu University College, P.O.BOX 6-60100
Embu, Kenya
3.Institute of Biotechnology Research, Jomo Kenyatta University of Agriculture and Technology, P.O.BOX 62000-00200, Nairobi, Kenya

Abstract

Africa supports a population of over 1 billion people with over half of them depending on maize for food and feed either directly or indirectly. Maize in Africa is affected by many stresses, both biotic and abiotic which significantly reduce yields and eventually lead to poor production. Due to the high demand for maize in the region, different improvement strategies have been employed in an effort to improve production. These include conventional breeding, molecular breeding, high throughput phenotyping techniques and remote sensing-based techniques. Conventional breeding techniques such as open pollination have been used to develop drought avoiding maize varieties like the Kito open pollinated variety (OPV) of Tanzania and Guto OPV of Ethiopia. A combination of conventional breeding and molecular biology techniques has led to improved breeding strategies like the Marker Assisted Back Crossing (MABC) and Marker Assisted Recurrent Selection (MARS). These techniques have been used to improve drought tolerance in existing inbred maize lines like the CML 247 and CML 176. Through genetic engineering, different genes including *C4-PEPC*, *NPK1*, *betA*, *ZmNF-YB2*, *cspB*, *ZmPLC1* and *TsVP* have been cloned in maize. Transgenic maize crops expressing these genes have shown increased tolerance to drought stress. Although there is substantial progress towards developing drought tolerant maize, many African farmers are yet to benefit from this technology. This is due to lack of an enabling policy framework as well as a limited financial investment in biotechnology research.

Keywords: Maize, Drought tolerance, Genetic engineering; Biotechnology; Transgenic crops

1. Introduction

Maize is a staple food to more than 1.2 billion people in Sub-Saharan Africa (SSA) and Latin America. The whole plant can be used for either food or non-food purposes. It is the second most widely grown cereal crop for human consumption after wheat in many parts of the world hence its importance towards achieving food security cannot be overlooked. In Africa, maize production is affected by both biotic and abiotic stresses (Mugo and Hoisington, 2001). Africa largely depends on agriculture hence the need for improvement in technology to achieve food security. In SSA, maize contributes up to 51% of all the consumed calories in the diet. However, yields are variable and sometimes may be as low as 2t/ha (FAO, 2015). Most developed countries use maize as an animal feed and for the industrial production of ethanol, soaps, paints, corks, powders and pharmaceuticals. The main use of maize in developing countries remains food consumption.

Agricultural production in Africa faces many challenges as a result of biotic and abiotic stress factors affecting crop production. Since these stress factors affect maize yield, attempts to reduce their effects and improve yields have been made through the concerted efforts of conventional breeding and genetic engineering. Through genetic improvement, genetics and plant breeding, biotechnology principles have been consolidated to increase the ability of maize to tolerate drought. Drought tolerant traits that have been identified in maize plants by either conventional breeding or molecular means have been manipulated and delivered to maize to enhance tolerance to drought.

2.0 Genetic improvement of maize

Genetic improvement of maize refers to the application of any genetics, plant breeding as well as biotechnology principles with the aim of identifying and exploiting the genetic variation within maize populations. Genetic improvement of maize for tolerance to drought involves first, the identification of genetic traits which are heritable and variants which respond to drought stress in any breeding population. This is the most important requirement to achieving any meaningful progress towards drought stress tolerance. It has been suggested that drought stress alleles occur at very low frequencies in a breeding population, hence the importance of evaluating these populations before any work can commence (Blum, 2011). Although natural populations may possess unique alleles, they are often limited by undesirable traits such as low yields and poor adaptability (Edmeades, 2013). The most important primary trait in any breeding population is grain yield under stress and unstressed conditions. Other important secondary traits include; leaf erectness, canopy temperature, the number of kernels per cob as well as other important cob and grain traits like kernel weight, which contributes to grain yield (Jansen, 2012).

Earlier improvement attempts in maize involved selection based on plant phenotype, where seeds from the best maize plants would be stored and used to improve subsequent generations. Today, genetic improvement is based on the genotype and involves the use of genetic markers which identify polymorphisms between individuals (Mitra, 2001). The bulk of maize genetic improvement, therefore, is based on techniques which improve the genotype such as marker assisted breeding and genetic engineering since improving the genotype can result in the desired phenotype.

3.0 Status of maize biotechnology in Africa.

Biotech crops were commercialized in 1996 with 1.7 million hectares of genetically engineered (GE) crops being cultivated. By 2011, a 94 fold growth rate had been experienced resulting in about 160 million hectares of GE crops. Of all the countries which had planted GE crops in 2014, 71% were developing countries with a total area of 181 million hectares (James, 2014). However, Africa's share of GE crops was less than 1.6% with only South Africa, Burkina Faso, Egypt and Sudan having commercialized crop biotechnology. Only South Africa and Burkina Faso are involved in substantial plant biotechnology with the cultivated land under GE totaling to 2.7 and 0.5 million hectares respectively (Falck-Zepeda *et al*., 2013). The main targets for improvement in African maize are agronomic and morphological factors such as yield, drought tolerance, insect resistance, disease tolerance and resistance to the parasitic weed Striga (Mugo and Hoisington, 2001). By 2014, confined field trials (CFT) for insect resistance, drought tolerance and herbicide tolerance in maize were going on in Uganda, South Africa and Kenya (Karembu *et al.,* 2014). The Water Efficient Maize for Africa (WEMA) project seeks to deliver the first biotech maize in Africa with resistance to insects (Bt) and drought by the year 2017 (James, 2014).

