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Abstract

Maize is the most-produced cereal worldwide. In Africa alone, more than 300 million people depend on maize as their main food crop. In addition, maize is also very important as feed for farm animals. Currently, approximately 1 billion tons of maize are grown in more than 170 countries on about 180 million hectares of land. 90% of the world's production is yellow maize, but in Africa, 90% of the total maize production is white maize. Maize production in Africa is very low: while the average yield worldwide is approximately 5.5 tons/hectare/year, production in Africa stagnates at around 2 tons/hectare/year.

In Africa, maize production is continuously and severely affected by a number of threats, such as weeds, insects, bacteria, viruses, nematodes, fungi, low quality seed, low levels of mechanization, suboptimal post-harvest management, drought and climate change. Currently, damage caused by stem borers, grain borers, fall armyworms and *Striga* can completely destroy the maize yield, and drought also has an enormous impact on yield. Erratic rain patterns, inadequate farming methods and drought stress can lead to 70-100% crop loss, which is dramatic for both farmers and consumers, as the whole food chain is affected.

To continue to guarantee maize food and feed security in Africa, good agricultural practices, intercropping, new hybrids obtained by conventional and marker-assisted breeding, and genetically modified (GM) plants are valuable tools to develop varieties with increased yield and resistance to pests, weeds, diseases and drought.

Many maize kernel components such as starch and proteins are very nutritious. However, (white) maize lacks provitamin A and the important amino acids lysine and tryptophan. Therefore, several breeding programs develop new maize varieties with enhanced lysine and tryptophan levels. Children consuming this maize variety showed a clear increase in growth rate and weight. Vitamin A-enriched maize varieties have already been developed and commercially released.

Facts and figures

Maize is the main staple food crop of more than 300 million Africans.

90% of Africa's total maize production is white maize, whereas 90% of the world's production is yellow maize.

Maize occupies approximately 24% of farmland in Africa and the average yield stagnates at around 2 tons/hectare/year.

In 2013, 20% of global maize flour exports originated from Africa.

In all African farmlands, stem borers cause significant yield losses ranging from 15 to 40%. In optimal insect infestation conditions, total crop failure can occur.

In 2016, the fall armyworm arrived in Africa. This insect spread in only one year to 12 African countries and is an enormous threat to local maize production.

Maize Lethal Necrosis Disease is a devastating and widely spread viral disease in Sub-Saharan Africa: yield losses of up to 90% have been reported.

Striga, a parasitic weed, has already infested 40 million hectares of land in Sub-Saharan Africa, resulting in a yield loss of 20 to 80%. The seeds of this weed remain viable and dormant in the soil for at least 20 years.

Contamination of maize kernels with mycotoxins is a major problem for the African maize trade and economy. More than 4.5 billion people in the developing world are chronically exposed to excessive levels of mycotoxins, resulting in adverse health effects.

Occasional drought stress affects approximately 40% of Africa's maize-growing areas. Yield losses of 10-25% were measured. Approximately 25% of the maize suffers frequent drought, with harvest losses of up to 50%.

Maize proteins in the endosperm are deficient in the amino acids lysine and tryptophan, and white kernels additionally lack provitamin A. Because maize is the main food source of many people, a large portion of them suffer from "hidden hunger", a pandemic related to vitamin and protein deficiencies.



The diversity of maize

Maize originates from Mexico and was domesticated about 10,000 years ago in the Tehuacán Valley in the Mexican highlands by indigenous people.^{1,2} Later, it was spread to the Mexican lowlands, other regions of Latin America, the Caribbean, New Mexico and Arizona.³ During the first millennium CE, maize cultivation spread more widely to northern America and Canada. After European contact with the Americans in the late 15th – early 16th centuries, explorers and traders carried maize to Europe, and from there to Asia and Africa.⁴ Nowadays, maize grows between latitude 58°N in Canada and Russia and latitude 40°S in Chile and Argentina. The crop grows best at moderate altitudes but is also found below sea level in the Caspian Depression and at up to 3,800 meters in the Andean mountains.

Figure: Maize field in Africa (source: Johnnie Van den Berg, North-West University, South Africa).

Plant one seed and you get 500 kernels per cob in return!

Maize is the domesticated variant of teosinte, although both plants have a very different appearance: teosinte is a short, bushy plant with one small cob of 25 mm long, whereas the maize we know today has a single tall stalk with multiple leaves. Both plants are able to cross-breed and to produce fertile progeny.⁵

Modern maize is a tall plant of approximately 2 to 3 meters high with a fine, profusely branched root system (Figure 1.1). This root system plays an important role in the absorption of water and nutrients from the soil, and can, under optimal conditions, be 1,500 m long. The stem of the maize plant varies in height from less than 0.6 m to, in extreme cases, more than 5 m. Each plant contains 8 to 20 small, parallel-veined leaves typical of grasses.

Maize is a monoecious plant: male and female flowers grow on the same plant as separate inflorescences (Figures 1.1 and 1.2). Male germ cells are produced in the tassel at the top of the plant, whereas female ones are located in one or more

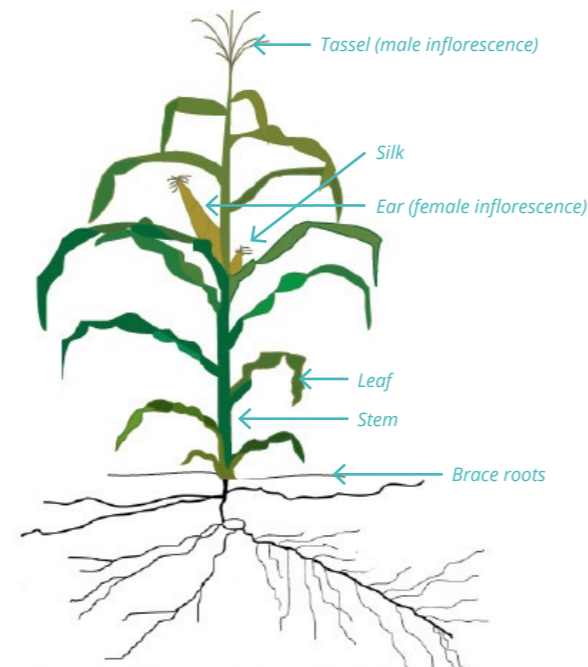


Figure 1.1: Schematic representation of a maize plant.

ears, which grow from the bases of the leaves to the midsection of the plant.⁶ The tassel contains anthers that open upon maturation and release up to 100 million wind-dispersed pollen grains. About 95% of the flowers on a cob receive pollen from nearby plants, whereas only 5% of the kernels are produced through self-pollination (pollen from the same plant). Pollen grains, which live for about 12 to 18 hours, are very small, lightweight,



Figure 1.2: Male (A) and part of female (B) inflorescence of maize plants (source: Johnnie Van den Berg).

easily carried by the wind and barely visible to the naked eye. One ear, enclosed by bract leaves, contains several hundred egg cells, and each of them can develop into a kernel after fertilization. The kernels are about the size of peas, and grow in regular rows around a white, pitchy substance that forms the cob. Depending on the maize variety, the number of kernel rows may vary between 4 and 40, with the number of kernels per row between 36 and 40. This implies that a single ear can easily produce 500 to 1,000 kernels.⁷

The maize plant grows optimally in deep, well-drained, rich soils and areas with average day temperatures of 18-21°C. Frost and flooding will kill the plant. Maize can grow in conditions with annual rainfall ranging from 230 to 4,100 mm, but less than 750 mm is not optimal.

MAIZE, CORN OR YET ANOTHER NAME?

In literature, the terms "maize" and "corn" are often used to identify the same plant. In general, the only difference between the two words depends on which form of English you use. In the United States and Canada and a few other countries, "maize" and "corn" are one and the same: a tall grain plant with seeds, called "kernels", often used for cooking. In British English, the word "corn" refers to a cereal crop or grain, including a single kernel. "Maize" is the proper name of the plant itself, whereas "corn" refers to whatever common local grain is most harvested for its edible seeds. The term "maize" is accepted for scientific and international applications because it refers to the particular grain, whereas corn suggests different meanings by context and geographical location. In South Africa, maize is commonly called "Mielie" (Afrikaans) or "Mealie" (English), words that are all derived from Portuguese word "milho".



Figure 1.3: Yellow maize kernels.

Which maize kernel do you prefer: White, yellow, red or black, flint or dent, containing more or less sugar?

The maize kernel, which is botanically a fruit, is frequently referred to as a seed. It consists of 73% starch, 9% protein, 4% oil and 14% other components such as fiber, and supplies an energy density of 365 Kcal/100 gr.^{2,7,8} This energy density is very similar to that of other staple crops such as rice (360 Kcal/100 gr) and wheat (340 Kcal/100 gr). The endosperm, which surrounds the embryo, is largely starch (approximately 90%) and the embryo contains high levels of oil (30%) and protein (18%). Maize endosperm contains dif-

ferent protein fractions: albumins (3%), globulins (2%), zeins (60%) and glutelin (34%), while embryo proteins are mainly albumins (60%). Because the endosperm protein is deficient in lysine and tryptophan, two very important amino acids, maize needs to be eaten alongside different protein sources such as legumes or animal products (see Chapter 6). Finally, maize provides many of the B vitamins and essential minerals, but lacks other important nutrients such as vitamin B12, vitamin C, folate and iron.²

Maize oil (4%) contains predominantly unsaturated fatty acids (60% linoleic acid, 24% oleic acid and 11% palmitic acid). Due to its high linoleic acid content, maize oil is marketable as a high-value product, because it is both essential and “heart healthy”.



Figure 1.4: Temperate (yellow) and tropical (white) maize (source: Mieke Van Lijsebettens, VIB-UGent Center for Plant Systems Biology, Belgium⁹).

There are about 50 different varieties of maize grown throughout the world, and classification can be done on the basis of kernel shape, size, color, taste, etc. There are two major kernel shapes: round (flint maize) or tooth shape (dent maize). White, yellow and red are the most common colors for maize kernels, but varieties with red-brown, light red, pale yellow, orange and black kernels also exist (Figures 1.3 and 1.4).²

African producers and consumers only began to accept white maize instead of the yellow variety in the early twentieth century. Despite this late adoption, white maize rapidly spread throughout Africa. In fact, yellow maize is not very popular nowadays in Africa for the following 3 main reasons: (1) yellow maize is associated with food-aid

programs and therefore perceived as being consumed only by poor people, (2) yellow maize is associated with animal feed and (3) yellow maize is too sweet.^{2,10}

Maize varieties can be classified into 5 major groups according to specific food, feed and production needs: sweet corn, popcorn, flour corn, dent or field corn, and flint corn. Sweet corn is harvested immaturely, before the conversion from sugar to starch takes place. These kernels have therefore more sugar than starch content. This maize is most commonly found in stores as a food component. Popcorn is also a variety that is used for human consumption. The popcorn kernel has a tough outer shell which encapsulates a small amount of soft starch content. When the

Country	Maize Consumption
Lesotho	120
Malawi	107
Zambia	89
Zimbabwe	88
South Africa	81
Kenya	62
Togo	58
Swaziland	55
Tanzania	47
Namibia	46
Benin	43
Mozambique	42
Burkina Faso	39
Ethiopia	34
Angola	29
Botswana	28
Cameroon	27
Cape Verde	26
Central African Republic	26
Mali	25
Seychelles	25
Senegal	22
Nigeria	22
Ghana	19
Uganda	19

Table 1.1: Maize consumption in Africa (kg/person/year) (adapted from ²).

These values are an underestimation, as they were corrected for an 80% average extraction rate (this varies in each country by type of flour milled as well as by the maize type used).

THE GENETIC CODE OF MAIZE

The maize B73 genome is diploid (=2 copies of each chromosome), and consists of 20 chromosomes (n=10).¹¹ There are approximately 40,000 annotated (= identified) protein-encoding genes.^{11, 12} Nearly 85% of the genome is composed of hundreds of transposable elements (= jumping elements), which are inconsistently spread across the genome. During the evolution process, the maize genome has undergone several rounds of genome duplication, and although maize behaves as a simple diploid, large portions of the genome are duplicated and are thus even tetraploid (= four copies).^{11, 13} Maize is closely related to other important grains and crops, such as sorghum, rice, and wheat.¹²

Five major loci (= DNA regions) account for the enormous morphological differences between teosinte and modern maize⁵, but many other genes have also played a role in domestication.¹⁴

kernel is heated, the moisture turns into steam which expands, splits the pericarp and causes the endosperm to explode, turning the kernel inside out. Flour corn is made of soft kernels consisting of soft starch content. This maize is easy to grind and is very often used in baked goods. Dent or field corn accounts for approximately 99% of all corn produced in the US. This corn is mainly used as livestock feed, a source of natural corn syrup and in industrial products (see below). Dent corn is much starchier than sweet corn and therefore has a bland flavor and a mealy texture. Flint corn, which has a hard and glassy outer shell, is mainly grown in Central and South America for food and feed. Finally, a sixth, smaller maize group, the pod or Indian Corn, is more often used for ornamental purposes. This maize has unique elongated kernels with varied color patterns. White dent and flint varieties are commonly grown in developing countries, while yellow maize is more commonly grown in the rest of the world.



Figure 1.5: Women (A) harvesting maize kernels in the field (source: Johnnie Van den Berg), and (B) grinding the grains into flour, (C) homemade flour drying in the sun (source: Bruno Tinland, personal collection).

Maize, a component of many food and feed products

All over the world, maize is a major food source due to its excellent properties: it is easy to propagate from single plants or small nurseries to hundreds of hectares, and the ears with their kernels are easy to harvest. Maize is an important staple food for about 1.2 billion people around the world, provides over 20% of the total calories in human diets in 21 countries and is Africa's most important staple crop, feeding more than 300 million people on the continent.^{15, 16} Africa uses 95% of its maize production as food.^{9, 16, 17} The per capita consumption of maize is highest in southern Africa, averaging 120 kg per person per year in Lesotho, 107 kg in Malawi and more than 80 kg in Zambia, Zimbabwe and South Africa (see Table 1.1).² By comparison, in Mexico, one person consumes 97 kg of maize products per year on average.

