CHALLENGES OF MAIZE BREEDING UNDER TROPICAL CONDITIONS OF BRAZIL

Abstract – Tropical maize production represents 49% of the global maize harvested area. The annual productivity increase rates in tropical regions are twice as high as in temperate regions. In Brazil, the increase in maize grain yield was expressive in the last 41 years. Such achievement was possible due to the joint research efforts in many areas of science, especially plant breeding. However, although the notable success in maize breeding, the maize breeders still face several challenges in developing high-yielding hybrids, since tropical conditions provide intense pests and diseases pressures, as well as water deficit conditions. In addition, the constant increase of breeding programs costs requires the development and application of new techniques to improve the genetic gain per time unit, increasing the maize breeding programs’ efficiency. This review discusses the main challenges faced by maize breeding programs under tropical conditions, highlighting topics related to the development of hybrids for first and second growing seasons; breeding for biotic and abiotic stresses conditions; specialty maize breeding; use of transgenics; and genomic tools to maximize the efficiency of maize breeding programs under tropical conditions.

Keywords: *Zea mays*, water deficit stress, biotic stresses, genomic prediction, genotypes-by-environments interaction

DESAFIOS DO MELHORAMENTO DE MILHO EM CONDIÇÕES TROPICAIS DO BRASIL

Resumo - A produção de milho tropical representa 49% da área global de milho colhido. Em termos de produtividade, a taxa de aumento anual nas regiões tropicais é duas vezes maior do que a taxa de aumento nas regiões temperadas. O aumento da produtividade de grãos de milho foi expressivo nos últimos 41 anos no Brasil. Tal conquista foi possível devido ao esforço conjunto de diversas áreas da ciência, especialmente, o melhoramento vegetal. Apesar do sucesso do melhoramento do milho, muitos são os desafios enfrentados pelos melhoristas de milho para desenvolver híbridos de alta produtividade. As condições tropicais proporcionam desafios mais intensos em termos de pressões de pragas e doenças e, sobretudo, condições de déficit hídrico. Além disso, o aumento constante dos custos dos programas de melhoramento exige o desenvolvimento e aplicação de novas técnicas para melhorar o ganho genético por unidade de tempo, aumentando a eficiência dos programas de melhoramento de milho. Esta revisão discute os principais desafios enfrentados pelos programas de melhoramento de milho em condições tropicais, destacando tópicos relacionados ao desenvolvimento de híbridos de milho para primeira e segunda safras; melhoramento para condições de estresse biotico e abiotico; melhoramento de milho especial; uso de transgênicos em milho; e ferramentas genômicas para maximizar a eficiência dos programas de melhoramento de milho em condições tropicais.

Palavras-chave: *Zea mays*, estresse hídrico, estresses bióticos, predição genômica, interação genótipos por ambientes
Originated from mid-altitude areas of South-Central Mexico, maize was cultivated as early as 8700 years before the present. Two significant paths spread maize through the American continent: (i) from northern Mexico to the southern USA and subsequently to northern USA and Canada; (ii) from the low lands of Mexico to Central America, the Caribbean, and Andes (Edmeades et al., 2017). Maize was then introduced into Europe, Africa, and Asia between the fifteenth and seventeenth centuries.

Tropical maize production represents 49% of the global maize harvested area. Even though temperate maize yield average (7.2 t ha\(^{-1}\)) is higher than tropical maize (3.3 t ha\(^{-1}\)), the annual increase rate in tropical regions is 2.3%, while the annual increase rate in temperate regions is only 1% (Edmeades et al., 2017).

In the last 41 years, the increase in maize grain yield was expressive in Brazil: 109 e 133 kg ha\(^{-1}\) year\(^{-1}\), first and second growing seasons, respectively (Conab, 2021). These improvements were possible due to the joint efforts of many areas of science, such as crop science, agricultural mechanization, phytopathology, entomology, and plant breeding. However, although maize breeding success, many are the challenges the breeders face to develop high-yielding, biotic and abiotic stresses resistant-tolerant tropical maize hybrids.

Hence, this review aims to address the main challenges of maize breeding programs under tropical conditions. Therefore, it will be highlighted topics related to the development of maize hybrids for first and second growing seasons; maize breeding for biotic and abiotic stresses conditions; the main challenges of specialty maize breeding; transgenics in maize; the use of genomic tools to maximize the efficiency of maize breeding programs under tropical conditions.