4.0 Abiotic and biotic stresses affecting maize production.

4.1 Abiotic stresses

Abiotic stress in reference to plants refers to an environmental condition that reduces the growth and eventually yields to levels below optimum. It is difficult to quantify how these stresses affect plants but their impact cannot be ignored. The most common abiotic stresses affecting maize production in Africa include; water stress, high temperatures and unfavourable soil conditions.

Water stress is in most cases manifested as drought and affects vast regions in SSA. About 22% of sub-tropical regions and 25% of lowland tropical regions experience drought annually. Drought leads to yield losses of up to 15%, 53% and 30% in maize when it occurs at pre-flowering, flowering and post flowering stages respectively (Heisey and Edmeades, 1997). When drought stress occurs at maize seedling establishment, it results in elongation of meristematic cells and reduced cell division, which eventually results in a reduction in growth rate of the maize seedling. At vegetative stage, maize plants are sensitive to water limitation. Drought at this stage leads to a reduction in fresh root weight, root length, root-shoot ratio and shoot elongation rate. In photosynthetic mechanisms, drought affects photosystem-II more than photosystem-I, leading to the production of high-energy electrons in the leaf, which results in photo-oxidation of chlorophyll. As drought severity increases, the plant's production of Reactive Oxygen Species (ROS) increases, leading to damage of chloroplasts and cell membranes hence subsequent cell and plant death (Aslam *et al.,* 2013). The occurrence of drought stress a week to and after silking in maize has been observed to initiate kernel and ear abortion (Heisey and Edmeades, 1997). Although it is difficult to measure the economic impact of drought stress, the obvious effects include; reduced maize yields, which may reach 100% under severe drought (Ali *et al.,* 2011).

Heat stress refers to the increase in temperatures beyond a certain point sufficient to cause irreversible damage to plant growth and development. Tolerance to heat, on the other hand, refers to the plant's ability to produce significant yields irrespective of the high ambient heat (Wahid *et al*., 2007). Heat causes crop damage as a result of the destruction of cellular organization. The damage may be direct or indirect and includes; protein denaturation, an increase in fluidity of lipid membranes, inactivation of enzymes and inhibition of protein synthesis. Maize has an upper heat threshold of 38ºC. Towards and beyond this threshold, many physiological aspects are affected and eventually yield decreases. Known physiological changes in maize include; increased pollen damage, reduced growth rate and reduction in the rate of grain filling as a result of a reduction in the rate of cell division, reduced starch biosynthesis and sugar metabolism (Cairns *et al.,* 2012). The photosynthetic activity of leaves growing at optimal temperatures has been shown to decrease when heat rises above 35ºC. This has been attributed to the reduction in quantum yield of electron transfer in photosystem-II due to a decrease in the photochemical quenching factor (Sinsawat *et al.,* 2004).

Soil stress in crop production is diverse. However, there are some common soil stresses encountered in SSA such as excess salts, water logging, and metal toxicity. Salts pose a challenge to plants since their excessive accumulation causes cytotoxicity and prevents the synthesis of enzymes and proteins. Excess salts also affect membranes structure and permeability. A salinity induced growth reduction model in maize which mimics the biphasic model of salinity-induced growth reduction described by Munns (1993) has been observed in maize (Fortmeier and Schubert 1995). High salt levels reduce the rate of seed germination by lowering the osmotic potential of the soil solution. This inhibits water uptake by the seed, causing sodium and chloride toxicity to the embryo. Carbon fixation in maize is inversely proportional to salt stress. In salty soils, therefore, maize plants have salt-induced reduction in photosynthesis as a result of denaturation of carbon fixation enzymes, reduction in stomatal conductance and destruction of photosynthetic apparatus (Sinsawat *et al.,* 2004).

4.2 Biotic stresses

Any stress that occurs due to substantial damage to a plant by a living organism is referred to as biotic stress. This leads to yield reduction especially in Africa where many farmers cannot afford the cost of controlling the stress causing agent. Pests and diseases are the most common biotic stresses in any crop production system in the world (Cairns *et al.,* 2012). In 2013, 25% of maize crop yield in Africa was lost to diseases alone. Most microbial pathogens causing maize crop diseases of economic importance in Africa are of fungal origin although viruses and bacteria also cause significant losses. Maize diseases such as the Turcicum leaf blight, Grey leaf spot (Northern leaf blight), Crazy top disease (Downy mildew), Head smuts and the common rusts are all caused by fungal pathogens. The maize streak virus, maize dwarf mosaic virus and the maize lethal necrosis are caused by viruses. The Stewart's disease (Bacterial wilt) and bacterial leaf spots are caused by bacteria (Guantai *et al.,* 2010).

Insects such as moths, for example, the African maize stalk borer and earworms, beetles including the grain borers and weevils, leaf bugs, maize fleas, and aphids cause maize yield reduction. Maize yield losses due to the parasitic weed Striga in Africa is enormous, with up to 60% of crop loss in SSA being attributed to Striga species (Kim *et al.,* 2002). This accounts for annual losses of over 7 billion US dollars in revenue (Suleiman and Rosentrater, 2015).