In Africa, (white) maize is used in a number of products, such as bread, porridges, tortillas, are-



Food	Country
Porridges, thin fermented	
Ogi	Nigeria
Uji	East Africa
Mahewu	South Africa
Porridges, thick	
Tô, tuwo, asida, akoumé	West Africa, North Africa, Horn of Africa
Ugali	Kenya, Tanzania
Steamed foods	
Couscous, cuzcuz	Africa
Bread, unfermented	
Corn bread	Africa
Bread, fermented	
Injera	Ethiopia
Fermented Dough	
Kenkey, ablo	Ghana, Benin, Togo
Alcoholic beverages	
Urawga, mwenge	Kenya, Uganda
Chibuko	Southern Africa
Pito	Nigeria
Talla	Ethiopia
Busas	Kenya
Opaque beer	Zambia
Munkoyo	Zambia

Table 1.2: Traditional foods made from maize in Africa (adapted from ?).



A



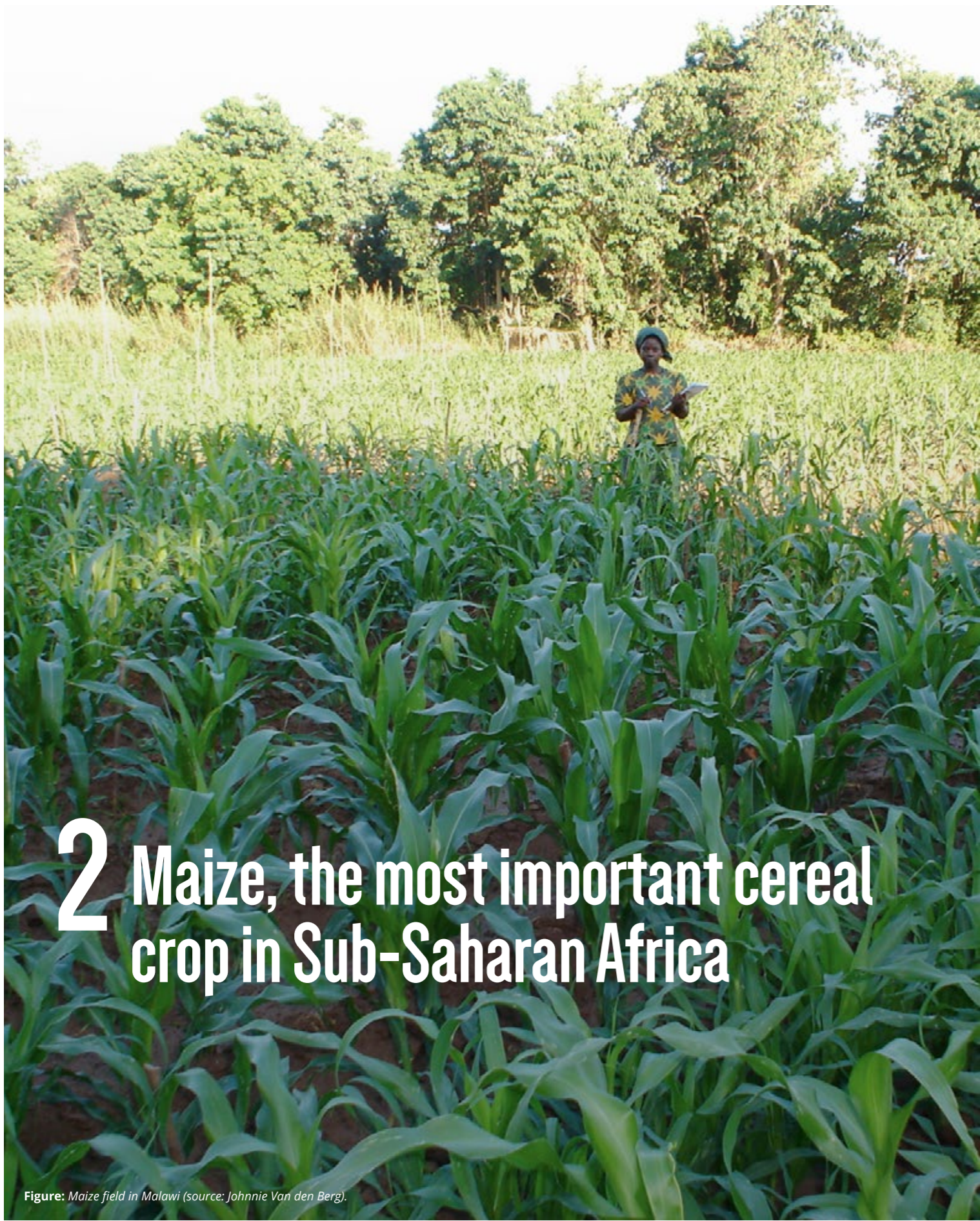
B

Figure 1.6: Food based on maize in Africa: (A) Ugali (Tanzania) and (B) corn bread (South Africa).

pas, cornbread and couscous (Table 1.2). African women dedicate significant labor and time to harvesting the maize, producing flour and preparing traditional meals (Figure 1.5).

In Sub-Saharan Africa, the whole grain can be roasted or cooked and served as food. *Githeri* is a typical Kenyan dish, with maize kernels and beans mixed and boiled together. Dried kernels or grain are often processed into flour, which is used in several dishes. Very famous in Kenya is *ugali*, a dish made of maize flour cooked in boiling water or milk to a porridge- or dough-like consistency (Figure 1.6). Traditionally, one rolls a lump into a ball with the right hand and then dips it into a sauce or stew of vegetables and/or meat. This dish is also known in other African countries such as Tanzania (*nguna*), Uganda (*posho*), Rwanda and Burundi (*ubugali*) and Democratic Republic of Congo (*bugali*), while in Burkina Faso and Mali, *tô*, a paste of hulled cereals, is a traditional meal. In South Africa, *mush*, a similar cornmeal, is often consumed. Maize is also used to produce unconventional local brews in Kenya, known as *changaa* or *busaa*, which are often served during traditional ceremonies. Finally, the oil of the embryo is used in cooking oils, margarine and salad dressings.

Also animals have relied on maize as a food source for many years. Maize is the world's number one feed grain and is used as the main source of calories in animal feed and feed formulation in both developed and developing countries. Approximately 60% of the maize produced globally is used for animal feed. Rapid increases in poultry consumption in Africa contribute to the higher use of maize for livestock feed.² For farmers, maize is easy to grow, harvest and store as feed for their animals.



2 Maize, the most important cereal crop in Sub-Saharan Africa

Figure: Maize field in Malawi (source: Johnnie Van den Berg).

Maize production and yield figures differ dramatically by country

Maize is the most-produced cereal worldwide. In 2014, more than 1,022 million tons of maize were produced in more than 170 countries on about 181 million hectares of land (Figure 2.1).¹⁸ The top producers were the United States of America with 361 million tons, China with 216 million tons, Brazil with 80 million tons, and Argentina and Ukraine with 33 and 28 million tons, respectively. India is the sixth-largest producer with around 24 million tons, followed by Mexico and Indonesia (both 23 million tons), South Africa (14 million tons) and Romania (12 million tons). These 10 countries account for almost 80% of the world's total maize production, and more than 60% originates from the top three countries (Figure 2.1).

Maize is the basis for food security in some of the world's poorest regions in Africa, Asia and Latin America. In Africa, 51 countries produced approximately 75 million tons of maize in 2014 (7.4% of the total world production) on 37 million hectares (20.44% of the total area planted worldwide). Maize occupies approximately 24% of farmland in Africa, which is more than any other staple crop, and is a food crop accounting for 73% and 64% of the total demand in Eastern and Southern Africa and Western and Central Africa, respectively.¹⁶ South Africa is currently the main maize producer of the African continent, and almost half of its production consists of white maize meant for human consumption.

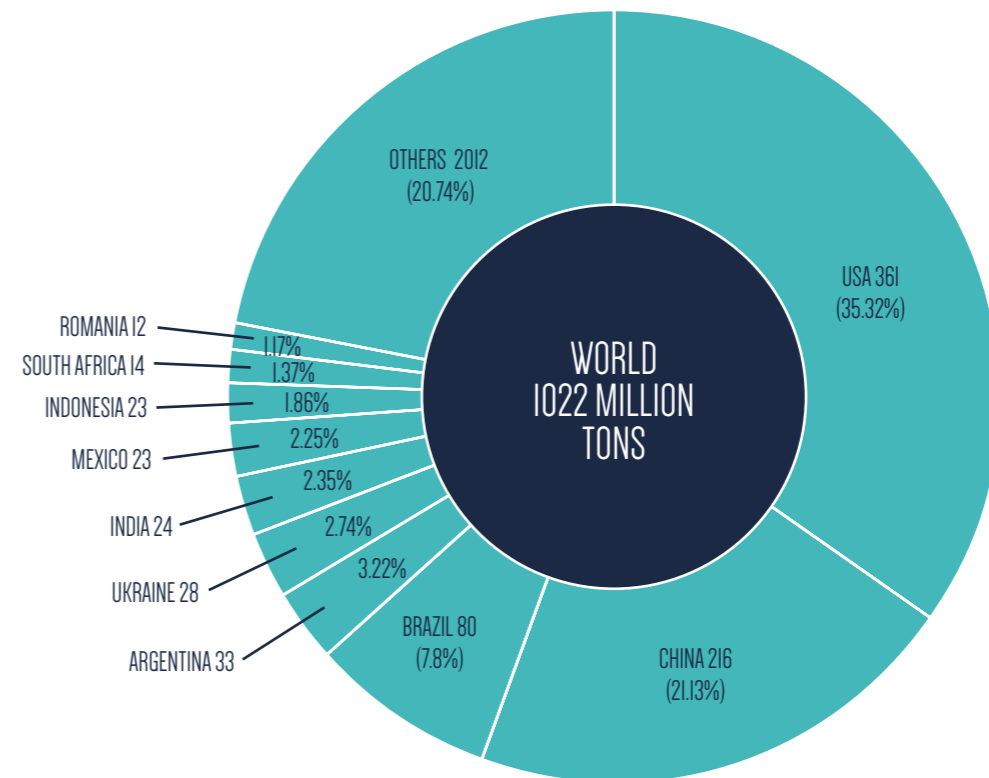


Figure 2.1: Overview of the worldwide production of maize in 2014 ¹⁸.

The major problem for maize production in Africa is very low yield: maize yields in Africa have stagnated at around 2 tons/hectare/year¹⁹ resulting in the need for Africa to import more than 20% of the required maize from non-African countries. Among the top 10 producers of maize worldwide, there is a large variation in production yield between the different areas: while yield is approximately 10.7 tons/hectare/year in the USA, yield is only 2.75 and 3.30 tons/hectare/year in India and Mexico respectively (Table 2.1). The situation is even more dramatic in Africa: while the yield was still reasonable in countries like Egypt (7.73 tons/hectare/year), South Africa (4.54 tons/hectare/year) and Ethiopia (3.42 tons/hectare/year),

the majority of countries have yields of less than 2 tons/hectare/year (35 of the 51 African countries producing maize) and even less than 1 ton/hectare/year (15/51 African countries, such as Zimbabwe, South Sudan and Gambia) in 2014 (Table 2.2).¹⁸ Maize plays an important role in the livelihoods of millions of (small) farmers, who grow maize for food, animal feed and income. For instance, about 9 million households in Ethiopia are currently engaged in maize cultivation.²⁰ However, these farmers are very often too poor to afford irrigation and pest control and apply good agricultural practices, and are therefore exposed to significant risks of production and income failure (see Chapter 4).

Country	Million tons	Production yield per hectare (tons/hectare/year)
USA	361	10.7
China	216	6
Brazil	60	5.2
Argentina	33	6.6
Ukraine	28	6.2
India	23	2.7
Mexico	23	3.3
Indonesia	19	4.9
South Africa	14.9	4.5
Romania	11.9	4.8

Table 2.1: Production yield per hectare for the top 10 producers worldwide ¹⁸.

Country	Million tons	Production yield per hectare (tons/hectare/year)
South Africa	14.9	4.5
Nigeria	10.8	1.8
Ethiopia	7.2	3.4
Tanzania	6.7	1.6
Egypt	5.8	7.7
Malawi	3.9	2.3
Kenya	3.5	1.7
Zambia	3.3	2.8
Uganda	2.8	2.5
Ghana	1.7	1.7

Table 2.2: Maize production and yield per hectare in 2014 in the top ten African countries ¹⁸.

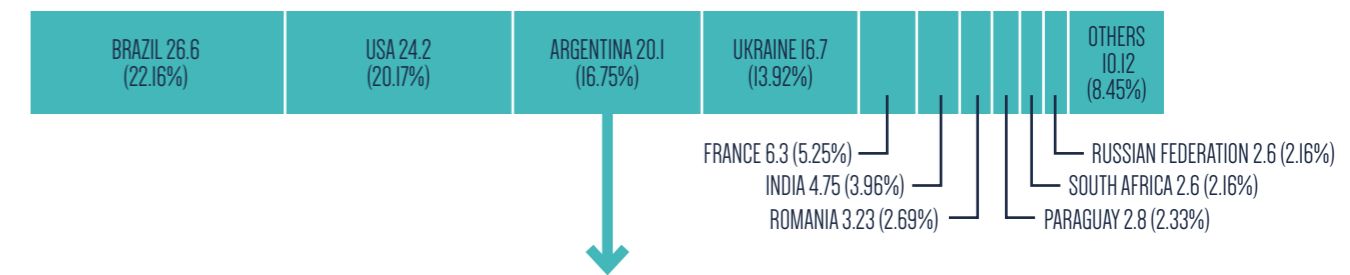
Approximately 122 million tons of maize were traded in 2013

Maize is very important for the economy of Brazil, which exported almost 27 million tons in 2013, making it the largest exporter of that year. The USA (the largest producer of maize in 2014), Argentina and Ukraine are also major exporters of maize, followed by France, India, Romania, Paraguay, South Africa and the Russian Federation (Figure 2.2).¹⁸

Japan produces almost no grain itself, but is a very important importer of maize for food, feed and industrial use. In 2013, Japan imported approximately 14.4 million tons, and was therefore number 1 in maize import. Remarkably, while Mexico is the seventh-largest producer of maize, it still imports much of its grain (7.1 tons in 2013). This is mainly because Mexico processes much of its production of white maize for human food

products, but has to import yellow maize for its livestock feed in order to support increased meat production.² The import and export figures for maize from China are more complex. In some years, China is the second-largest exporter of maize, but in other years, it has to import a significant amount of maize. This was also the case in 2013: China was the third largest importer that year, with approximately 7.3 million tons of maize. China's maize exports are largely a function of government export subsidies and tax rebates because prices in China are higher than those in the world market.¹⁹

EXPORTING COUNTRIES



IMPORTING COUNTRIES

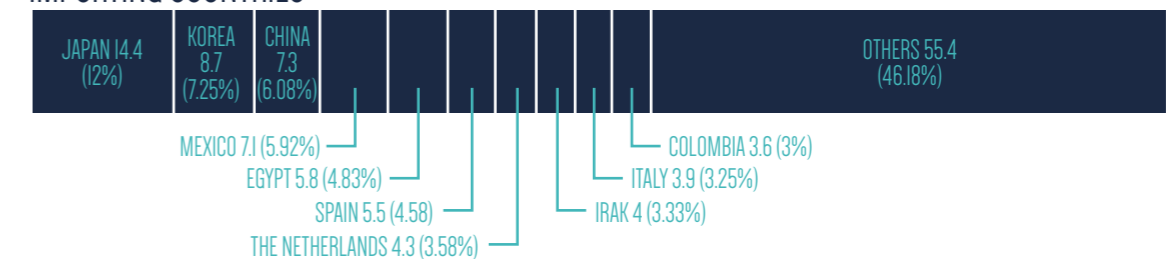


Figure 2.2: Overview of export and import of maize in 2013 ¹⁸.