**National panorama of maize breeding**

It is undeniable that the discovery and exploitation of heterosis were responsible for changing the entire chain of maize breeding. Proposed by Shull in 1909, the use of maize hybrids was entirely restricted due to the poor agronomic performance of maize inbred lines.

As Troyer (2006) reported, until 1930, the use of open-pollinated varieties was dominant in the USA. However, due to the feasibility of obtaining double-cross hybrids, in 1939, maize hybrids cultivars represented 75% of the total maize harvested area in the USA.

In Brazil, the commercial use of maize hybrid seeds was later. In this scenario, Benjamin H. Hunnicutt, founder, and director of the Escola Agrícola de Lavras, deserves mention due to the publication of the first book about maize written in Portuguese language, entitled “O Milho: Sua Cultura e Aproveitamento no Brasil”, in 1924.

In 1932, the first national maize breeding program was initiated by the Instituto Agronômico de Campinas (IAC). Three years later, in 1935, led by Antônio Secundino de São José, the Escola de Agricultura de Viçosa also started its maize breeding program. The first double-cross maize hybrid was released in 1939 by the IAC, and since then, Brazilian maize breeding programs have presented continuous evolution in almost nine decades of existence.

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Brazilian maize grain yield increased by 99.06 kg ha\(^{-1}\) year\(^{-1}\) (Figure 1). Considering only the last 20 years, the increase in maize productivity was even higher, 145.1 kg ha\(^{-1}\) year\(^{-1}\) (\(r^2 = 0.8251\)). The contribution of maize breeding is clear, and the increase in maize productivity is highly associated
with the adoption of single-cross maize and transgenic maize. Private companies dominated the national maize breeding program market. However, public breeding programs are essential in obtaining and offering lower-cost maize hybrids to low investment farmers. A better characterization of 2019/20 maize cultivars can be found in Pereira Filho and Borghi (2020).

In Brazil, maize is cultivated in two different growing seasons: the first growing season, or summer season (Figure 2A), and the second season or off-season (Figure 2B). Each season presents specific environmental conditions. For example, while the summer season maize is sown during periods of better water availability, between September to December, off-season maize faces more intense climatic challenges due to its seeding window between January and April. Furthermore, it is necessary to consider specific environmental conditions of the first and second growing seasons in Brazilian maize breeding programs. Indeed, environmental conditions represent additional challenges for Brazilian maize breeders. Notoriously, the second growing season presents more significant risks, such as weather conditions, intense pests and, diseases pressures, as well as water deficit stresses (Andrea et al., 2018).

Although maize production is well-established across Brazilian territory, marginal regions have emerged. Accounting for a total production of 5.3 and 2.4 thousand tons of first and second growing season maize, respectively (Conab, 2021), the MATOPIBA region has increased its importance over the years due to high agricultural investments in the region (Von Pinho et al., 2014). Even though its significant development, the MATOPIBA region lacks infrastructure, mainly in maize production flow to industry and exportation regions.

Although locally restricted to a few states, a third growing season has been practiced in Brazil. Together, the states of Roraima, Amapá, Pernambuco,
Figure 2. Total maize production (in thousand tons) of first (A) and second (B) growing seasons in 2019/2020. Data from Conab (2021).

Alagoas, Sergipe, and Bahia produce 1.8 million tons of maize grain in the third growing season (Conab, 2021). The SEALBA region has a high agricultural potential and was responsible for 90% of the third growing season of maize production in 2019/2020 (Conab, 2021).

Developing well-adapted maize hybrids is necessary to increase crop productivity and profitability in the SEALBA region. In addition, due to the uncertainties of the rainfall regime (Pacheco et al., 2018; Procópio et al., 2019), drought tolerance should be prioritized by maize breeding programs.

Maize breeding for the first growing season

The first maize growing season corresponds to 25% of all Brazilian maize production. In 2019/20, this season was responsible for a total production of approximately 25.8 million tons (Conab, 2021). Sown and cultivated in rainy seasons, the maize crops find favorable climatic conditions to express their genetic potential. Andrea et al. (2018) highlight that water deficit stress is the major limiting factor in tropical maize. Therefore, summer maize breeding programs may direct their efforts and resources to develop high-yielding maize cultivars.

Due to maize breeding efforts, modern tropical germplasm resembles temperate germplasm. Therefore, modern tropical maize hybrids are smaller in size, resistant to lodging, possess more erect and numerous leaves, present shorter intervals between pollen release and the emergence of style-stigma, produce only one large and vigorous ear per plant (Borém et al., 2017; Edmeades et al. 2017). These are must-have traits in all tropical maize breeding programs and, in conjunction with the correct spatial arrangement of the maize plants, allow to raise plants population per area unit, increasing maize crops productivity (Bernhard and Below, 2020).