5.0 Approaches to improved maize production

5.1 Conventional breeding technique

All the early maize improvement efforts were directed towards increasing yield. The first significant contributor to maize yield increase in the early 18th century was the introduction of hybrids. A hybrid is a resulting cross of two different varieties with an aim to get the desirable attributes of each variety such as resistance to diseases, tolerance to drought, fruit size, colour, taste or any other attribute that may be considered important. Through crossing inbred lines resulting from inbreeding, maize breeders produced hybrids which had higher yields due to heterosis (Fraley, 2009). The use of hybrids combined with other emerging improvement techniques and good husbandry techniques increased the yields of maize and have continued to be used to date. Conventional breeding, however, is greatly limited by the existing low genetic variation, high costs and the long generation time it takes to produce a single improved selection (Gao *et al*., 2013). Grain yield is influenced by different factors such as growth rate and nutrient supply. Since many conventional breeding programs include grain yield as a parameter for selection, there is a need to understand the components that contribute to higher yields so as to improve the entire selection process.

5. Molecular breeding

Molecular breeding techniques used in maize improvement include Quantitative Trait Loci (QTL) mapping, Marker Assisted Selection (MAS) and transformation. Among the first techniques to be applied to improve maize populations was the use of molecular markers. Through these markers, various generations of DNA could be detected using techniques such as Restriction Fragment Length Polymorphism (RFLP), Simple Sequence Repeats (SSR'S), Amplified Fragment Length Polymorphism (AFLP) as well as Single Nucleotide Polymorphisms (SNP'S) (Prasanna and Hoisington, 2003). Genetic engineering of maize is now possible due to advances in technology involving two major gene delivery techniques; the direct DNA delivery technique and *Agrobacterium*-mediated transformation (Wang *et al.,* 2009). These biotechnological approaches have brought precision and speed in the breeding process.

5.2.1 Direct DNA delivery

There are several direct DNA delivery techniques used in plant transformation. These techniques deliver either physical or chemically derived DNA into intact cells, whole plants or protoplasts. They include particle bombardment, where a high-density tungsten or gold particle is coated with the nucleic acid to be delivered to the plant and accelerated by a helium pulse to penetrate several cell layers at high velocity. When these particles are delivered to or near the nucleus, the DNA comes off the coat and can be integrated into the plant chromosomes (Sanford, 2000). The use of Carbide silicon fibers is commonly referred to as silicon carbide mediated transformation. In this technique, a packed cell volume is mixed with a sterile suspension of silicon carbide whiskers and the DNA to be delivered into these cells and vortexed (Frame *et al.,* 1994). Since the surface of these whiskers is negatively charged as the DNA in the mixture, there is a little affinity between the DNA and the whiskers which act as sharp needles puncturing the cells and enhancing DNA delivery (Asad and Arshad, 2011). Transfection techniques use either a polymer (polyfection) or a liposome (lipofection). The genetic material is coated onto the (poly) cationic carrier or the liposome carrier aggregate due to their differences in surface charge and is delivered to the target cells once cell membranes of the target cells fuse with these vesicles (Wagner 1998). Microinjection is another direct DNA delivery approach which involves the use of a glass microcapillary-injection pipette to inject either a plasmid or sometimes whole chromosomes into a plant cell. This technique has the advantage of precision in delivery of the transgene and high transformation efficiency but is not commonly used due to the expensive nature of the equipment needed (Darbani *et al.,* 2011). The wave and beam-mediated transformation utilize ultrasound waves to increase the permeability of cell membranes and increase the ease with which micro molecules enter the cells during transformation (Wyber *et al.,* 1997).

5.2.2 Agrobacterium-mediated transformation

A. tumefaciens-mediated genetic transformation in plants is a six step process which begins by *Agrobacterium* attachment and ends when a certain segment called the T-DNA from the Tumour inducing (Ti) plasmid is transferred into the nucleus of the infected cell (Gelvin, 2010). During attachment or colonization, it is thought that capsular polysaccharides produced by the *att* region play a very important role (Reuhs *et al.,* 1997). After attachment, induction of the bacterial virulence system follows. The *vir* region contains six essential and two non-essential operons. Vir A proteins detect phenols produced by the wounded plant such as acetosyringone and other monosaccharides which act synergistically with it (Winans, 1992). *Vir G* controls the expression of the other *vir* genes after phosphorylation by Vir A. The third step involves generation of the T-DNA transfer complex. At this stage, there is generation of a single-stranded nucleic acid molecule which is just a copy of the bottom T-DNA strand controlled by *vir D1* and *Vir D2* gene. Once the ssT-DNA-VirD2 complex is ready, the next step involves its transfer into the nucleus of the host plant. During the transportation process, Vir B proteins form a channel through which the T-DNA-protein complex passes from the bacteria to the host while Vir D4 generates ATP for the transfer process (De La Riva *et al*., 1998). Once the T-DNA is inside the plant host cell, it has to find its way into the nucleus. This is facilitated by a number of *Vir* and host plant proteins. The *Vir* proteins involved are VirD2 and VirE2 which code for plant-active nuclear localization signal (NLS) sequences. Importin α and cyclophilin family of proteins are the plant proteins involved in this process (Pitzschke and Hirt, 2010). Once the T-DNA is inside the nucleus, stable integration into the host genome is directed by VirD2 which ligates the T-strand 5' end of the host chromosome. In maize transformation, this technique was developed in 1996 and is considered superior to direct delivery techniques due to its high frequency of transformation and its ability to insert single copies of inserts (Ishida *et al.,* 1996).