The maize value chain in Africa

Maize is a major food crop in Africa, where it accounts for nearly half of the calories and proteins consumed. The US agency for international development calculated that the poorest quarter of the Kenyan population spends 28% of its income on maize. Each country produces its own maize, but still has very often to import additional maize for food and feed consumption (see above). Nevertheless, maize is also a very important cash crop in Africa. Many African countries, such as South Africa, Uganda, Tanzania, Rwanda and Namibia, are important exporters of maize flour. In 2013, 20% of the worldwide export of maize flour came from Africa, while the USA and France accounted for 14.9% and 10.5%, respectively.²¹ However, it is often very difficult to obtain a good overview of processing and export figures for Africa, because 70-80% of the maize is traded informally (Figure 2.3).²¹⁻²³

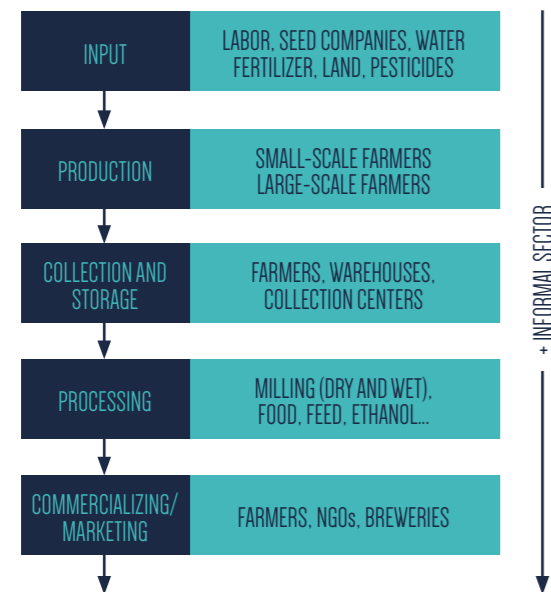


Figure 2.3: The maize value chain in Africa (adapted from ^{21, 23, 24}).

The maize value chain can be divided into different actors, but in general, the most common are input providers, farmers, traders, processors and partners involved in retail, feed and ethanol production (Figure 2.3).²¹⁻²³ Key inputs for maize production are land, water, chemicals such as fertilizers, pesticides and herbicides and high-quality seed. Maize production in Africa is mainly (>75%) done by small-scale farmers (less than 0.5-0.7 hectares of land), while some large-scale farmers mainly work for global export. Once maize is harvested, proper cleaning, drying and storage (14% moisture and 12-20°C) are needed before processing starts. The major raw kernel processing methods are (1) storage of kernels, (2) making whole-kernel products, (3) nixtamalization*, leading to masa flour, tortillas and chips and (4) wet and dry milling.⁷

Both dry and wet milling procedures lead to different end products (Table 2.3). During the dry milling process, the corn kernels are milled into a medium to finely ground meal with a pin, a hammer or disk mills, without the additional use of water or chemicals. This meal is used particularly for the production of feed, ethanol or other non-food items. These (mainly manual) mills can be found in African villages and markets, while major companies will invest more in wet milling. Wet milling is a more intensive process that will extract the highest use and value from each component of the maize kernel. First, the kernels are soaked in steep tanks containing a dilute aqueous sulfur dioxide solution. Afterwards, the germ is removed and further processed to collect the high-value oil. The remaining fiber and proteins are separated and used as feed. A part of the starch will be used in the food, paper and textile industries, while the other part will be used as sweetener or for the production of ethanol. While wet milling is a more

versatile, large-scale process that results in more products, dry milling (especially for ethanol production) is much cheaper and more efficient.

Maize can be processed into a variety of food and industrial products, including sweeteners, oil, beverages, starch, industrial ethanol and fuel ethanol.² Through enzymatic conversion, starch will result in products such as dextrin, sorbitol, sorbic and lactic acid, and appears in many household items such as mustard, syrup, ice cream, beer, aspirin, cosmetics, shoe polish, glue, fireworks, batteries, ink and paint. Maize is also used as filler for plastics, paper, yarn, cigarette papers, insulation and adhesives, and for making explosives, dyes, synthetic rubber, nylon, etc.^{2,7} Approximately 28% of the maize grown in Uganda will end up in the domestic industry, of which 60% will be processed into flour, 37% used for animal feed, and 3% used as input to make beer.²¹ The remaining 72% goes to on-farm consumption (18%), seed saving (2%), export market (22%) and post-harvest losses.²¹ The commercial maize market is controlled mainly by a small number of very strong, influential dealers and processors. Although the maize value chain is growing and expanding in

Africa, there are still many constraints that should be resolved, such as low-quality seeds, low use of fertilizers and insecticides, post-harvest losses, inappropriate storage conditions, lack of collection and transport infrastructure, poor markets, etc.

Worldwide, and especially in the USA, a significant portion of the maize produced is used to generate ethanol fuel. This fuel is often used as motor fuel, mainly as a biofuel additive for gasoline. This added demand together with low production levels, especially in Africa, have more than doubled maize grain prices over the past ten years and made it less affordable for the most vulnerable consumers.

Table 2.3: Overview of the end products obtained after milling (adapted from ⁷).

Dry milling	Wet milling
Brewer's grits	Feed products
- <i>Liquor</i>	Ethanol
- <i>Animal feed</i>	Corn oil
Corn flour	Sweeteners, syrups
- <i>Breakfast cereals (after extruding)</i>	Starch
Corn grits	
- <i>Flakes => arepa flour</i>	
- <i>Breakfast cereals (after extruding)</i>	
Non-food products	

* Nixtamalization is a process in which the maize grains are soaked in an alkaline solution, such as limewater, and hulled.

3 Maize has many enemies

Maize is grown all over the world, but yield differs and fluctuates very much depending on the area where and the season during which maize is grown. In many African countries, the average maize yield per hectare is very low. The main reason for this is that maize production is continuously affected by a number of biotic and abiotic stresses. Biotic stresses occur as a result of damage done to the plants by other living organisms. For maize, threatening organisms are stemborers, nematodes, bacteria, viruses, fungi and weeds. Abiotic stresses are the negative impacts of non-living factors on plants in a specific environment. For maize, abiotic stresses are poor soil fertility, drought and extreme temperatures (see Chapter 5), and poor post-harvest management.

Figure: African boll worm on maize (source: Johnnie Van den Berg).



Figure 3.1: The parasitic weed *Striga hermonthica* in a maize field in Kenya (source: Johnnie Van den Berg).

The battle between maize and weeds: Who will win?

A major maize pest in Sub-Saharan Africa is *Striga*, a parasitic flowering weed that attacks several crops, resulting in retarded plant growth, stunted and withered crops, and reduced grain yield (figure 3.1).^{25, 26} There are several *Striga* species, but for cereals, purple-flowered *Striga hermonthica* and to a lesser degree the red flowered *Striga asiatica* have large economic impacts.^{27, 28} After perceiving specific signals from the host plant, in this case maize, the haustorium** of the germinating *Striga* seed attaches itself to the root, penetrates into the vascular system of the plant and leeches water, nutrients and minerals intended for maize growth.^{29, 30} One single crop plant can support over a hundred parasitic weeds, each capable of producing thousands of seeds.²⁹ *Striga* seeds are very small and are mainly spread through the use of contaminated maize seed, eroded soil, wind, surface run-off, equipment, animals and humans. The seeds remain dormant in the soil for at least 15-20 years. At every planting season, some of the dormant seeds germinate, infest

the maize plant and reproduce. In this way, the problem intensifies. *Striga* infections flourish in low-fertility soils, in areas where rainfall is low and during monocropping.^{31, 32}

Striga is most damaging to the crop before young *Striga* weeds even emerge from the soil. Early signs of infections are folded leaves and wilting, even when there is still sufficient soil moisture. Therefore, *Striga hermonthica* is also known as the most destructive “witch” weed. Because *Striga* plants become intertwined with the maize roots, the weeds are almost impossible to remove through conventional weeding.

In Sub-Saharan Africa, season-long weed competition causes yield losses of 30-100% and affects the livelihoods of about 100 million people, resulting in annual crop losses estimated at USD 1 billion.^{26, 28, 33-36} Almost 40 million hectares of land in Sub-Saharan Africa, and 76% of the farmland in Kenya, is infested with *Striga*.^{28, 29} Moreover, at least 30% of farm-saved seeds are *Striga*-infested.³⁷ In Kenya, farmers refer to it as *kayongo*, *oluyongo* and *imoto*, while in Tanzania, it is known as *kiduha*.

**A haustorium (haustoria) is a structure that grows into or around another structure to absorb water or nutrients.



Figure 3.2: Infection of maize with maize stem borer larvae (A-C) (source: Belay Garoma).

Stem borers and other insects make life very difficult for maize

Stem borer damage is one of the main causes of low maize yields in Africa.^{28, 38} These insects seriously limit the potential yield by infesting the crop during its growth, from seedling stage to maturity (Gressel et al., 2004). Eggs can be found on maize leaves. The newly emerged larvae enter the whorls of the young maize leaves and feed on the tender leaves. In older plants, the larvae bore into the stem and make tunnels. As a consequence, infected plants experience stunted and poor growth, reduced yield, and are more susceptible to wind lodging and secondary disease infestations (Figure 3.2).^{28, 38}

Yield losses of 20-50% and greater due to stem borers are observed at the smallholder level in

several African countries. In Kenya, an average national crop loss of 13.5% (or approximately 400,000 tons), valued at more than USD 90 million, was reported.³⁹ These losses can even increase to up to 100% during dry years or when no pest management measures are taken. Field surveys revealed natural infestations of 2-19 larvae per plant. There are many different stem borers, but some of the most common ones are the African stem borer *Busseola fusca*, the spotted stem borer *Chilo partellus*, the coastal stem borer *Chilo orichalcociliellus*, the pink stem borer *Sesamia calamistis* and the African bollworm *Helicoverpa armigera* (Table 3.1).^{38, 39}

In 2016, the fall armyworm (*Spodoptera frugiperda*) invaded Africa from the West African tropics up to the South African Highveld and infiltrated at least 12 African countries (Togo, Ghana,

Zambia, Zimbabwe, South Africa, Malawi, Mozambique, Namibia, Kenya, Rwanda, Uganda and Tanzania).⁴⁰ In one year, 300,000 hectares in Sub-Saharan Africa have already been severely damaged by this armyworm. Spreading happens quickly, because each adult moth lays up to 2,000 eggs during its 2-week lifespan. Both maize leaves and cobs are eaten by this armyworm, resulting in dramatic yield losses (Figure 3.3).⁴⁰ Farmers are desperate because this insect is resistant to most chemicals, even when pesticides are used in doubled or even tripled doses. This new invasion gives African farmers new challenges in terms of control of this pest, which was previously never encountered on the continent (Johnnie Van den Berg, personal communication).

Figure 3.3: Fall armyworm in damaged maize shoot (A) and on a maize leaf (B).

Other pests, such as ear borers, armyworms, cutworms, beetles, weevils, grain borers, rootworms and white grubs can also be found on maize in Sub-Saharan Africa.

Table 3.1: Overview of important insect pests found in African maize fields.

Insect	Damage caused on infected plants
African maize stem borer^a (<i>Busseola fusca</i>)	Larvae feed in whorls, cause “small windows” after the leaf grows out, and some leaves are even rolled up. The larvae tunnel into the stem and inside the stem. Larvae can also tunnel into the ears, leading to direct yield losses. Feeding on kernels may also lead to downgrading of the harvest.
Spotted stem borer^a (<i>Chilo partellus</i>)	Leaf and stem damage are comparable to that of the maize stem borer. The observed yield losses due to ear damage are less comparable to that of the maize stem borer.
Coastal stem borer^a (<i>Chilo orichalcolielus</i>)	Damage occurs as a series of small holes in younger leaves and/or transparent patches in older leaves. Larvae can tunnel into the stem, resulting in broken and/or drying stems, and eventual death of the growing point.
African pink stem borer^a (<i>Sesamia calamistis</i>)	The larvae penetrate the stem shortly after they emerge from their eggs. The larvae can tunnel into the stem, resulting in broken and/or drying stems, and eventual death of the growing point. During ear filling, the majority of the larvae are in the ears.
Fall armyworm^a (<i>Spodoptera frugiperda</i>)	The symptoms of crop damage are very similar to other armyworm species and maize borer damage. Most plants recover from foliar feeding, but when grains consumed by larvae, the damage is more severe. Yield losses of 15-55% and even 100% are reported.
African bollworm^a (<i>Helicoverpa armigera</i>)	The larvae prefer ears, but can also eat large holes through the whorl leaf roll. The presence of fecal granules near the feeding sites is an indication of the presence of this bollworm. Infection can prevent pollination because the larvae feed on silks of young ears. The larvae can also open the tips of pollinated ears, leading to ear rot when rain enters the ear.
Larger grain borer^b (<i>Prostephanus truncates</i>)	Maize grains are damaged by both larvae and adults, but only the adults produce tunnels. After 3 to 6 months of grain storage, 30% weight losses due to infection with the larger grain borer were reported. Just before harvest, plants in the field can also be infected by this grain borer.
Maize weevil^b (<i>Sitophilus zeamais</i>)	The eggs, larvae and pupae develop inside intact maize grains. The adult beetle makes holes with irregular edges in the grains. Infestations produce heat and moisture.