Once defined the essential traits, maize breeders may focus on introducing the so-called value-added traits into the germplasm. Notoriously, tropical regions provide climatic challenges and biotic adversities, such as higher pathogens and pests pressures. For example, Savary et al. (2019) highlighted an overall reduction ranging from 19.5 to 41.1% in global maize crop productivity due to pests and diseases. In the panorama of tropical maize, these losses are even more remarkable since temperature...
and humidity favor the development of pests and pathogens.

Due to the increasing demand for high-yielding hybrids, maize breeders face a significant challenge to improving the breeding program’s efficiency in increasing the genetic gains per time unit. Therefore, breeding companies have been adopting new strategies to reduce the breeding cycle and thus increase the efficiency of the breeding program.

The inclusion of double haploid (DH) technology into the breeding pipeline has been widely adopted in tropical maize breeding. Although this technology allows obtaining 100% homozygous maize lines rapidly, haploid inducers lines adapted to the tropical region climate represent a bottleneck in public sector breeding programs.

The CIMMYT Global Maize Program (GMP) has been developing tropicalized haploid inducer lines to optimize the use of DH technology since 2007 (Maqbool et al., 2020). These efforts were successful; therefore, tropical maize breeders find at their disposal second-generation haploid inducer lines (CIM2GTAILs) that present high haploid induction rates under tropical and subtropical conditions. Meanwhile, in Brazil, several works were performed to optimize the use of DH technology, especially in breeding programs of Brazilian public universities (Battistelli et al., 2013; Couto et al., 2015; Pires et al., 2019; Ribeiro et al., 2020).

With the constant increase of phenotyping costs and the reduction of genotyping costs, breeding companies are increasingly seeking to introduce genomic prediction (GP) tools into their programs (Bernardo and Yu, 2007). GP is a powerful tool available to maize breeders to maximize the genetic gains per time unit (Heslot et al., 2014). Pires (2017) presents a breeding scheme in which it is possible to observe the use of genomic selection within a breeding pipeline (Figure 3).

Maize breeding for the second growing season

The second maize growing season, known as off-season maize, is responsible for placing Brazilian agriculture in a prominent position at a global level. The second crop season corresponds to 73% of Brazilian maize production, representing

![Figure 3](https://example.com/figure3.png)

**Figure 3.** Scheme of a maize breeding pipeline adapted from Pires (2017).
75 million tons harvested in 2019/20 (Conab, 2021).

Off-season maize accounts for 13.7 million hectares, which is well divided among Middle-West/South states. Mato Grosso, Mato Grosso do Sul, Goiás and Paraná correspond to 39%, 13%, 12% and 17% of the total area in 2019/20. However, due to the heterogeneity and complexity of the environments, off-season maize breeding programs face more intense challenges.

The second growing season presents more significant crop risks, mainly due to lower water availability in the most critical stages of the maize crop (flowering and grain fill periods), which is the primary limiting factor of crops productivity (Andrea et al., 2019). Therefore, second crop cultivation requires more seed sowing and crop management planning.

Despite high-yielding, second growing season, maize hybrids must present earliness. Since the plant completes its cycle as fast, the crop will be exposed to abiotic and biotic stresses for a short period. Therefore, the off-season maize grown in a succession of the soybean crop increases the demand for developing early-maturing maize hybrids. According to Pereira Filho and Borghi (2020), on an average of the last seven crop years, 68.51% and 25.29% of the cultivars available in the seeds market were early and super early.

In addition, it is crucial a good connection between maize breeding programs and maize farmers’ interests and needs. Cantelmo et al. (2018) present some of the challenges faced by off-season maize farmers. For example, in the Cerrado environments of Mato Grosso, Goiás, and the Minas Gerais States, leaves and grain diseases tolerance, and water-deficit stress tolerance, are highly desired traits by maize farmers. On the other hand, high stability and tolerance to ear rot are essential traits for Western Paraná and Southern Mato Grosso do Sul regions.

The genotypes-by-environments interaction (GxE) is a primary challenge in plant breeding, especially in tropical regions. GxE becomes even more challenging in the second growing season due to unpredictability over the environments and crop years.