5.2.3 Other techniques

The breeding strategy has been improved by the introduction of high throughput phenotyping techniques. Since yield is a function of many processes in the plant cycles such as vegetative cover and abiotic stresses, studying these parameters has provided a pointer on expected yields. New tools have been developed based on remote sensing using spectral reflectance indices, for example, the Normalized Difference Vegetation Index (NDVI) (Marti *et al.,* 2007). Other tools are based on thermal sensing, fluorescence imaging and multi-sensor imaging (Jones and Schofield, 2008). Combining these phenotyping techniques and molecular tools has provided a higher precision and speed in plant breeding and improvement. Early season NDVI in maize was used to predict end season grain yields while fluorescence and multi-sensor imaging have been used to phenotype maize in the field (Zaman-Allah *et al.,* 2015).

6.0 Progress in transferring drought tolerance to maize

In maize, drought stress alone triggers the expression of about 163 expression sequence tags (ESTs) which might or not correspond to individual genes expressed under drought conditions (Zheng *et al.,* 2004). Whichever the case, the expression of some of these genes like poly(ADP-ribose) lead to the use of NAD+ as a substrate for DNA repair or cell defense, an energy consuming process which can lead to cell death as a result of ATP depletion (Briggs and Bent, 2011). The most commonly applied mitigation strategy to inhibit crop death through activation of stress induced lethal genes has been through silencing of these genes (Vanderauwera *et al.,* 2007). Other alternatives include altering other genes within the same expression cascade. For example, with *PARP*, over-expressing the nudix hydrolase (*NUDX*) group of genes ensures increased synthesis of the substrate hence more NAD+ for the required process (Briggs and Bent, 2011). Other strategies used include conventional breeding especially crossing drought susceptible varieties with tolerant ones. This is, however, an old practice highly dependent on chance and with high uncertainty compared to biotechnological techniques.

To achieve tolerance to drought in maize, different traits can be targeted for improvement. Targeting genes which will reduce the Anthesis Silking Interval (ASI) ensures maize plants set seeds even under drought conditions. Manipulating genes within the carbohydrate regulation pathway has also been shown to enhance seed set under limited water. The Abscisic Acid (ABA) pathway is thought to turn on many genes involved in drought stress tolerance hence targeting genes within his pathway or other genes which can affect this pathway might trigger tolerance to drought (Ali *et al.,* 2011). Commonly used strategies that have been employed to transfer drought tolerance to maize include; classical breeding, marker-assisted selection and genetic engineering.

6.1 Classical breeding

Breeding in maize for drought stress tolerance has been achieved through strategies such as intra-population and inter-population improvement schemes as well as backcross breeding. Through these approaches, drought tolerance and increased yields in tropical maize populations have been achieved at the International Maize and Wheat Improvement Centre (CYMMYT) (Edmeades, 2013). From these improved populations, inbred lines which are tolerant to drought and with other desirable traits such as reduced Anthesis Silking Interval (ASI), barrenness and senescence are picked and used as parents in breeding programs in the strive to develop drought-tolerant hybrids (Banziger *et al.,* 2004). After this, drought-tolerant hybrids are constantly selected after being subjected to controlled drought conditions.

Classical breeding has resulted in early maturing maize varieties which take advantage of short rain periods hence avoid drought. These have been developed through the open pollination strategy where certain desirable varieties are planted in an open field and the designated female parent de-tasseled hence pollinated by the other variety. This is usually repeated over time to result in a variety that is uniform and with the desirable traits. This technique was used in the development of the Kito OPV maize in Tanzania and Guto OPV maize of Ethiopia which were released in 1983 and 1988 respectively by CIMMYT. The Guto variety matures 20 days earlier than other local varieties in Ethiopia and 35 days earlier than the Bako composite maize hence avoids drought (Lopez-Pereira and Morris 1994). In Africa, breeding for drought tolerance gained prominence in 2007 with the advent of the Drought Tolerant Maize for Africa (DTMA) project (Fisher *et al.,* 2015). Through this project, 15 very early maturing maize OPVs and three very early maturing hybrids which can avoid drought from their short growth cycle have been bred and released in 13 countries since 2007 (CIMMYT, 2016).

6.2 Marker Assisted Selection (MAS)

Molecular mapping for tolerance to drought has been in practice since the 1990s, addressing plant traits which are related to drought such as high yields under drought, desirable root morphology as well as osmotic adjustments (Blum, 2011). In maize, this technique started with the discovery of the association between markers and traits in the early 1970s which later led to the development of genetic maps. In addition to these marker technologies, phenotyping and statistical tools are essential in the development of quality marker-trait associations, commonly referred to as Qualitative Trait Loci (QTL) (Ribaut and Ragot, 2007). In MAS, therefore, genetic markers are used instead of phenotypic traits to select desirable plants which eventually ends up accelerating the breeding cycle and the eventual release of new cultivars. The commonly used MAS approaches to improve drought tolerance in maize are Marker Assisted Back Crossing (MABC) and Marker Assisted Recurrent Selection (MARS). MAS has enabled breeders to reduce significantly on the amount of field testing required to develop a new cultivar although the genotype-phenotype associations upon which this technique is based depends heavily on the accurate phenotyping (Edmeades, 2013).