^a Lepidoptera (moths); ^b Coleoptera (beetles)

During storage as well as growth, maize can be infected and destroyed by insects, especially when the maize is stored on-farm with no pesticide use or control over moisture content.⁴¹ Two major examples are the larger grain borer

Prostephanus truncates and the maize weevil *Sitophilus zeamais* (Table 3.1, Figure 3.4).⁴² The larger grain borer was accidentally introduced into Africa in the 1970s from its area of origin in Mexico and converts grain into powder with-

in a short period of time.⁴³ The resulting powder cannot be used anymore for livestock or human consumption, as it contains insect eggs, excreta and exuviate (Figure 3.4).⁴² The larger grain borer is currently recognized as the most destructive pest of farm-stored maize, with losses varying from 9-45%, depending on the period of

storage.⁴⁴ The maize weevil *Sitophilus zeamais* infections can also result in grain weight losses of 12-20% and even up to 80% when the untreated maize is stored in traditional cabins.⁴⁵ Additionally, the consumption of weevil-infested maize grain is very unhealthy, as these grains are prone to contamination by mycotoxins (see below).⁴⁶

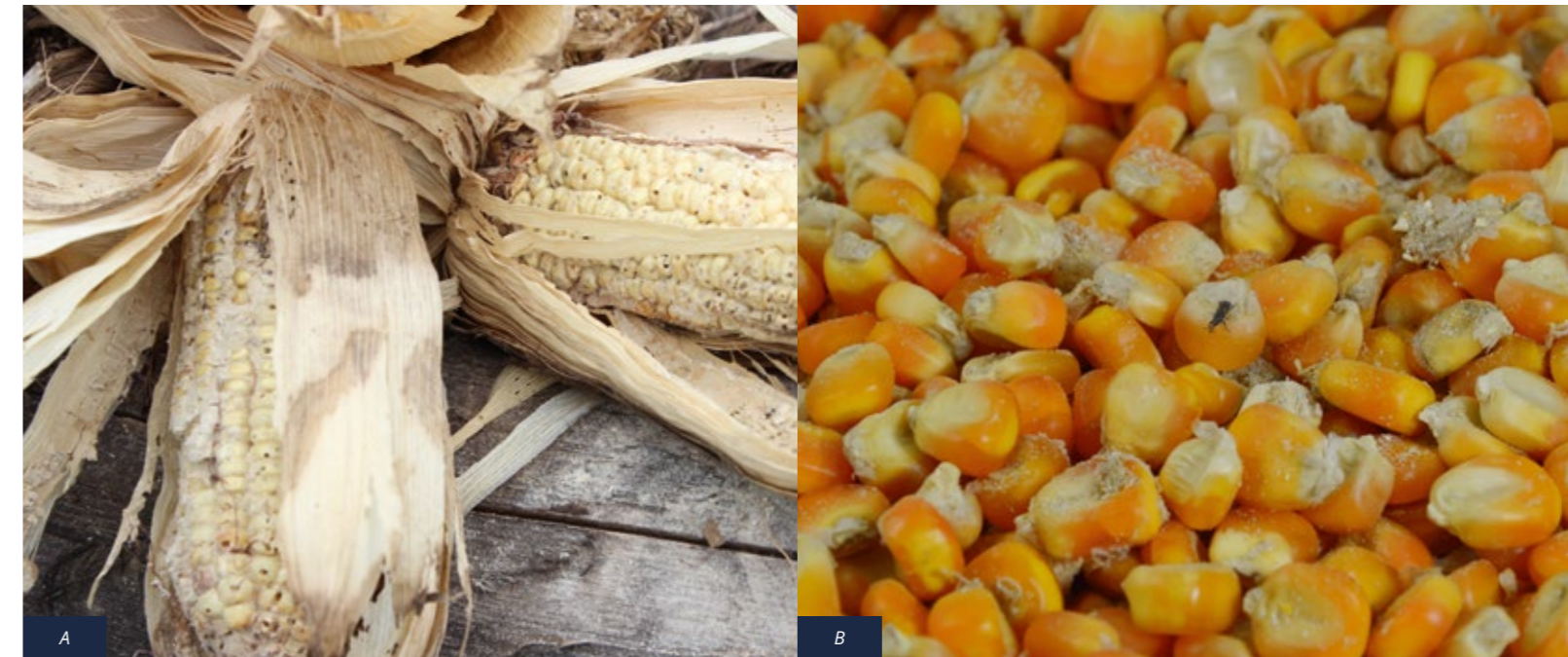


Figure 3.4: (A) Maize ear damaged by larger grain borer (source: Bernardo Muatinte), (B) maize kernels damaged by weevils.

Bacteria, viruses, nematodes and fungi: (Almost) invisible but so harmful

Bacterial diseases

Important diseases in maize caused by bacteria are bacterial leaf streak and bacterial whorl and stalk rot. Bacterial leaf streak is caused by *Xanthomonas campestris* pv. *zeae* and was reported for the first time in South Africa in 1949.⁴⁷ This bacterium is mainly found in warm and dry maize production areas and is mainly spread by irrigation water, wind and rain, aphids and by plant-to-plant con-

tact (Figure 3.5). The bacteria can penetrate the maize leaves through their natural openings, called stomata. The initial leaf symptoms appear as dark, oil-drenched streaks that will enlarge to form narrow yellow translucent streaks of 2-3 mm over the whole leaf area. As a result, photosynthesis* will be limited, leading to limited grain fill. Up to 40% of the leaves can become brown and dry prematurely, dramatically lowering the yield.

*Photosynthesis is a vital process in plants through which energy from light is used to convert carbon dioxide into energy-rich compounds (carbohydrates), such as glucose. This is how the plant is able to produce energy for itself. Most photosynthesis takes place in the leaves of a plant.

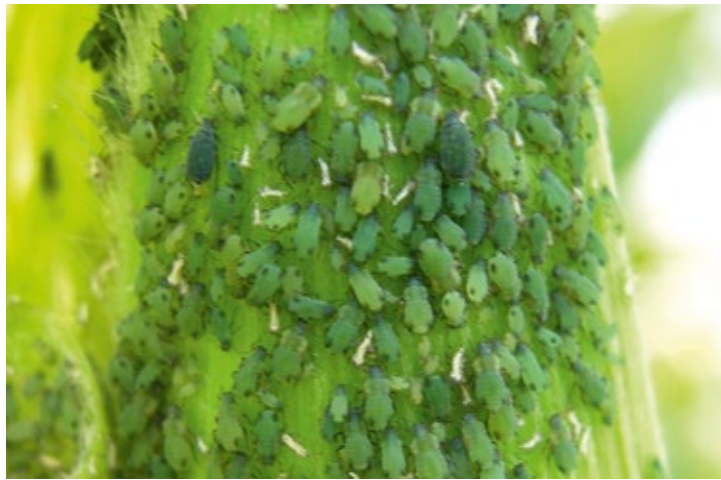


Figure 3.5: Aphids feeding on maize can spread *Xanthomonas bacteria* (source: Johnnie Van den Berg).

Bacterial whorl and stalk rot in maize are caused by *Erwinia chrysanthemi* pv. *Zea* (*Erwinia carotovora* s.sp. *Zea*). Bacteria enter the plants through stomata, but also through wounds caused by hail, strong wind and insects. The bacteria overwinter in stalk tissue on the soil surface. Bacterial whorl and stalk rot of maize is mostly observed in areas with high rainfall and/or irrigation, particularly in poorly drained soils.^{48, 49} Bacterial whorl rot symptoms can be observed from seedling to flowering stage, but mainly occur from the 6-8 leaf stage.

The uppermost leaves wilt and a slimy, soft rot that imparts a pungent, recognizable smell will appear in the base of the whorl. The loss of leaves will lead to lack of nutrients, and subsequently to reduced grain fill and yield. The growing point of the plant dies and the plant will collapse. The primary symptoms of bacterial stalk rot appear during the midseason when plants suddenly lodge. The infected stalk will become water-soaked, turns tan to brown and becomes soft and mushy. As a result, the stalk will collapse although the vascular strands are still intact. The infected stalk emits a very strong, typical rotting odor. Infection higher up in the plant will also have a negative effect on plume development and will strongly affect pollination.

Viral diseases

A very devastating and widely spread viral disease in Sub-Saharan Africa is maize (or corn) lethal necrosis disease.⁵⁰⁻⁵² This disease is the result of a simultaneous infection of two viruses, the maize chlorotic mottle virus and any of the cereal viruses in the *Potyviridae* group, such as the maize dwarf mosaic virus, although single infection with maize chlorotic mottle virus can already lead to serious crop losses.⁵⁰ Maize lethal necrosis disease is mainly spread by vectors, such as maize thrips, rootworms and leaf beetles, and affects all commercial maize varieties in Sub-Saharan Africa at every stage of development. Infection leads to tissue chlorosis and necrosis, severe plant stunting and plant death. When infected late in development, plants are barren, have small, partially filled, malformed ears and remain unproductive.⁵³ In 2011, there was a serious outbreak of this disease in Kenya, and since then, the disease has also been reported in Democratic Republic of Congo, Tanzania, Uganda, South Sudan and Ethiopia.^{50, 52, 54-56} In Kenya, yield losses of up to 90% resulted in an estimated grain loss of 126,000 metric tons in 2012, with a value of approximately 52 million USD.⁵⁰ This disease causes not only big economic losses for farmers and seed companies in Sub-Saharan Africa, but also affects consumers,

as there are no longer products on the market. Because maize lethal necrosis disease very often gives rise to secondary fungal infections (see below), these infected plants/grains are no longer suitable for human or animal consumption.

Another major viral pathogen constraint in Sub-Saharan Africa is maize streak disease, caused by the maize streak virus (Figure 3.6).⁵⁷ This disease has resulted in substantial losses throughout Sub-Saharan Africa and can result in major maize crop failures.⁵⁸ The virus is mainly transmitted by sap-feeding leafhoppers. Once inside the plant, it multiplies and moves within the sap above the point where infection occurred. This will result in parallel streaks on the leaves. Plants can be infected at any stage of development, but when infected very early, they will remain stunted, unable to produce complete cobs and seeds, and will die early. When the infection occurs later during plant development, the symptoms are not obvious and have no direct effect on vigor and plant productivity.⁵⁷

The impact of nematodes on maize yields is often underestimated

At least 17 nematode groups have been identified that damage maize. The two most important groups are the lesion nematodes (*Pratylenchus* spp.) and the root knot nematodes (*Meloidogyne* spp.). After infection, the upper parts of infected plants are stunted, yellow and patchy in growth, and plants may even die before harvesting. During drought, nematodes or their unhatched eggs can enter a resting phase and live for almost six months. This allows the nematodes to survive the period between two maize-growing seasons. Once the seeds start to germinate or the soil conditions are favorable again, the eggs will hatch and infect the plant. The nematodes can



Figure 3.6: Maize streak disease (source: Johnnie Van De Berg).

spread through water, plant and soil residues on tools or shoes used on infested fields.

Fungal diseases

Maize gray leaf spot is caused by the fungus *Cercospora zea-maydis* and was first observed in the US.⁵⁹ Nowadays, gray leaf spot is recognized as one of the most destructive and yield-limiting diseases of maize worldwide, and has also become pandemic in Africa.⁶⁰⁻⁶² In Africa, the first economic losses due to gray leaf spot were reported in South Africa and Zimbabwe in the 1990-1991 and 1995-1996 growing seasons, respectively. Since then, this disease has also been observed in Cameroon, Kenya, Uganda, Zambia, Democratic Republic of Congo, Ethiopia, Malawi, Mozambique, Nigeria, South Africa, Swaziland and Tanzania.^{61, 62} *Cercospora zea-maydis* only infects maize and produces spores following periods of high humidity.⁶² These spores are dispersed by wind and rain to the lower leaves where they start to form lesions of 1 to 3 mm long with chlorotic



Figure 3.7: Symptoms of gray leaf spot (A) and Turcicum leaf blight (B) on maize plants (source: Belay Garona).

borders and a rectangular shape. Upon development, mature lesions become gray and tan in color and run parallel with the leaf veins (Figure 3.7). The lesion numbers will rapidly increase and appear on higher developing leaves. Finally, the entire leaf will blight, resulting in stalk deterioration and severe lodging.⁶² This fungus is able to survive within infested maize crop residues present on the soil surface during intercrop periods. Gray leaf spot leads to yield losses of between 20% and 70%.^{62, 63}

Turcicum leaf blight, caused by the fungus *Exserohilum turcicum*, is also a very important maize disease worldwide.⁶⁴ The disease appears predominantly in wet and humid areas with moderate temperatures.⁶⁵ Infection leads to blighting of the leaves, reduced photosynthesis and less grain filling (Figure 3.7).⁶⁶ Yield losses of up to 70% have been reported.⁶⁵ The fungus can survive during winter in infected crop debris and is disseminated by wind and rain onto new plants. In areas with reduced tillage (= the agricultural preparation of soil by mechanical agitation) methods and increased use of nitrogen fertilizer, the fungus is more prevalent.

Mycotoxins, an additional problem for already infected maize plants

Once a (maize) plant is wounded or infected by bacteria, viruses or stem borers, it becomes very vulnerable to superinfection by fungi such as *Aspergillus flavus* and *Fusarium verticillioides* Sacc Nirenberg (Figure 3.8).^{67, 68} These fungi can grow on maize kernels and produce mycotoxins, such as aflatoxin and fumonisin.^{68, 69} Mycotoxins are toxic compounds that are mainly concentrated in the kernel fiber, the gluten protein and germ, and are very stable, even after kernel processing.⁶⁷ Toxin production is mainly caused by pre-harvest infection, delayed harvesting, wet conditions during harvest periods, insufficient grain drying, and high moisture levels during storage and transportation.