Therefore, the development of highly stable maize hybrids that mitigate the impacts of GxE is crucial. To this purpose, it is necessary to test and evaluate the genotypes in a great range of representative target environments, the so-called mega environments. In such a scenario, a broad characterization of the mega environments is crucial for the success of the breeding programs. For many breeding programs, there are four mega environments for second growing season: Region 1, corresponding to Southwestern Paraná and Mato Grosso do Sul; Region 2, Northern Paraná and Paranapanema region in São Paulo; Region 3, Southwestern Goiás and Southern Mato Grosso; and Region 4, Middle-North Mato Grosso.

There are several methodologies to study the GxE, such as one-and two-stage ANOVA; mixed models approach (Figueiredo et al., 2014; Santos et al., 2017); factorial regression; inclusion of environmental and genotypic covariables information; Finlay Wilkinson model; adaptability and stability indices (Figueiredo et al., 2014; Oliveira et al., 2014); AMMI and GGE models (Balestre et al., 2009a, 2009b; Oliveira et al., 2010); Bayesian approach (Eeuwijk et al., 2016; Bernardo Júnior et al., 2018), and more recently, the use of genomic information and GxE modeling for the prediction of maize hybrids, genomic association and genomic selection studies (Dias et al., 2018a).
From an organizational point of view, maize breeding programs for the second growing season follow the structure described in Figure 3. However, it is essential to emphasize that the initial in-nursery evaluations may vary according to the breeding program specificity, objectives, and target environments. During this time, the germplasm can be characterized for resistance to major diseases, drought, salinity, heat, and cold tolerance (Araus et al., 2012; Cabrera-Bosquet et al., 2012; Abreu et al., 2019).

Maize breeding for abiotic and biotic stress conditions

In conjunction with climate changes, the global population growth trend imposes enormous challenges for maize breeding. In addition, global climate changes, drought, greater incidence of plant pests, and diseases increase agriculture risks (IPCC, 2014). Therefore, maize breeding is crucial to overcome abiotic and biotic stress conditions to mitigate global climate changes impacts on agriculture. Furthermore, with the expansion of crop cultivation throughout the Brazilian territory, the concern with the incidence of extreme temperatures, whether heat or cold, has increased. In addition, the nature of a large part of Brazilian soils, which are rich in aluminum and poor in nutrients, is a challenge for maize production in Brazil.

The selection of heat or cold-tolerant plants adapted to low-nutrient stresses has been considered the best alternative to increase maize production in new agricultural areas. Fortunately, the existence of a wide genetic variability for several abiotic tolerances has been described in maize (Rodrigues et al., 2017; Noor et al., 2019).

In this context, several strategies can be employed to improve selection efficiency, such as indirect selection through high heritable traits. For example, the anthesis-silking interval is a trait of interest in selecting heat-tolerant genotypes (Mendes et al., 2015; Rodrigues et al., 2017; Noor et al., 2019). In addition, studies have been performed in other to better understand heat-tolerance inheritance and effects (Andrade et al., 2013; Dutra et al.; 2015; Abreu et al., 2016).

For cold tolerance, studies have focused on developing vigorous and earliness maize seedlings, which allow early selections. Root morphology evaluations are helpful for the selection of cold-aluminum-tolerant genotypes (Hund et al., 2008; Coelho et al., 2015). Plant height, biomass production, prolificity, pigment content, and photosynthetic efficiency can also be evaluated (Hund et al., 2008; Rodrigues et al., 2017).

Some genes and QTLs have been associated with abiotic stress tolerance in maize. QTLs associated with seedling emergence, vigor, root traits, and chlorophyll content have been reported as desired traits in cold stresses conditions (Presterl et al., 2007; Silva Neta et al., 2015, 2020). On the other hand, QTLs related to traits of interest in heat stress conditions were found (Inghelandt et al., 2019). QTLs for phosphorus and nitrogen use efficiency and related traits continue to be identified in maize (Mandolino et al., 2018; Chibesa and Tembo, 2020). MATE genes are primarily responsible for aluminum tolerance in maize (Vasconcellos et al., 2021).

Lack of fully understanding of abiotic stress tolerance mechanisms and genetic inheritance, limitations of molecular techniques (Gilliham et al., 2017), GxE (Dias et al., 2018b; Santos et al., 2020), low heritability of most traits under biotic and abiotic stress conditions (Dias et al., 2018b) are dominant...
challenges faced by tropical maize breeders.