MABC involves the transfer of only a few genes such as a drought tolerance transgene or a loci into an adapted variety. The backcrossing parent often has a large number of desirable characteristics but lacks few characteristics of interest to the breeder. MABC technique was used to convert a normal maize inbred line V25 to a hard endosperm quality protein maize (QPM) variety by crossing it with a donor parent CML176 which contains the *opaque 2* gene. The recipient parent was extra-early early maturing and high yielding but with a low nutritive value normal endosperm while the donor parent had the desirable QPM but was low yielding. After several backcrosses, the *opaque 2* gene was introgressed to the V25 parent while retaining its desirable agronomic traits (Babu *et al.,* 2005). This technique was also used in the improvement of an inbred line CML 247 which is susceptible to drought by backcrossing it with a drought tolerant line Ac7643. CML 247 is a tropical inbred maize line which is high yielding under good watering conditions but has a very large ASI under drought conditions which might lead to the loss of the desired traits. After backcrossing, the resulting hybrid was evaluated and found to yield at least 50% higher yield than the control hybrids under high water stress although less than the controls at low stress (Ribaut and Ragot, 2007). Genotype-environment interaction effects, as well as the limitation of MABC to improving single traits, have limited the use of this technique in maize improvement hence breeders prefer the use of MARS technique (Beyene *et al.,* 2016).

MARS involves combining several genes into one genotype by identifying and selecting genomic regions with relatively complex traits like drought tolerance and yield as well as disease resistance within a population. This technique is mostly used to breed for disease resistance in maize with an aim of developing stable disease tolerant genotypes since it has been observed that most pathogens overcome disease resistance driven by single genes as a result of the emergence of new pathogen races (Collard and Mackill, 2008). A pathogen might not easily overcome more than one effective genes by just mutating but may easily overcome the resistance of a single gene after it mutates. Previously, it was difficult to combine more than one resistance genes in a single genotype because these genes have a similar phenotypic resemblance. However, with the advent of DNA markers, the number of resistance genes in a single plant can now be identified with ease. MARS technique was used in the improvement of maize line NSE331 by crossing it with line NSE626 and using NSE331 as the recurrent parent. NSE331 inbred line has superior agronomic characteristics but with late flowering, while NSE626 has average to low agronomic performance although it flowers 8 to 12 days earlier than NSE331. Therefore, by crossing NSE331with NSE626 and backcrossing using NSE331 as the recurrent parent, breeders observed that the progeny flowered several days earlier than the parents while maintaining the desirable agronomic potential of NSE331 (Ragot *et al.,* 2000). Recently, MARS technique has been used to improve drought tolerance in the commercial CIMMYT inbred lines currently planted in eastern and southern Africa regions. These commercial lines with prefixes CML were crossed with other drought tolerant donor lines from CYMMIT with prefixes CZL or VL to obtain 10 good populations which were recurrently selected using SNP markers to come up with drought tolerant commercial lines (Beyene *et al.,* 2016).

MAS technique in plant breeding experiences a number of drawbacks. It lacks the ability to predict the phenotype of a given genotype in a segregating population based on the constitution of alleles at a given loci (Ribaut and Ragot, 2007). Of the two MAS techniques, MARS seems to be more advantageous than MABC as MARS allows the improvement of more than one maize trait while MABC can only allow improvement of one or at most two traits at a time.

6.3 Genetic engineering

Several attempts have been made to enhance maize tolerance to drought through the genetic engineering approach. Transgenic maize highly expressing *C4 phosphoenolpyruvate carboxylase* (*C4-PEPC*) gene showed an increased water use efficiency and dry weight when subjected to moderate drought conditions (Jeanneau *et al.,* 2002). Shou *et al.* (2004) produced transgenic maize plants expressing the *Nicotiana Protein Kinase* (*NPK1*) gene which exhibited improved tolerance to drought. The production of the NPK protein consequently induces the expression of genes responsible for the production of Heat Shock Proteins (HSPs) and Glutathione-S-transferases (GSTs) in maize which protect the photosynthetic machinery from drought-induced damage. Engineered maize with *betA* gene by Quan *et al.* (2004) showed drought tolerance at seedling, germination and reproductive stages. Quan *et al.* (2004) attributed osmotic stress tolerance in transgenic maize line DH4866 to the accumulation of glycine betaine in the maize leaves. Maize transformed with the maize transcription factor *ZmNF-YB2* exhibited an improved tolerance to drought stress and an increase in yield under severe water stress (Nelson *et al.,* 2007).

Transgenic maize referred to as DroughtGard was engineered with a gene *cspB* which codes for proteins that stabilize the mRNA under stresses like low temperature in the common soil bacteria *Bacillus subtilis*. The transgenic maize exhibited increased productivity during the vegetative growth and grain yield under water-limited conditions (Castiglioni *et al.,* 2008). The *ZmPLC1* gene cloned in maize in the sense or antisense orientation in maize inbred line Ye 7922 increased the expression of Phosphoinositide phospholipase C, thereby protecting transgenic plants from cell membrane damage. Additionally, damage to PSII which is caused by drought was less in *ZmPLC1* transgenic lines than in wild-type hence the gene was thought to protect the plant's photosynthetic machinery to drought stress damage (Wang *et al.,* 2008). Transgenic maize plants expressing transgenes *betA* and *TsVP* as a result of pyramiding the two transgenes from cross-pollination exhibited improved tolerance to drought as a result of increased accumulation of soluble sugars and proline under drought conditions. An even more desirable aspect of these transgene pyramided plants was their short ASI and reduced leaf curling under drought conditions (Wei *et al.,* 2011).