Contamination of maize kernels with mycotoxins is a major problem because they are very toxic to humans. In 2004, some maize samples in Kenya had 220 times the allowed limit of aflatoxin concentration allowed by Kenyan authorities, resulting in 125 deaths.⁶⁸ Fumonisin is often fatal to humans and farm animals as well. Although

dairy cattle can still tolerate relatively high levels of aflatoxins in their feed^{68, 69}, some of these toxic compounds can be excreted in the milk, which is then consumed by humans. The FAO estimated that more than 4.5 billion people in the developing world are chronically exposed to mycotoxins, resulting in adverse health effects such as impaired growth of young children, modulated infections, reduced immunity and liver carcinogenesis.^{70, 71} It was calculated that 10% of all adult deaths in Sub-Saharan Africa could be due to liver cancer caused by mycotoxin exposure. Contamination of maize kernels with mycotoxins is also dramatic for the African maize trade and economy. In 2010, 2.3 million 90-kg bags of maize in Kenya were declared unfit for consumption by humans and animals due to aflatoxin contamination.



Figure 3.8: Maize kernels infected by fungi producing mycotoxins (source: Bruno Tinland, personal collection).

Maize grains are lost both before and after harvest

The low yields of maize harvests in Africa are mainly caused by several pests and infections by viruses, fungi and bacteria as described above. However, inadequate post-harvest management in Africa also leads to the loss of 14-36% of maize grains harvested.⁷² These losses impact the food supply and food security and also contribute to in-

creasing food prices.^{72, 73} Post-harvest losses can occur at different stages: harvesting and drying (6-16%), threshing and shelling (1-4%), transport to store on the farm (1-2%), on-farm storage (4-10%), transport to the market (1-2%) and marketing itself (1-2%).⁷² During threshing and shelling, losses might be caused by grain cracking, breaking and partial or total consumption by insects. These losses are mainly observed on large farms because of mechanical shelling, compared to small-scale farmers who shell ears manually.⁷²

Smallholder farmers store their grain at their farms in structures made of wood or clay (Figure 3.9). However, these traditional storage structures still expose the harvested grain to rodents and insect attacks. Furthermore, unfavorable weather conditions might prevent the grains from drying sufficiently.⁷⁴



Figure 3.9: Storage of maize on the farm in structures made of (A) wood (source: Bernardo Muatinte) and (B) clay (Source: Bruno Tinland).



4 Both breeding and modern biotechnology can protect maize against attacks

A broad spectrum of biotic and abiotic stresses, lack of access to quality seed and fertilizers, low levels of mechanization and suboptimal post-harvest management lead to low maize yields in Africa. To overcome these problems, good agricultural practices, intercropping, use of hybrid varieties, new open-pollinated varieties and biotech-enhanced maize crops may help to overcome these problems.



Figure: Different maize varieties in a shade house (source: Johnnie Van den Berg).

First actions to improve yield and to counteract infections

Many problems begin with the quality of the land on which maize is cultivated in Africa: very often, maize is grown on degraded, nutrient-starved soils, whereas the most suitable soil for maize should have good effective depth, an optimal moisture regime, good internal drainage, and a good balance of chemical and nutritional content. Smallholder farmers often only apply a small quantity of fertilizers on their fields, of which only a tiny portion will be used by the maize plant. Farmers are also perfectly aware that adding fertilizer to the soil will lead to more weeds, which, as a consequence, increases the need for hand-weeding. To guarantee a successful and high-yielding maize growing season, efficient and drastic weed control is needed. Weeding is predominantly a task for women, often occupying 80% of their time.⁷⁵ Maximum maize yields are achieved when the maize is kept weed-free for the first 56 days after planting. A one week delay in the first weeding will decrease the maize yield by one-third. Other good agronomic practices to fight against *Striga* infestations are, for instance, uprooting and burning *Striga* plants before they flower, the utilization of *Striga*-free planting material and clean tools, crop rotation, intercropping (see below), good irrigation, and the application of herbicides.²⁹

Good agronomic practices are also essential to combat infestations by nematodes and insects as well as by bacteria, viruses and fungi. The first actions to be taken are to grow the maize on well-drained irrigated fields, to avoid excessive flooding and movement of infected plants to other areas, to remove and even burn infected plants, to leave the field fallow for some time, to apply trap crop-

ping or crop rotation with non-cereal crops such as beans, sweet potatoes or cassava, and to apply crop protection products to the field.^{50, 51, 53, 54} A lot of problems can be avoided if farmers start with healthy seeds, or if they treat their seeds with an insecticide to provide early-stage protection against thrips, aphids or other pathogens. However, diseases are often ranked low on the farmer's priority list, because the symptoms of diseases are frequently confused with damage from abiotic stresses and insect pests.⁵¹ More than 75% of farmers in Africa do not control both insect pests and diseases, while the rest use some chemicals and everyday household remedies for control. Some farmers try to avoid diseases and pests by planting early-maturing varieties.⁵¹

To control stem borer pests in Africa, chemical control methods are the most effective, and recommended by the national agricultural extension agencies.^{41, 76} Several insecticides, formulated as either granules or spray applications, are registered for stem borer control. In many African countries, such as Kenya, Ethiopia and Mozambique, large-scale commercial farmers rely on these insecticides, but only about 5% of smallholder farmers use them because they are often very costly and not easily available.^{28, 42, 77-79} Some smallholder farmers developed methods to make the treatments as inexpensive as possible by using very small amounts of insecticide. However, due to these very low doses, the insecticides are not always effective. In Kenya, 90% of farmers apply wood ash, soil and tobacco snuff to control stem borers, but only about 2% found them to be very effective.⁸⁰ Other farmers in Kenya tried to combat the stem borers through biological control by introducing the histerid beetle *Teretrius nigrescens*, with varying degrees of success.⁸¹ Most of the farmers use cheap, everyday



Figure 4.1: Maize-Desmodium intercropping (source: Johnnie Van den Berg).

household products, such as dish washing liquid, general disinfectants (Jeyes fluid), spices (pepper), salt and paraffin oil, to combat stalk borers and cutworms.⁵¹ Apparently, dish washing liquid seems to be effective against aphids and spider mites, and black and red peppers against a number of grain storage pests.⁵¹

Intercropping maize with other crops may lead to increased yield and decreased stem borer and *Striga* infestations

To increase productivity per unit of land, minimize infection risks, reduce weeds and stabilize yield, intercropping can be an effective solution.⁸² Intercropping is the cultivation of two or more crops at the same time in the same place, and was already performed by the Native Americans: they planted maize in a complex system together with beans and squashes, a combination called “the three sisters”. Maize provides support for beans, and the beans provide nitrogen derived from nitrogen-fixing rhizobia bacteria living in the roots of the beans. The squashes provide ground cover to stop weeds and inhibit evaporation by shading the soil.² Nowadays, maize is very often

intercropped with groundnut, cowpea or bean, but the choice of the compatible crop depends on the plant growth, habit, land, light, water and fertilizer utilization.⁸²⁻⁸⁴ Particularly in the absence of nitrogen fertilizer, cereal-legume intercropping is commonly practiced, because the legumes will fix the nitrogen from the atmosphere and will not compete with maize for nitrogen in the soil.^{85, 86}

A very successful strategy to combat stem borers and *Striga* is habitat management or the “push-pull” system. This system combines the simultaneous growth of maize with *Desmodium uncatum* (Figure 4.1).⁸⁷ *Desmodium*, a nitrogen fixing crop, seems to control both *Striga* and stem borer pests on the maize field, as it produces a smell that repels the adult stem borer moths and a chemical that prevents *Striga* from attaching to maize roots.^{88, 89} Furthermore, *Desmodium* will also act as a cover crop within the inter-row area, protecting soil against erosion.

Classical breeding: Performing optimal crossings

Ancient farmers from Mexico were the first to domesticate maize. They noticed that the plants were not all the same in appearance, that some grew higher than others and some kernels were tastier than others. So, they selected the best plants with the desired characteristics and cultivated them for the next harvest season. These selections and cultivation were already the first steps in plant breeding, and until today, conventional and marker-assisted breeding (see text box p.34) are commonly used to create improved varieties with resistance to insects, viruses, bacteria and/or herbicides. During conventional and marker-assisted breeding, the breeder manually performs crosses between two selected

parental plants. The male and female reproductive organs are separable and easy accessible (Figure 1.1) making controlled crosses easy to perform.⁶ Before manual pollination in the greenhouse is performed, silks and tassels of different plants are covered with white and brown paper bags, respectively. After some time, the breeder will gently shake this brown bag to collect the pollen into the bag.^{6, 9}



Figure 4.2: Elisabeth Njuguna, PhD student at the VIB-UGent Center for Plant Systems Biology, is covering a pollinated ear with the brown tassel bag (source: Mieke Van Lijsebettens).

When the uniform growth of silks is observed, the white paper bag is removed, the tip of the young cob is cut to allow the silks to regrow overnight and in the morning, the freshly collected pollen in the brown bag is shed over the short, regrown silks of the young ear. All the information about the male and female plants and the date of the cross are indicated with a pencil on the brown bag (Figure 4.2) that will cover the pollinated ear. When the ear is fully matured, it can be harvested and dried (Figure 4.3).

Experienced maize breeders can perform 300 to 500 crosses in one day. With each cross yielding several hundred seeds, maize can quickly generate large numbers of offspring for the genetic



Figure 4.3: After maturation, the ears are dried in a separate drying room (source: Mieke van Lijsebettens).

analysis of DNA markers.⁶ Although maize breeding is a commonly applied technique, it has some major drawbacks: the breeding programs proceed rather slowly because maize has a relatively long life cycle (approximately 13 weeks, depending on the variety) and is space-consuming. Due to its size and the high light intensity required for growth, maize needs to be grown in large chambers. Additionally, genes encoding for major disease and pest resistance are not all identified yet.^{6, 9, 90} Tropical maize flowers much later in long daylight regimes in temperate regions because it is adapted to a day length of 12-13 hours.

Before commercialization, the most appropriate hybrids or open pollinated varieties (see text box p.34) will be evaluated in several field trials at different locations and in different agro-ecological regions (Figure 4.4).



Figure 4.4: Evaluation of new hybrids in a field trial (source: Belay Garona).

CROSS-BREEDING AND MARKER-ASSISTED BREEDING⁹¹

Since the origin of agriculture, people have sought to produce plants that are stronger and have higher yields than their parents. These new and improved plants are obtained by continuous selection of the most performant varieties. Two parent plants carrying different traits are crossbred in order to combine the traits into one plant. Subsequently, the offspring that contains the advantageous combination of the traits of the two parents is used.⁹¹ In some breeding programs, only one desirable trait from parent 1 is sought to be transferred to parent 2. To achieve this, several consecutive crosses are necessary, where the offspring with the desirable trait is repeatedly crossbred with the original (commercially appealing) parent plant 2. This process, called backcrossing, is repeated several times to obtain a new plant with as many traits as possible from the original beneficial parent 2, but with the new trait from parent 1.⁹¹ For maize, as much as 17 generations may be needed.⁹² Each maize variety has a different generation time, but if you consider an average generation time of 6 months, this means that breeding will take at least 9 years (without calculating any research or screening time in between).

Selection steps during crossbreeding are particularly time-consuming. Certain traits cannot simply be evaluated on the basis of plant phenotype (= observable traits) and for other traits, such as disease resistance, plants need to be infected and evaluated in the field. Furthermore, traits such as drought, yield and growth speed are determined by multiple genes. Enormous progress in plant genomics has unraveled the genetic specification of a number of traits. By detecting a specific DNA fragment (marker) in a crossing product, which is linked to a certain trait, the presence of the trait can be determined and selected at a very early stage. When the DNA regions with a direct influence on the trait are known, cross-breeding selection or marker-assisted selection is facilitated. For each offspring of a certain cross-breed, DNA markers allow the determination of which combination of genes is present, and the most beneficial combination can be identified very quickly.^{91, 93} Hence, reduced numbers of backcrosses are required. Nowadays, marker-assisted breeding has almost become standard in crop improvement programs.⁹¹

OPEN POLLINATED VARIETIES (OPV) VERSUS HYBRIDS

New maize varieties can be generated as open pollinated varieties (OPV) or hybrids. However, their offspring have quite different characteristics.

“Open pollinated” refers to seed that will “true breed”, and uncontrolled pollination occurs by an insect, bird, wind, humans or any other natural mechanism. Because pollen can come from different maize varieties, open pollination will create a lot of diversity. For instance, seedlings from OPV will not be uniform in height, color and might mature at different times. Seeds of OPV are saved by the farmers across generations or even across decades. Therefore, these OPVs might be well-adapted to the farmer's environment. Advantages of OPVs are that the cost of the seed is not as high as that of hybrid seed (see below), seeds can be saved and can easily be exchanged among farmers. On the contrary, OPVs have lower yield potential than hybrid seeds and are not uniform in development. A hybrid (F1 hybrid) results from a controlled cross between 2 parents that are first obtained through self-pollination (inbreeding). In this way, each parental inbred line in the cross will have identical copies of each chromosome (homozygous). When both homozygous parents are crossed, all obtained F1 hybrids will be genetically identical and have uniform characteristics. In addition, these F1 hybrids will usually perform better than the average of the two parents for one or more traits (hybrid vigor).⁹⁴ This hybrid vigor is very important for African smallholder farmers, because the hybrids will provide a uniform and higher yield and harvesting of all plants can occur at the same time, making farming easier. Hybrid seeds are not only much more expensive than OPV seeds, but farmers have to buy new F1 seeds every year. The reason for this is that the offspring of the F1 hybrids will again show very diverse traits and can have reduced yields.