**Maize breeding for drought tolerance**

Drought is the major abiotic limiting factor of tropical maize productivity (Andrea et al., 2019). In vegetative stages, water deficit stress causes a reduction in plant height, leaf expansion, and plant dry matter. Although the plant root/aerial part ratio slightly increases under some level of water deficit stress, the root growth and nutrient uptake are drastically reduced when the water deficit stress becomes more severe (Bänzinger et al., 2000). In reproductive stages, drought may cause anthesis-silking desynchrony, ears reduction, poor pollination, reduction in grain filling, and other disturbances according to water deficit stress severity (Maazou et al., 2016).

In this scenario, there are two practical approaches to maize breeding: i) breeding for drought tolerance (Abreu et al., 2014; Santos et al., 2017; Abreu et al., 2019); or ii) breeding for water use efficiency (WUE). A drought-tolerant plant maintains its functions in the condition of severe water deficit stress. Thus, a drought-tolerant plant can survive and reproduce in drought conditions. On the other hand, a water-use efficient plant requires less volume of water to maintain or increase its production than an inefficient genotype (Fritsche-Neto and Borém, 2011).

According to Cooper et al. (2014), drought-tolerant plants often perform poorly in favorable conditions. Therefore, some maize breeders have focused on developing water-use efficient genotypes to obtain agronomically superior genotypes under optimal and limiting conditions.

Due to its low heritability, grain yield direct selection has been considered inefficient (Câmara et al., 2007). Therefore, indirect selection, performed in high heritable traits, can improve selection efficiency (Bänzinger et al., 2000; Abreu et al., 2017). In addition, morphological root traits, stay-green, plant and ear heights, prolificity, among other traits, can be used to perform drought-tolerant genotype selections (Câmara et al., 2007; Kamphorst et al., 2019).

In water-restricted environments, the anthesis-silking interval presents a significant negative correlation with grain yield (Santos et al., 2020). The selection of shortens anthesis-silking intervals can improve genetic gains in maize breeding programs for drought tolerance (Musvosvi et al., 2018). In addition, several other methods have been proposed to select water-tolerant maize genotypes, such as stress tolerance index and harmonic mean (Santos et al., 2020).

Dias et al. (2018b) highlighted that phenotypic selection promoted genetic gain for drought tolerance. However, there are still many challenges in maize breeding programs for drought tolerance, such as precise phenotyping and accurate understanding of drought-tolerance traits inheritance.

Many small-effect genes control most drought tolerance-related traits in maize (Dias et al., 2018a). Also, some genes that have essential roles in drought tolerance have been identified over the year, such as $AOX2$, related to the antioxidant defense system (Marques et al., 2019a), $ZmVPP1$ and $ZmTIP1$, associated with rooting modifications under water deficit stress conditions (Liu and Qin, 2021), $ZmDBP3$ and $ZmAN13$ (Marques et al., 2019b).

**Maize breeding for pest and disease resistances**

Diseases and pests are limiting factors of maize production under tropical and subtropical conditions. Production losses can be quantitative and qualitative,
Tropical maize breeding programs face many challenges to overcome several significant diseases of maize, such as white leaf spots, maize rusts, ear rots, leaf blights, and stunt diseases. Even though the inheritance of most maize diseases is quantitative with many small-effect genes, vertical resistance, conferred by one or a few genes, has been successful in managing some maize diseases, such as rust. \textit{Rpp} genes are known to confer resistance to specific races of the phytopathogen \textit{Puccinia polysora} (Wu et al., 2015). Wang et al. (2020) identified new candidate genes with potential use in maize breeding programs.

Even though vertical resistance provides a specific high level of disease resistance and is easier to work within breeding programs, horizontal resistance has been prioritized by breeders mainly due to its greater durability and protection to a wide range of races and species of phytopathogens. In this case, recurrent selection can be a great breeding strategy to improve the frequency of desirable diseases resistance alleles in the breeding population.

QTLs associated with resistance to 21 fungal diseases and 13 viruses have been identified (Rossi et al., 2019). Genomic regions associated with resistance to white leaf spot, caused by \textit{Pantoea ananatis}, have also been found and can be used for marker-assisted selection (Lana et al., 2017), which is particularly useful due to the low chemical control efficiency of the disease. The qRfg1 and qRfge QTLs increase the resistance to Gibberella stalk rot by 32-43\% (Zhang et al., 2012). In addition, the inheritance of resistance to \textit{Fusarium} spp. has been reported as a complex trait, and some QTL with moderate effects were detected (Prasanna et al., 2021).

Although necessary, studies on the inheritance of maize stunt disease are still scarce (Teixeira et al., 2013; Oleszczuk et al., 2020). However, Costa et al. (2019b) has shown high genetic variability for maize stunt resistance in tropical hybrids.