7.0 Hurdles to genetic improvement of maize in Africa

There has been a considerable effort towards improving tropical maize through biotechnology in Africa. However, the passage of genetically modified (GM) maize from experimental stages to commercial production remains negligible hence little commercial GM maize is in the market (Thomson, 2004). GM crops are subjected to strict regulations and processes in many countries. Although these regulations aim at exploiting the benefits which come with biotechnology and at the same time safeguarding humans and the environment from the potential risk, these regulations may be a bottleneck to GM crop development in Africa. In most African countries, adopting a GM crop highly depends on political good will and the existing policy framework.

Before biotechnology research can commence in a country, biosafety regulations must be in place so as to create conducive environments in which GMO research can be carried out. To date, 2016, 170 countries in the world have ratified to the Cartagena protocol on biosafety, out of which 48 are from Africa. The requirement to develop a biosafety protocol as stipulated in the Cartagena protocol has been achieved in 27 African countries. Furthermore, only 24 of the 27 countries have national biosafety authorities hence can conduct agricultural biotechnology and research. A major challenge to biotechnology research is the heavy capital investment required to fund this expensive technology. A considerable amount of money is needed to build research facilities, furnish these facilities with expensive equipment and purchase required consumable. Substantial investment is also needed for manpower training to produce specialists in various aspects of biotechnology such as molecular biology and tissue culture.

8.0 The future of GM drought tolerant maize in Africa

Most of Africa has environmental challenges, especially drought and will benefit from having drought tolerant maize, which will result in an increase in the acreage under maize. The future of maize biotechnology in Africa is bright and there is optimism that GM drought tolerant maize will be commercialized in the next few years. Before this is achieved, however, there is a need for the inauguration of biosafety frameworks as well as political good will from African leaders to ensure that drought-tolerant maize for Africa is commercialized. Additionally, agricultural biotechnology must be tailored towards making the drought tolerant maize affordable to the resource challenged African farmers. For this to be achieved, both private and public sectors will need to invest heavily in maize biotechnology research. Finally, there is a need for a policy framework that puts into consideration the benefits accrued from the GM technology especially drought tolerant GM maize for Africa.

References

Ali, Q., Elahi, M., Hussain, B., Hussain, N., Ali, F., & Elahi, F. (2011). Genetic improvement of maize (Zea mays L.) against drought stress : An overview. *Agricultural Science Research Journals*, ***1***, 228–237.

Asad, S., & Arshad, M. (2011). Silicon Carbide Whisker-mediated Plant Transformation. *Properties and Applications of Silicon Carbide*. INTECH Open Access Publisher.

Aslam, M., Zamir, I., Afzal, I., Yaseen, M., Mubeen, M., & Shoaib, A. (2013). Drought tolerance in maize through Potassium: Drought stress, its effect on maize production and development of drought tolerance through potassium application. *Cercetări Agronomice În Moldova*, ***XLVI***, 16.

Babu, R., Nair, S. K., Kumar, A., Venkatesh, S., Sekhar, J. C., Singh, N. N., … Gupta, H. S. (2005). Two-generation marker-aided backcrossing for rapid conversion of normal maize lines to quality protein maize (QPM). *Theoretical and Applied Genetics*, ***111***, 888–897.

Banziger, M., Setimela, P. a., Hodson, D., & Vivek, B. (2004). Breeding for improvement drought tolerance in maize adapted to southern Africa. In *Proceedings of the 4th International Crop Science Congress*.

Beyene, Y., Semagn, K., Crossa, J., Mugo, S., Atlin, G. N., Tarekegne, A., … Banziger, M. (2016). Improving maize grain yield under drought stress and non-stress environments in sub-saharan africa using marker-assisted recurrent selection. *Crop Science*, ***56***, 344–353.

Blum, A. (2011). Plant Water Relations, Plant Stress and Plant Producción. In *Plant Breeding for Water-Limited Environments* (pp. 11–52). Berlin, Germany: Springer Science+Business Media.

Briggs, A. G., & Bent, A. F. (2011). Poly(ADP-ribosyl)ation in plants. *Trends in Plant Science*, ***16***, 372–380.

Cairns, J. E., Sonder, K., Zaidi, P. H., Verhulst, N., Mahuku, G., Babu, R., … Prasanna, B. M. (2012). *Maize production in a changing climate. impacts, adaptation, and mitigation strategies*. *Advances in Agronomy* (1st ed., Vol. 114).

Castiglioni, P., Warner, D., Bensen, R. J., Anstrom, D. C., Harrison, J., Stoecker, M., … Heard, J. E. (2008). Bacterial RNA chaperones confer abiotic stress tolerance in plants and improved grain yield in maize under water-limited conditions. *Plant Physiology*, ***147***, 446–455.

CIMMYT. (2016). The Drought Tolerant Maize for Africa initiative. Retrieved June 22, 2016, from http://dtma.cimmyt.org/index.php/varieties/dt-maize-varieties

Collard, B. C. Y., & Mackill, D. J. (2008). Marker-assisted selection: an approach for precision plant breeding in the twenty-first century. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, ***363***, 557–572.

Darbani, B., Farajnia, S., Toorchi, M., Zakerbostanabad, S., Noeparvar, S., & Stewart, C. N. (2011). DNA-delivery methods to produce transgenic plants. *Biotechnology*, ***10***, 323–340.