Many interesting hybrids and open pollinated varieties have already been developed and research is still ongoing

Imazapyr-resistant hybrid maize

The International Maize and Wheat Improvement Center (CIMMYT), the Weizmann Institute of Science, the Kenya Agriculture Research Institute and the company BASF developed an herbicide-resistant maize variety, StrigAway®, which is able to decrease *Striga* infestation in the field. StrigAway® is also known as *ua kayongo* in Kenya and *komesha kiduha* in Tanzania. The StrigAway® maize is resistant to the herbicide imazapyr. Via conventional breeding, a naturally occurring imazapyr-resistance gene of maize, identified by BASF and made available to CIMMYT, was incorporated into a selected maize variety. Subsequently, the obtained herbicide-resistant maize seeds were coated with low doses of the herbicide. When StrigAway® maize seeds germinate, the seedlings absorb some of the herbicide used in the seed coating, and stimulate *Striga* to germinate and to attach to the maize roots.^{29, 34} However, before *Striga* can damage the maize plant, it will be killed by the herbicide. Some herbicide that is not absorbed by the maize seedling will diffuse into the soil and kill the remaining *Striga* seeds/plants. The herbicide will have no additional effect on soil quality, because only small amounts of the herbicide are used for coating and 2-3 months after planting, the herbicide is completely degraded. When using these StrigAway® seeds, the maize yield is increased at least threefold. This is not only due to the resistance against *Striga* infestations, but also due to incorporated resistance against maize streak virus and the Turicum leaf blight. StrigAway® seeds are now available in Kenya and

Tanzania and field trials are ongoing in Uganda, Ethiopia, Malawi, Zambia, Zimbabwe, Lesotho and Swaziland.

Insect-resistant maize varieties

Host resistance to stem borers and post-harvest insect pests is determined by plant genes. Morphological factors such as increased leaf fiber, surface wax and high hemicellulose content have been associated with resistance against stem borers.^{77, 95-97} Multiple borer-resistant maize plants have tougher leaf tissues due to thick epidermal cell walls that prevent larvae from feeding.⁹⁷ Within the framework of the “Insect Resistant Maize for Africa” (IRMA) project, CIMMYT developed and deployed insect-resistant, high-yielding maize hybrids and open-pollinated varieties with multiple borer resistance through conventional breeding.^{77, 78, 98} Hybrids that provide resistance to stem borers, the large grain borer and/or the maize weevil have been developed and tested in regional trials in Kenya, Uganda, Tanzania and Mozambique.^{42, 77, 78} Some of these hybrids have already been released in different countries and are also used in breeding programs to transfer the resistance genes into their locally preferred and adapted varieties.

A difficult and intensive search for new resistance genes

More than 95% of the maize hybrids grown in Africa are highly susceptible to the maize lethal necrosis disease (see Chapter 3). Because this disease is devastating to maize fields in Africa, CIMMYT and the Kenyan Agricultural and Livestock Research Organization evaluated nearly 25,000 maize varieties between 2012 and 2014 in order to map the DNA regions associated with maize lethal necrosis resistance so that they could be transferred into local varieties via



Figure 4.5: Screening for resistance to gray leaf spot in a field trial; the inbred lines on the right and left are resistant to gray leaf spot, those in the middle are susceptible (source: Belay Garoma).

crossings.⁵² Further fine mapping and validation of these genetic regions are currently ongoing. Nevertheless, some inbred maize lines that are resistant to maize lethal necrosis were reported a long time ago, but they have yellow kernels and are therefore not suitable for hybrid combinations in Sub-Saharan Africa because consumers prefer white kernels. Currently, CIMMYT is transferring this resistance into white kernel lines, but these new hybrids are not on the market yet.⁵² Similar breeding experiments have also been undertaken both in Kenya and Ohio to create some maize lethal necrosis-resistant varieties for East Africa.^{50,99}

Infections with the maize streak virus can also lead to 100% yield losses (see Chapter 3). Some genomic regions associated with maize streak virus resistance have already been mapped in several populations,^{100,101} and are now being evaluated by

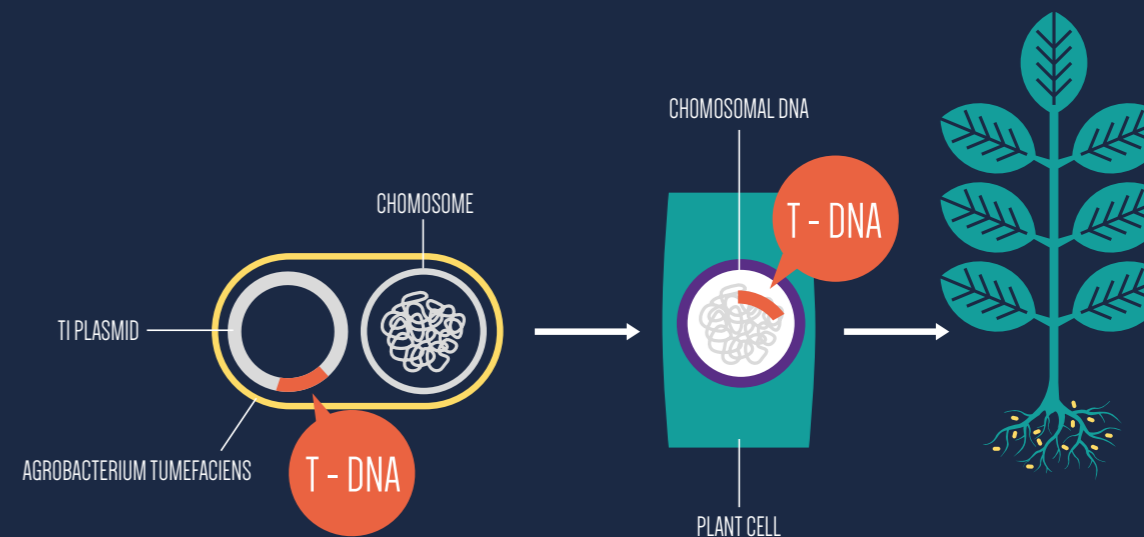
CIMMYT. The major disadvantage of these maize streak virus resistant genotypes is that they are not as yield productive as sensitive genotypes in non-epidemic years.³⁶

Although huge susceptibility differences are recorded in the field, no commercial hybrids with resistance against bacterial whorl and stalk rot, gray leaf spot and Turcicum leaf blight are on the market yet. Some inbred lines were recently tested in Ethiopia for their resistance against gray leaf spot and Turcicum leaf blight.¹⁰² Out of 25 hybrid lines, 4 showed resistance to Turcicum leaf blight, 3 to gray leaf spot (Figure 4.5) and the majority to both diseases.¹⁰² The aim is now to identify these genes and use the inbred lines in breeding programs with local varieties. Breeding programs are also underway to overcome ear rot caused by fungi, which is also associated with mycotoxin contamination.¹⁰³

GENETIC MODIFICATION VIA DIRECT GENE TRANSFER AND AGROBACTERIUM TUMEFACIENS-MEDIATED TRANSFORMATION¹⁰⁴

Plants can be genetically modified by both direct gene transfer and Agrobacterium-mediated transformation. Both techniques make use of a unique feature: individual plant cells are able to regenerate into a completely new plant. Two examples of direct gene transfer methods are electroporation and particle bombardment. Electroporation is a technique that uses short electric pulses of high field strength to deliver the gene of interest to a protoplast (plant cells without cell walls). These short pulses create pores leading into the plasma membrane of the cell through which the DNA can enter the cell. Particle bombardment is a process by which tiny particles of gold, coated with DNA, are “shot” under high pressure into the plant tissue. In some cases, the DNA will penetrate the nucleus of the plant cell, where it will incorporate itself into the plant’s DNA. Both electroporation and particle bombardment are low-efficiency techniques, and multiple copies and/or truncated parts of the desired DNA are frequently integrated into the plant DNA, which can’t be used for further selection.^{91,104}

A much more efficient transformation method makes use of the natural ability of the soil bacterium *Agrobacterium tumefaciens* to transfer DNA to plant cells.^{91,104} In nature, *Agrobacterium tumefaciens* can infect certain plants, and during this process, transfers a piece of its own DNA (the transfer DNA or T-DNA) to the nucleus of the plant cell where it incorporates into the plant DNA.



Schematic representation of Agrobacterium tumefaciens' gene transfer mechanism

In the 1970s, molecular biologists, including professors Marc Van Montagu and Jeff Schell (University Ghent) unraveled the DNA transfer mechanism and discovered that the original genes included in the T-DNA were not essential for DNA transfer. They replaced the original genes with the genes of interest. The transfer of the new engineered T-DNA to the plant cells continued to work, provided it was flanked by the correct T-DNA border sequences and in the presence of bacterial proteins for T-DNA transfer and integration. Because this transformation process does not work with 100% efficiency, the presence of the T-DNA carrying the gene of interest in the plant is confirmed by selection for resistance to antibiotics or herbicides encoded by the respective resistance markers near the gene of

interest. The selection markers can then be subsequently removed.^{91,104,105} In contrast to direct gene transfer, only a few intact T-DNA copies are integrated into the plant genome. The only disadvantage of genetic engineering is that the T-DNA cannot be targeted to a specific predetermined position in the plant genome.

Important advantages of genetic modification compared to conventional breeding and marker-assisted breeding are that multiple traits can be incorporated in one transformation step, and that not only traits from the same plant variety but also from other plant varieties, and even other species, can be transferred to a plant of interest.¹⁰⁴

Genetic transformation of maize

The maize varieties used for food and feed applications in Africa have white and yellow kernels, respectively. In South Africa, approximately 65% of the maize produced for human consumption is white, whereas the remaining 35% is yellow and used for animal feed. Although all these varieties might benefit from additional/improved characteristics, such as disease resistance, the improvement of maize by genetic engineering has mainly focused on the yellow varieties.

In the early days, maize was mainly transformed via direct gene transfer (see text box p.37) because maize is not a natural host of *Agrobacterium*. Both white and yellow varieties were transformed via electroporation of protoplasts or particle bombardment, with varying and low efficiencies.^{6, 106-110} Furthermore, almost no fertile plants could be obtained.¹¹⁰

The first successful *Agrobacterium*-mediated transformation of maize was reported in 1996¹¹¹ and soon afterwards, many other studies followed.¹¹²⁻¹¹⁵ Research for successful transformation of maize has mainly been focused on maize varieties adapted to temperate zones, while less attention was given to those varieties adapted to tropical regions in Sub-Saharan Africa. For these varieties, genetic transformation is still a challenge.

Several maize protocols starting from various parts of the maize plant were already described in literature (Figure 4.6).^{113, 115-118} Freshly isolated immature zygotic embryo explants have been reported to be the most suitable material for *Agrobacterium*-mediated transformation.^{111, 112} Isolation and co-cultivation with the bacteria of

these healthy immature embryos at the correct developmental stage are the most critical factors in the transformation protocol.^{115, 116} These embryos can only be collected from vigorous plants grown in well-conditioned glasshouses. After co-cultivation of the embryos with the *Agrobacterium*, transformed calli (= growing mass of unorganized plant cells) are allowed to grow on medium containing an antibiotic to kill the remaining bacteria and an herbicide to select the transformed plants. Three to four months after co-cultivation, GM-rooted shoots are transferred to soil, acclimatized in a growth chamber for several weeks and subsequently transferred to the greenhouse. The female and male flowers of the transformed plant are usually fertile. The T-DNA is also stably transmitted to the progeny.^{113, 115, 116}

Due to a lack of efficient transformation and good regeneration procedures from one plant cell to a complete plant,¹¹⁹ no stable GM tropical maize varieties with improved traits are available yet. Because it is very time-consuming and costly to introduce transgenes from temperate genotypes into local tropical varieties by backcrossing, efforts to optimize this transformation process were undertaken with tropical maize genotypes available in Kenya.^{9, 117, 120, 121} Until now, no GM plants have been obtained yet.¹¹⁷

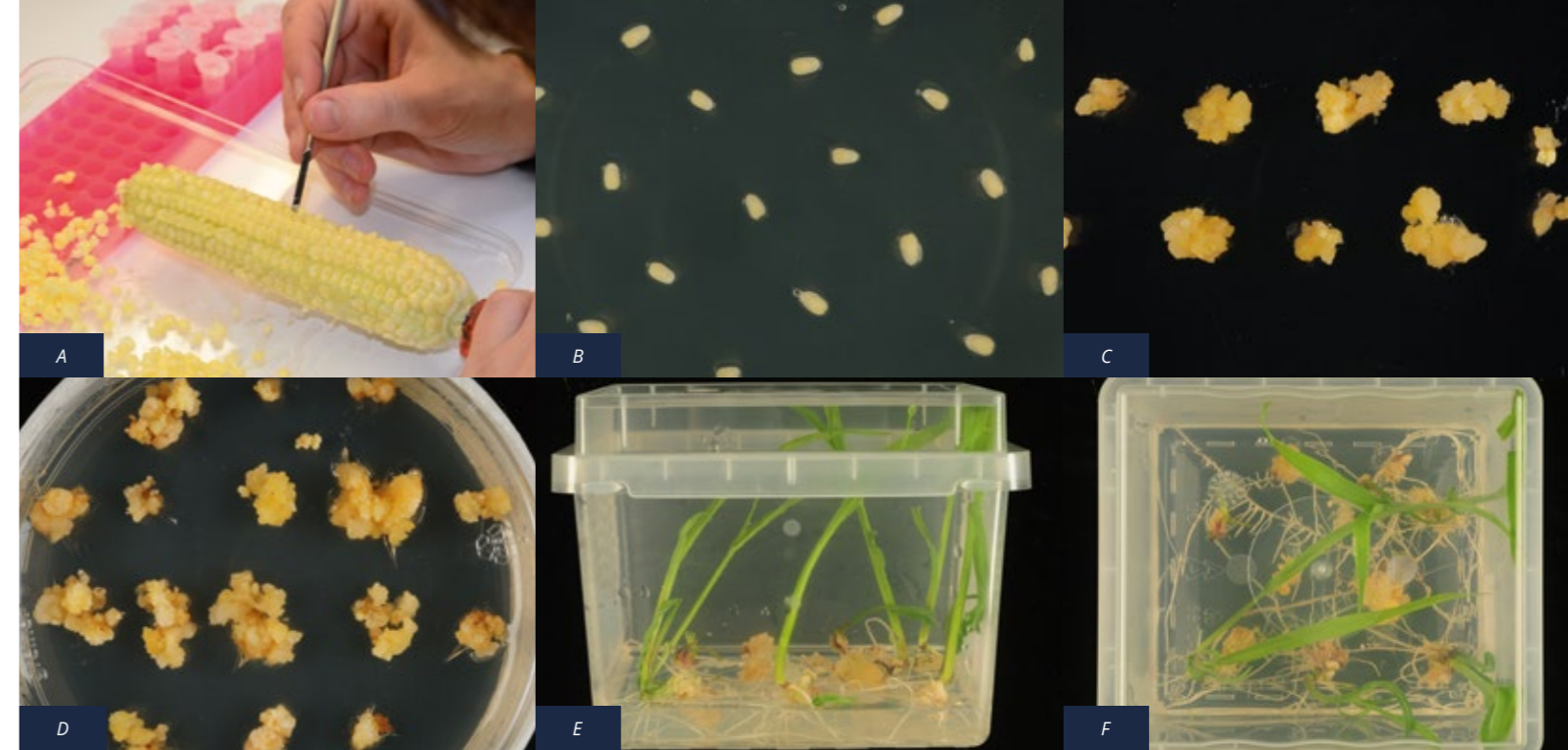


Figure 4.6: *Agrobacterium*-mediated transformation of maize. (A) Isolation of immature embryos, (B) immature embryos; (C-D) regenerating calli on selective medium, (E-F) development of GM shoots (source: Mieke Van Lijsebettens).