De La Riva, G. A., González-Cabrera, J., Vázquez-Padrón, R., & Ayra-Pardo, C. (1998). Agrobacterium tumefaciens: A natural tool for plant transformation. *Electronic Journal of Biotechnology*, ***1***, 25–48.

Edmeades, G. O. (2013). Progress in Achieving and Delivering Drought Tolerance in Maize - An Update. *The International Service for the Acquisition of Agri-Biotech Applications*.

Falck-Zepeda, J., Gruère, G., & Sithole-Niang, I. (2013). *Genetically Modified Crops in Africa: Economic and Policy Lessons from Countries South of the Sahara*. (J. Falck-Zepeda, G. Gruère, & I. Sithole-Niang, Eds.)*International Food Policy Research Institute Report*. Washington: International Food Policy Research Institute.

FAO. (2015). FAOSTAT database collections. Retrieved December 15, 2015, from http://faostat.fao.org

Felgner, P. L., Gadek, T. R., Holm, M., Roman, R., Chan, H. W., Wenz, M., … Danielsen, M. (1987).

Lipofection: a highly efficient, lipid-mediated DNA-transfection procedure. *Proceedings of the National Academy of Sciences of the United States of America*, ***84***, 7413–7.

Fisher, M., Abate, T., Lunduka, R. W., Asnake, W., Alemayehu, Y., & Madulu, R. B. (2015). Drought tolerant maize for farmer adaptation to drought in sub-Saharan Africa: Determinants of adoption in eastern and southern Africa. *Climatic Change*, ***133***, 283–299.

Fortmeier, R., & Schubert, S. (1995). Salt tolerance of maize (Zea mays L.): the role of sodium exclusion. *Plant, Cell and Environment*, ***18***, 1041–1047.

Fraley, R. T. (2009). Molecular Genetic Approaches to Maize Improvement – an Introduction. In *Biotechnology in Agriculture and Forestry* (Vol. 63, pp. 3–7). Berlin.

Frame, B. R., Drayton, P. R., Bagnall, S. V, Lewnau, C. J., Bullock, W. P., Wilson, H. M., … Wang, K. (1994). Production of fertile transgenic maize plants by silicon carbide whisker-mediated transformation. *The Plant Journal*, ***6***, 941–948.

Gao, L., Cao, Y., Xia, Z., Jiang, G., Liu, G., Zhang, W., & Zhai, W. (2013). Do transgenesis and marker-assisted backcross breeding produce substantially equivalent plants? A comparative study of transgenic and backcross rice carrying bacterial blight resistant gene Xa21. *BMC Genomics*, ***14***, 738.

Gelvin, S. B. (2010). Plant proteins involved in Agrobacterium-mediated genetic transformation. *Annual Review of Phytopathology*, ***48***, 45–68.

Guantai, S. M, Seward, P., Ochieng, J. A., Njoka, E., Ogwora, E., Collins, S., & Walker, S. (2010). Maize Diseases and thier Control. In S. Wanjala & G. s M (Eds.), *Maize handbook* (second, Vol. 1, pp. 91–98). Nairobi: Kenya Maize Development Programme.

Heisey, P. W., & Edmeades, G. O. (1997). *World Maize Facts and Trends 1997 / 98 Maize Production in Drought-Stressed Environments : Technical Options and Research Resource Allocation*. *Agricultural Economics*. Mexico.

James, C. (2014). *Global status of Commercialized biotech / GM Crops : 2014*. *ISAAA Brief No. 49:* Ithaca, NY, USA.

Jansen, C. (2012). *Breeding for cob traits in maize*. Iowa state university.

Jeanneau, M., Gerentes, D., Foueillassar, X., Zivy, M., Vidal, J., Toppan, A., & Perez, P. (2002). Improvement of drought tolerance in maize: Towards the functional validation of the Zm-Asr1 gene and increase of water use efficiency by over-expressing C4-PEPC. *Biochimie*, ***84***, 1127–1135.

Jones, H. G., & Schofield, P. (2008). Thermal and other remote sensing of plant stresses. *Genaral and Apllied Plant Physiology*, ***34***, 19–32.

Karembu, M., Nguthi, F., Ogero, K., & Odong', J. (2014). Progress with Biotech Crops in Africa 2013. *International Service for the Acquisition of Agri-Biotech Applications (ISAAA) AfriCenter*.

Kim, S. K., Adetimirin, V. O., Thé, C., & Dossou, R. (2002). Yield losses in maize due to Striga hermonthica in West and Central Africa. *International Journal of Pest Management*, ***48***, 211–217.

Lopez-Pereira, M. ., & Morris, M. L. (1994). *Impacts of International Maize Breeding Research in the Developing World, 1966-1990. CIMMYT*. Mexico D.E.

Marti, J., Bort, J., Slafer, G. A., & Araus, J. L. (2007). Can wheat yield be assessed by early measurements of Normalized Difference Vegetation Index? *Annals of Applied Biology*, ***150***, 253–257.

Mitra, J. (2001). Genetics and genetic improvement of drought resistance in crop plants. *Current Science*, ***80***, 758–763.

Mugo, S., & Hoisington, D. (2001). Biotechnology For The Improvement Of Maize For Resource Poor Farmers : The cimmyt approach. In *Second National Maize Workshop of Ethiopia* (pp. 203–213).

Munns, R. (1993). Physiological processes limiting plant growth in saline soils: some dogmas and hypotheses. *Plant, Cell & Environment*, ***16***, 15–24.

Nelson, D. E., Repetti, P. P., Adams, T. R., Creelman, R. A., Wu, J., Warner, D. C., … Heard, J. E. (2007). Plant nuclear factor Y (NF-Y) B subunits confer drought tolerance and lead to improved corn yields on water-limited acres. *Proc Natl Acad Sci U S A*, ***104***, 16450–16455.

Pitzschke, A., & Hirt, H. (2010). New insights into an old story: Agrobacterium-induced tumour formation in plants by plant transformation. *The EMBO Journal*, ***29***, 1021–1032.

Prasanna, B. M., & Hoisington, D. (2003). Molecular Breeding for Maize Improvement : An Overview. *Indian Journal of Biotechnology*, ***2***, 85–98.

Quan, R., Shang, M., Zhang, H., Zhao, Y., & Zhang, J. (2004). Engineering of enhanced glycine betaine synthesis improves drought tolerance in maize. *Plant Biotechnology Journal*, ***2***, 477–486.

Ragot, M., Gay, G., Muller, J.-P., & Durovray, J. (1999). Efficient Selection for Adaptation to the Environment through QTL Mapping and Manipulation in Maize. In *Molecular Approaches for the Genetic Improvement of Cereals for Stable Production in Water-limited Environments* (pp. 128–130). Mexico D.F.: CIMMYT.

Reuhs, B. L., Kim, J. S., & Matthysse, A. G. (1997). Attachment of Agrobacterium tumefaciens to carrot cells and Arabidopsis wound sites is correlated with the presence of a cell-associated, acidic polysaccharide. *Journal of Bacteriology*, ***179***, 5372–5379.

Ribaut, J. M., & Ragot, M. (2007). Marker-assisted selection to improve drought adaptation in maize: The backcross approach, perspectives, limitations, and alternatives. *Journal of Experimental Botany*, ***58***, 351–360.

Sanford, J. C. (2000). Turning point article: The development of the biolistic process. *In Vitro Cellular & Developmental Biology - Plant*, ***36***, 303–308.

Shou, H., Bordallo, P., & Wang, K. (2004). Expression of the Nicotiana protein kinase (NPK1) enhanced drought tolerance in transgenic maize. *Journal of Experimental Botany*, ***55***, 1013–1019.

Sinsawat, V., Leipner, J., Stamp, P., & Fracheboud, Y. (2004). Effect of heat stress on the photosynthetic apparatus in maize (Zea mays L.) grown at control or high temperature. *Environmental and Experimental Botany*, ***52***, 123–129.

Suleiman, R. A., & Rosentrater, K. A. (2015). Current Maize Production , Postharvest Losses and the Risk of Mycotoxins Contamination in Tanzania. In *Agricultural and Biosystems Engineering Conference Proceedings and Presentations* (p. 127).

Thomson, J. A. (2008). The role of biotechnology for agricultural sustainability in Africa. *Philosophical Transactions Of The Royal Society B*, ***363***, 905–913.

Thomson, J. A., & Shepherd, D. N. (2010). Developments in Agricultural Biotechnology in Sub-Saharan Africa. *AgBioForum*, ***13***, 314–319.

Vanderauwera, S., De Block, M., Van de Steene, N., van de Cotte, B., Metzlaff, M., & Van Breusegem, F. (2007). Silencing of poly(ADP-ribose) polymerase in plants alters abiotic stress signal transduction. *Proceedings of the National Academy of Sciences of the United States of America*, ***104***, 15150–15155.

Wagner, E. (1998). Effects of membrane-active agents in gene delivery. *Journal of Controlled Release*, *53*, 155–158.

Wahid, A., Gelani, S., Ashraf, M., & Foolad, M. (2007). Heat tolerance in plants: An overview. *Environmental and Experimental Botany*, ***61***, 199–223.

Wang, C.-R., Yang, A.-F., Yue, G.-D., Gao, Q., Yin, H.-Y., & Zhang, J.-R. (2008). Enhanced expression of phospholipase C 1 (ZmPLC1) improves drought tolerance in transgenic maize. *Planta*, ***227***, 1127–1140.

Wei, A., He, C., Li, B., Li, N., & Zhang, J. (2011). The pyramid of transgenes TsVP and BetA effectively enhances the drought tolerance of maize plants. *Plant Biotechnology Journal*, ***9***, 216–229.

Winans, S. C. (1992). Two-way chemical signaling in Agrobacterium-plant interactions. *Microbiological Reviews*, ***56***, 12–31.

Wyber, J. A., Andrews, J., & D'Emanuele, A. (1997). The use of sonication for the efficient delivery of plasmid DNA into cells. *Pharmaceutical Research*, ***14***, 750–756.

Zaman-Allah, M., Vergara, O., Araus, J. L., Tarekegne, A., Magorokosho, C., Zarco-Tejada, P. J., … Cairns, J. (2015). Unmanned aerial platform-based multi-spectral imaging for field phenotyping of maize. *Plant Methods*, ***11***, 35.

Zheng, J., Zhao, J., Tao, Y., Wang, J., Liu, Y., Fu, J., … Wang, G. (2004). Isolation and analysis of water stress induced genes in maize seedlings by subtractive PCR and cDNA macroarray. *Plant Molecular Biology*, ***55***, 807–823.