Mon810, THE MOST RENOWNED GM MAIZE IN THE WORLD¹²²

In 1998, Mon810 was one of the first GM crops allowed for cultivation worldwide. This maize, developed by Monsanto, produces its own insecticide, which was initially specifically directed against the European maize borer *Ostrinia nubilalis*. Since the 1950s, the soil-borne bacterium *Bacillus thuringiensis* was used in sprays as microbial pest control against these insects. *Bacillus thuringiensis* produces a Bt toxin, which is inactive and harmless in the bacteria, but becomes active and toxic in the digestive tract of certain insects. This Bt toxin attaches itself to the cell membranes of the gut of the insect, where it induces changes in the cell membrane that are lethal to the insect. Because the larvae of the moth live mostly inside the stem of the maize plant, it is hard to kill them by spraying insecticides on the crop. Therefore, scientists transferred the genetic information of the Bt toxin (the gene *Cry1aAb*) to maize and developed Mon810. Mon810 produces the toxin itself and therefore kills the insects when they feed on the plant. Because Bt toxin specifically binds to the intestinal cells of some insects and not to mammalian intestinal cells, this protein is not toxic for animals and humans. Mon810 offers several advantages: the certainty of harvest for the farmers, lower environmental impact because of fewer sprayed insecticides and lower levels of mycotoxins in the maize plants due to reduced pest damage to maize ears. Mon810 is currently approved for import in many countries (Argentina, Australia, Brazil, Canada, China, Columbia, Japan, Mexico, The Philippines, South Africa, Switzerland, South Korea, Taiwan, USA, Uruguay, and the European Union) but can only be cultivated in a few countries (USA, Brazil, Canada, South Africa, Argentina, the Philippines, Uruguay, Spain, Czech Republic, Slovakia and Portugal).¹²³ To derive as many different local varieties as possible, the Mon810 variety was used in breeding programs to transfer the Bt trait into local maize varieties adapted to the local soil and climatic circumstances. In Spain for instance, more than 100 Mon810 maize varieties are grown today.

Biotechnological innovations are needed to secure maize production in Africa

During a meeting organized by the United Nations Industrial Development Organization (UNIDO) in Nairobi in 2013, agricultural experts made up a list of the most important transgenic maize varieties that could ensure maize production and food security in Africa.³⁶ This list comprises five main traits: (1) insect-resistance with a *Bacillus thuringiensis cry* gene (Bt maize), (2) resistance to African viruses, such as the maize streak virus, (3) resistance to the parasitic weed *Striga*, (4) drought tolerance (see chapter 5) and (5) decreased levels of mycotoxins.³⁶

Bt maize is only commercially available in South Africa

In 2016, South Africa was the only African country growing biotech maize (see text box p.39).¹²³ In 1998, insect-resistant Bt maize was planted for the first time and since 2003, herbicide-tolerant GM maize is also cultivated.¹²³ Currently, approximately 2.16 million hectares of biotech maize is planted in South Africa: 420,000 hectares of Bt maize, 407,000 hectares of herbicide-tolerant (HT) maize and 1.33 million ha of stacked (=combined) Bt/HT maize. Approximately 86% of the white and 92% of the yellow varieties are genetically modified.¹²⁴

During the 1998/1999 season, farmers started to grow Bt yellow maize, whereas Bt white maize for human consumption was grown for the first time during the 2001/2002 season.³⁶ The majority of small-scale farmers will only plant white Bt maize for home consumption, whereas only a limited number will plant yellow Bt maize to feed their

animals.³⁶ However, most of the Bt maize in South Africa is grown by large-scale farmers.¹²⁵

Originally, all Bt maize plants contained the MON810 event (see text box p.39), but the Bt11 and Mon89034 varieties were also commercially released in 2006 and 2011, respectively. Bt11 contains insect-resistance whereas Mon89034 produces two different insecticidal proteins from *Bacillus thuringiensis*, Cry1A105 and Cry2Ab2.¹²⁵ As a result, Mon89034 provides enhanced benefits in the control of a wider spectrum of Lepidopteran insect pests such as maize borers and earworms, and will also have greater durability than plants producing only one Cry protein.¹²⁵

The main impact of Bt maize in South Africa is the strong reduction of pest damage. Although the largest yield impact (32%) was seen in 2002, the estimated yield impact since 2008 was approximately +10.6%.¹²⁶ The higher costs of this technology are compensated by the savings on insecticides to control maize stem borers. The increased yield even created extra income.¹²⁶ A survey indicated that 42.5% of the farmers growing Bt maize perceived this maize as more environmentally friendly.¹²⁵ An important additional positive effect of growing Bt maize is the reduction of mycotoxin contamination, as the maize grains on the ear are no longer infected by the insects and thus no longer superinfected by fungi (See chapter 3).^{125, 127} Additionally, recent field trials demonstrated that the only maize variety that was resistant to the fall armyworm was Bt maize.⁴⁰

Until now, Bt maize seed has been supplied to smallholders through government-sponsored interventions; therefore, smallholders have not yet experienced the real costs of the seed.¹²⁸

One major obstacle to successful adoption by smallholders seems to be the lack of information about Bt maize technology. Farmers should, first of all, be informed that these Bt seeds provide resistance to insects and, secondly, they need information on the importance of planting a refuge of non-Bt maize next to their Bt crop. This refuge is an area in which non-Bt maize plants are growing and where stem borers can easily feed and reproduce. In this way, the refuge strategy prevents stem borers from developing resistance to Bt maize. However, large commercial South African farmers are often unaware of the purpose of refugia and thus don't plant them.^{129, 130}

The Kenya Agricultural Research Organization and the African Agricultural Technology Foundation have also developed an insect-resistant maize variety with the MON810 event. In January 2016, the Kenyan National Biosafety Authority approved this GM variety for limited environmental release to allow national performance trials, which is a first step towards the acceptance for commercialization of this variety on the Kenyan market (Dorington O. Ogoyi, National Biosafety Authority Kenya, personal communication).

Resistance against *Striga* and maize streak virus

Herbicide-tolerant maize plants have been grown in South Africa since 2003, but development of maize plants resistant to *Striga* infestations is still in the research stage. Genomic tools are now being used to identify potential resistance genes against *Striga* in maize and its wild relatives. Recently, several wild sorghum varieties were found to have resistance against *Striga*.¹³¹ After the identification of the genes responsible for the resistance, these resistance genes can be transferred to maize by genetic transformation, or the

existence of potential homologous resistance genes in maize can be investigated. Furthermore, it seems that during infestation, RNA moves freely between the parasitic weed plants and their hosts.¹³² Researchers are now convinced that the RNAi technology,¹⁰⁴ based on a biological process in which RNA molecules inhibit gene expression, can become a powerful tool that interferes with the lifecycle of the weed. This could be achieved by transforming a maize plant with an RNAi construct that targets gene sequence(s) specific to the parasite.²⁹ Currently, such strategies are already used to develop GM tomatoes and sorghum, but the strategy has not worked in maize yet.¹³³⁻¹³⁶

Research on the generation of GM maize plants resistant to maize streak virus began more than 10 years ago.^{137, 138} The aim was to interfere with the infection cycle of the virus upon infection. The virus-resistant GM plants displayed a significant delay in symptom development, a decrease in symptom severity and higher survival rates than non-GM plants after a challenge with maize streak virus.^{137, 138} The GM construct was further improved, and the newly generated GM plants are currently being tested in the field for their resistance against the virus.⁵⁷

5 The fight of maize against climate change and drought

Drought is a major abiotic stress factor in Africa that seriously affects the productivity of maize. In combination with erratic rain patterns and inadequate farming methods, drought can lead to 70-100% crop loss, and climate change will only worsen the problem. Therefore, several research organizations are looking for new maize varieties that are tolerant to drought.

Figure: Maize affected by drought.

Maize yield is dramatically decreased by drought

Annually, drought causes up to 24 million tons of yield loss in maize worldwide, with the biggest impact occurring in Sub-Saharan Africa. Indeed, 90% of its maize is grown under rain-fed conditions, and up to 25% of the farmland suffers from frequent drought.^{139, 140} Due to climate change, maize yields in Africa have already declined by 3.8% since the 1980s, and further declines of 5-10% are expected by 2050.¹⁴¹⁻¹⁴⁴



Figure 5.1: Drought in South Africa has an enormous effect on maize yields (source: Johnnie Van den Berg).

Jones and Thornton postulated that there are 3 major types of maize crop responses to climate change: (1) crop yields decrease, but to an extent that can be solved by breeding and agronomy; (2) crop yields increase, for instance, in certain regions of Ethiopia and (3) maize yields decline so rapidly that agriculture has to undergo major changes.¹⁴⁵ By 2055, yields are expected to decrease dramatically in nearly three-quarters of African countries.^{143, 145}

Maize is a versatile crop, but it grows best in wet and warm climates.¹⁹ Seeds will not germinate below 10°C and frost will damage all growth stages. On the other hand, temperatures above 32°C are

detrimental for highland tropical maize development. Currently, temperatures already exceed the optimal temperature for lowland tropical maize (34°C) in several countries, such as Burkina Faso, Gambia, Nigeria, Senegal and Sudan.¹⁹ Current maize varieties need 500 to 800 mm of water for growth, mainly from soil moisture reserves. It is also expected that by 2050, the temperatures will increase by an average of 2.1°C and rainfall patterns will dramatically change.¹⁹ There will likely be increased rainfall in East Africa and decreased rainfall in southern Africa.¹⁴⁶

Drought has an impact throughout maize development, but is especially harmful when it occurs during flowering and pollination.⁹ The onset of the reproductive state is the most sensitive stage, and drought might result in 100% yield loss, especially when coupled with high temperatures.⁹ If drought occurs during the vegetative stage, the size of leaves and of the plant will be reduced, and there will also be a reduction in the number of kernels. When drought stress occurs only later in development during the grain filling period, lower yields will result from reduced kernel size and increased abortion rates after pollination.⁹

Higher temperatures mean shorter periods between planting and harvesting (i.e. crop duration), which results in less time for the plants to accumulate biomass and yield. It is estimated that crop duration will become significantly shorter by as early as 2018 in some locations, and by 2031 in the majority of maize-growing regions in Africa.¹⁴⁷

In 2015, South Africa had its lowest rainfall since recording started in 1904, with seasonal rains delayed up to 50 days and temperatures higher than normal during the maize planting season. This is dramatic for agriculture that uses 85% of

the available water resources.^{141, 142, 144} South Africa produced 25% less maize in 2015¹²³, dramatically affecting Sub-Saharan Africa, because South Africa is the region's major producer (Chapter 2). In 2015-2016, South-Africa even had to import maize to cover its own needs. In other African countries, such as Zambia, Zimbabwe and Malawi, long dry periods have also had a very negative impact on the maize yield. In Zimbabwe, food production decreased by 50% in 2015, and in Malawi, there was a maize deficit for the first time in a decade. Drought is dramatic for both farmers and consumers, as the entire food chain is affected. Farmers are being hit even more, because they don't produce enough feed to keep their animals alive and they have less income to support their families.

Given the increasing evidence for climate change in Sub-Saharan Africa, there is an urgent need to develop new maize varieties with increased tolerance of heat and drought.¹⁴⁸ Several research groups are working on these developments, with two major projects for Africa highlighted below.

Water Efficient Maize for Africa (WEMA)

The WEMA project was launched in 2008 with the objective of improving food security and rural livelihoods among small-scale African maize producers. The aim is to develop and deploy low-cost, drought-tolerant and insect-protected maize, by conventional breeding, marker-assisted breeding and GM technology approaches (see Chapter 4), and to provide royalty-free seeds of these improved maize varieties to small scale farmers through local seed companies (www.wema.org). The WEMA project is a public-private partnership between the African Agricultural Technology Foundation, the International Maize and Wheat

Improvement Centre, Monsanto and five national agricultural research systems. Funds for this project are received from the Bill and Melinda Gates Foundation, the Howard G. Buffett Foundation, and the United States Agency for International Development (USAID) (www.wema.org).

The WEMA project develops plants that are drought-tolerant and insect-resistant. Indeed, the combination of drought and insects often leads to complete crop failure, as damage caused by insects reduces the ability of plants to optimally use already limited water and nutrient supplies. The entire process of developing new hybrids by conventional breeding, marker-assisted breeding and/or GM technology approaches took approximately 7 years.

The WEMA project has already released 40 conventional drought-tolerant maize hybrids, commonly known as DroughtTego™ hybrids, in the 5 participating countries (Kenya, Tanzania, Mozambique, Uganda and South Africa) (www.wema.org). The first WEMA conventional maize hybrid WE1101 was made available to farmers in Kenya in June 2013.

This WE1101 hybrid needs 4 to 5 months to mature, depending on the growing area, and produces white grains with flint to dent-flint textures. Furthermore, the grains contain a good husk cover on the cob, which protects from damage by birds, weevils and grain rotting due to water seeping into the ear. This hybrid maize is also resistant to major diseases such as Turicum leaf blight, gray leaf spot and maize streak virus, and gives an average yield of 4 to 5 tons per hectare (www.wema.org).

Drought tolerance is not controlled by one gene but by a complex network of genes involved in metabolic and physiological pathways that are

influenced by the environment. Therefore, new drought-tolerant hybrids are developed through a combination of conventional, marker-assisted breeding and GM techniques.

The GM drought-tolerant maize technology (Mon87460) DroughtGard™, harboring the cold shock protein CspB from *Bacillus subtilis*, was donated to the project by Monsanto and is grown in many countries, with promising yields. Additionally, the WEMA GM plants contain the *Bt* gene from *Bacillus thuringiensis* (Mon810 in Kenya and Uganda and Mon89 in South Africa) and are therefore also insect-resistant. The first stacked (=combined) DT/Bt biotech maize hybrids are expected to be available for farmers and commercialization in 2017. These hybrids may yield up to 20-35% more grain than other commercial hybrids under moderate drought conditions (www.wema.org). South Africa will be the first country to grow these GM plants. In 2015, confined field trials for these hybrids were also approved in Kenya and Uganda.¹²³ In Tanzania and Mozambique, applications to perform confined field trials with these stacked events are also currently under review. The WEMA project is convinced that the new drought-tolerant and insect-protected maize varieties will provide agronomic, environmental and economic benefits for many farmers in Africa. These farmers will be able to produce more reliable harvests under moderate drought conditions and will obtain better grain quality due to reduced insect damage (www.wema.org).

Drought Tolerant Maize for Africa (DTMA)

The DTMA project, jointly implemented by the International Maize and Wheat Improvement Centre and the International Institute of Tropical

Agriculture, was launched in 2007 with the aim of increasing food and income security of small-holder farmers through the development and distribution of drought-tolerant maize varieties. DTMA received funding from the Bill and Melinda Gates Foundation, the Howard G. Buffett Foundation, the US Agency for International Development and the UK Department for International Development. During this project, approximately 200 drought tolerant maize varieties were developed with conventional breeding techniques. The DTMA maize varieties also exhibit resistance to some major diseases, display high protein content and some of them are also nitrogen use efficient (<http://dtma.cimmyt.org>). In 2013 alone, more than 33,000 metric tons of seed have been delivered to farmers in the 13 countries across Sub-Saharan Africa: Ethiopia, Kenya, Tanzania, Uganda, Angola, Malawi, Mozambique, Zambia, Zimbabwe, Benin, Ghana, Mali and Nigeria.¹⁴⁹ Surveys of 3,700 farm households in 6 countries (Ethiopia, Tanzania, Uganda, Malawi, Zambia and Zimbabwe) however revealed that only a small percentage of farmers work with these DTMA seeds.¹⁴⁹ The data showed considerable inter-country variation in farmer uptake. In Zimbabwe, only 9% of the maize plots are DTMA maize, in Tanzania 12%, in Ethiopia 15%, in Zambia 23%, in Uganda 26% and in Malawi 61%.¹⁴⁹ Farmers that received information on these seeds were apparently more likely to grow drought tolerant maize and less likely to grow a local maize variety, while without information, they grew their own local varieties.¹⁴⁹ Some farmers thought that these DTMA maize varieties were low yielding, late maturing, labor intensive to grow, and more prone to attacks by pests during storage. Therefore, it is important that these DTMA seeds are made available on local markets in affordable small packets of 1 to 2 kg, and that awareness is generated.¹⁴⁹

In 2016, a new project called “Stress Tolerant Maize for Africa” (STMA) was conducted for four years (2016-2019) by the same partners. This project aims to develop, via conventional breeding, new maize varieties capable of resisting environmental stress including drought, low soil fertility, heat, pests and diseases. Additionally, this project also seeks to enhance sustainable maize research and development systems in 12 focus Sub-Saharan African countries: Benin, Ghana, Ethiopia, Kenya, Malawi, Mali, Nigeria, Uganda, South Africa, Tanzania, Zambia and Zimbabwe (<http://www.cimmyt.org/project-profile/stress-tolerant-maize-for-africa/>).

Other drought-tolerant maize varieties are under development

Drought triggers significant plant responses, such as alterations in gene expression, accumulation of metabolites and the synthesis of specific proteins.^{150, 151} The functional genes involved in these pathways may help to generate drought-tolerant maize via genetic engineering.

After transfer of the tobacco-derived *npk1* gene, temperate maize showed significantly higher photosynthesis rates than non-transformed maize under drought conditions.¹⁵² This *npk1* gene was therefore transferred to tropical maize, and analysis of the GM plants under different drought conditions is ongoing.¹⁵³

Ethylene, a natural plant hormone, has been linked to numerous aspects of growth, and the maize protein *ARGOS8* seems to be a negative regulator of ethylene responses. Via biotechnological techniques, either the production of ethylene was reduced or the reaction of maize on ethylene was reduced. This resulted in increased yield under field drought conditions.¹⁵⁴⁻¹⁵⁶ In 400 different inbred maize lines, *ARGOS8* expression levels were too low to use them in breeding experiments.¹⁵⁵ Therefore, the genomic sequence of *ARGOS8* was altered via the CRISPR-Cas advanced breeding technique⁹¹ to create several *ARGOS8* variants. In a field study, these *ARGOS8* variants showed elevated levels of grain yield and had no yield loss under well-watered conditions.¹⁵⁵

6 How to make maize even healthier by adding micronutrients and vitamins

Figure: Collecting the cobs of quality protein maize hybrids at the Haramaya University research site (source: Bedasa Mekonnen Dosho, Haramaya University, Ethiopia).

Biofortification

All over the world, almost 1 billion people, of which 200 million are children, are affected by hunger, malnutrition and food insecurity. Additionally, “hidden hunger”, a pandemic related to vitamin and mineral deficiencies most commonly involving vitamin A, iron and iodine^{157,158}, currently affects the physical and mental potential of about 2 billion people.¹⁵⁹

As discussed in Chapter 1, a maize kernel contains approximately 73% starch, 9% protein, 4% oil and 14% other constituents, such as fiber. Maize is deficient in both lysine and tryptophan and a shortage in lysine can negatively affect human and animal growth.^{8,160} Because maize is the main food source in many (African) countries, maize and maize-derived food products are targets for nutritional enhancement. Improving the intrinsic nutrient content of a food crop by the addition of extra genes encoding for these nutrients is called biofortification. This is achieved by conventional breeding when possible, and by mutation breeding or GM technology in other cases.^{158,161,162}

Quality Protein Maize (QPM)

In maize, several mutations (= small changes in the DNA sequence) changing the amino acid composition in maize were discovered.^{158,160} One of them was the mutant maize “opaque-2”, which produces kernels containing the same amount of crude protein, but with a two-to-threefold increase in the level of lysine and tryptophan in the grain endosperm compared to normal maize.¹⁶³⁻¹⁶⁵ This increased concentration doubles the nutritional quality in the maize protein, implying that only half of the amount of “o2-maize” needs to be consumed to have the



Figure 6.1: Collecting the cobs of the Yellow and White Quality Protein Maize hybrids at the Haramaya University research site. For each hybrid, the number of seeds per plant, 1000 seed weight and tryptophan and lysine content will be determined (source: Bedasa Mekonnen Dosho).

same biologically usable protein. Therefore, a very rapid way to produce this biofortified maize would be to insert the o2-mutation into local varieties. However, this o2-maize appears to be more susceptible to diseases and fungi, has 8 to 15% lower yield lower than common maize, chalky and dull kernel appearance and poor milling characteristics. Additionally, the taste of this o2-maize was not at all appreciated by consumers.¹⁵⁸

In the late 1990s, quality protein maize (QPM) was developed at CIMMYT by Surinder Vasal and Evangelina Villegas, who received the World Food Prize

in 2000 for this achievement. Through extensive breeding programs, a range of tropical and subtropical maize populations were converted to o2 versions with much higher lysine and tryptophan levels using backcross recurrent selection while maintaining storage and agronomic qualities such as hard kernel, high yield, lodging, and disease and pest resistance (Figure 6.1).^{160,166,167} QPM maize has a positive impact on both humans and animals. Children consuming this maize showed a 9% and 12% increase in growth rate and weight, respectively, while animals grew faster, gained more weight and showed improved feed efficiency.¹⁶⁸ The first QPM variety was released in Ghana in 1992, and later in Ethiopia, Tanzania, Nigeria and Uganda via “the QPM Development and Dissemination Project for The Horn and Eastern Africa”.^{167,169}

Through genetic engineering, researchers try to develop maize varieties with increased lysine and tryptophan content. RNA interference¹⁷⁰ has been used to reduce zein proteins in maize.^{171,172} Reducing zein concentrations resulted in moderate increases of 15 to 20% in lysine content.¹⁷¹⁻¹⁷³

High provitamin A maize varieties

With the exception of vitamin B12, yellow maize contains many important vitamins, with provitamin A carotenoids and vitamin E the predominant fat-soluble vitamins in their kernels.⁷ However, in Africa, white maize is the predominant food maize and provitamin A carotenoids are absent in its kernels.¹⁷⁴ As a result, populations without access to a diversified diet in general chronically suffer from vitamin A deficiency. Chronically insufficient vitamin A intake is the main cause of blindness in children and increases the risk of disease and death from severe infections.¹⁶² In pregnant

women, vitamin A deficiency causes night blindness and increases the risk of maternal mortality. Additionally, carotenoids have a protective function in reducing the risk of cancer, cardiovascular diseases and other chronic diseases.¹⁷⁵

Because there is high variation in carotenoid content and composition among different maize varieties, some of these target genes could be used by classical breeding or genetic modification to improve the provitamin A content in white maize varieties.¹⁷⁶⁻¹⁷⁸ The carotenoid biosynthesis is complex, and therefore multiple genes should be taken into account during this breeding process.¹⁷⁹

In 2004, the HarvestPlus project was launched (www.harvestplus.org). This project aims to breed more nutritious cultivars of important staple crops, including maize, through marker-assisted breeding techniques (see chapter 5). This project was able to develop biofortified provitamin A-rich maize, which is also high-yielding, disease- and virus-resistant and drought-tolerant (www.harvestplus.org). As of 2015, this provitamin A-rich maize has officially been released by this project in Nigeria and Zambia, with plans to expand activities into Ethiopia and several other African countries (www.harvestplus.org).

Vitamin A-enriched maize varieties were engineered both through conventional breeding and genetic engineering.^{161,180} The white maize inbred line M37W lacks carotenoids in its endosperm. A GM variety could be generated which provides the recommended daily intake of provitamin A in 200 gr maize grain.¹⁸¹ An additional advantage of this GM maize is that the increased carotenoid content reduces mycotoxin (see chapter 3) contamination in the maize kernels.¹⁸⁰

7 Conclusion

More than 300 million Africans depend on maize as their main food crop. Unfortunately, compared to many other regions, the yield of maize production in many African countries is very low due to many biotic and abiotic stresses. Some of these hurdles can be overcome or at least avoided to some extent through improvements in agricultural practices, but the development of new maize varieties and hybrids via conventional breeding, marker-assisted breeding and/or biotechnological tools can further help. The development, dissemination and growth of these new varieties progress slowly mainly because of the lack of farmer awareness of the availability of these hybrids and the lack of an efficient regulatory system for the development and cultivation of GM crops in many African countries.

Most African smallholder farmers obtain very low maize yields that are sufficient only for auto-consumption. There are several reasons why yields for these farmers are so low: (1) Lack of information – most small scale farmers lack the appropriate information to implement optimal farming practices, such as crop rotation, the use of high-yielding hybrids, good manufacturing practices that reduce viral, bacterial and fungal infections, etc.; (2) Poor financial support – many smallholder farmers can't afford to buy fertilizers and use modern agricultural practices; and (3) lack of proper storage and transportation infrastructure and access to markets.

Integrating the best of conventional breeding with the best of modern biotechnology constitutes a realistic approach to improving maize varieties in Africa. There is, however, an urgent need for the establishment of a responsible and efficient regulatory system in most African countries. This regulatory system will ensure public confidence, encourage biotechnological research in Africa, and lead to higher yields and economic incomes. For the moment, only four African countries (Egypt, South Africa, Burkina Faso and Sudan) allow the cultivation of GM plants, but in 2016, only South Africa and Sudan planted GM crops.¹²⁴ In all other 51 African countries, no GM cultivars are grown and only 13 of them (including Egypt, South Africa, Burkina Faso and Sudan) allow confined field trials under bio-safety laws.¹²⁴ Establishing and implementing a workable regulatory system will encourage researchers and breeders to develop even more valuable (GM) maize varieties. It is also important that the biosafety regulatory processes do not make GM varieties so expensive that it becomes almost impossible for small scale farmers to purchase them.

8 References

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IPBO (International Plant Biotechnology Outreach), which forms part of the VIB, was set up in 2000 by Prof. Em. Marc Van Montagu and Ghent University. IPBO's mission is to promote knowledge and technology transfer in the area of plant biotechnology to developing countries, with a focus on a green and sustainable agriculture and agro-industry. To accomplish this mission, IPBO focuses on communication, training in plant breeding, green biotechnology and related biosafety, and fosters networking and project development to implement cooperation between developing countries and Flanders. More information: www.ipbo.vib-ugent.be



Basic research in life sciences is **VIB's** raison d'être. VIB is an independent research institute where some 1,500 top scientists from Belgium and abroad conduct pioneering basic research. As such, they are pushing the boundaries of what we know about molecular mechanisms and how they rule living organisms such as human beings, animals, plants and microorganisms. Based on a close partnership with five Flemish universities – Ghent University, KU Leuven, University of Antwerp, Vrije Universiteit Brussel and Hasselt University – and supported by a solid funding program, VIB unites the expertise of all its collaborators and research groups in a single institute. The VIB-UGent Center for Plant Systems Biology wants to gain insight into how plants grow and respond to the environment. Scientists study how leaves and roots are formed, which micro-organisms live on and around the plant and which substances the plant makes. This knowledge can lead to sustainable innovations in agriculture and food.



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The International Industrial Biotechnology Network (**IIBN**) was established in 2010 as a joint initiative of UNIDO, the Flemish Government (EWI) and IPBO. IIBN serves as a catalyst for advancing sustainable applications of agricultural and industrial biotechnology in developing and emerging economies in cooperation with Flanders and other international partners. IIBN is being developed along three tracks: (1) engage in advocacy to raise awareness for the development potential of esp. agricultural biotechnology by providing science-based information and case studies; (2) establish a formal network of like-minded institutions and organizations, and (3) foster R&D cooperation and capacity building in biosciences that addresses the needs of developing and emerging economies, in cooperation with stakeholders in Flanders and beyond.



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