The maize germplasm can be screened for diseases resistance in nursery evaluation in the breeding pipeline. However, it is also vital to perform in-field trials to understand the GxE interaction in the breeding population and make genotypes selections.

Several studies have been performed over the years to understand the various diseases in tropical maize germplasm as rust diseases (Von Pinho et al., 1999a, 1999b, 2000; Camargos et al., 2017), gray leaf spot (Brito et al., 2012; Veiga et al., 2012; Balestre et al., 2012), ear rots (Pereira et al., 2015, 2017; Camargos et al., 2017), and white leaf spot (Camargos et al., 2017).

While conventional breeding is employed mainly for diseases resistance, insect pest resistance is challenging to achieve through conventional techniques. Besides the complex nature of pest resistance, handling insects and infesting field experiments are the main challenges for conventional maize breeding programs (Dhillion and Sharma, 2012).

Resistance to insect pests is associated with a set of physical, chemical, and morphological
characteristics that negatively affect insects’ feeding and reproductive behavior (Boiça Júnior et al., 2014). In the context of insect pests, lesser cornstalk borer, stink bugs, fall armyworm, corn earworm, and maize leafhopper are considered the most important.

In this case, transgenic technology has primarily been employed. Pereira and Borghi (2020) present that most maize cultivars possess transgenic events for insect pest resistance, such as cry, vip, and cyt proteins, toxic to several insect orders (Baranek et al., 2020). It is pivotal to highlight the importance of managing the selection of resistant insect pests, to maintain transgenic event efficiency.

**Transgenic in maize breeding**

The contribution of transgenic events in maize to Brazilian agriculture is expressive. The use of transgenic maize cultivars has changed the entire management of maize crops, representing one of the primary factors to protect the agronomic potential of the crops. According to Rissi (2018), Brazilian maize production increased almost two-fold since the commercial release of transgenic maize in 2007/2008. The use of transgenic maize promotes a reduction in the number of insecticide applications and allows the chemical control of weeds, increasing the efficiency of the major crops (Silva and Von Pinho, 2018). In addition, Diniz et al. (2015) highlight that Bt transgenic maize hybrids present higher phenotypic stability than their respective conventional isogenic hybrids. Finally, Nascimento et al. (2020) demonstrated the effect of transgenic maize in the behavior of adult females of *Spodoptera frugiperda*.

According to data from the Associação Paulista dos Produtores de Sementes e Mudas (APPs), 46 transgenic maize events were approved in Brazil until 2019. It is important to emphasize that most new records refer to stacked or pyramided events.

Transgenic maize has improved the seed production system at the national level. Furthermore, the seed companies have significant concern in adopting procedures to guarantee the genetic, physical purity, and physiological and sanitary quality of seeds (Von Pinho et al., 2016).

According to Pereira Filho and Borghi (2020), in 2019/20, 67% of the available maize cultivars had some transgenic event, in which most are related to pest resistance or herbicide tolerance separately or stacked.

There have been several advances in transgenic maize technology since the first commercial transgenic maize cultivar was released in the USA in 1996. Yadava et al. (2017) present several advances and considerations about transformation technologies such as choice of genotypes, explants, and media for in vitro regeneration and transformation, transformation techniques, and selection systems.

Although the contribution of transgenics is expressive, breeding companies face an enormous challenge regarding the regulation and registration of new transgenic events. Therefore, efforts have been directed towards developing new breeding techniques, such as gene editing, cisgenesis, intragenesis, and RNA-dependent DNA methylation (Yadava et al., 2017).

**Genome-wide association and genomic selection in tropical maize breeding**

Genome-wide association and genomic prediction tools have been increasingly employed in maize breeding programs with the constant reduction in genotyping costs and increasing phenotyping costs. These approaches can be implemented in
several stages of the breeding program pipeline, from the initial stages of germplasm characterization, parental selection, maize hybrid prediction to the final stages of multi-environment trials and maize hybrids selection.

Cantelmo et al. (2017a) employed genome-wide association (GWAS) tools to characterize 470 maize lines and cluster the germplasm into different heterotic groups. Furthermore, using this tool, it was possible to identify ten markers related to ear weight traits in maize.


The use of GWAS tools is wide in the literature. GWAS information is crucial to employing marker-assisted selection (MAS), which boosts the efficiency of positive or negative selection of genotypes even in the early stages of the breeding pipeline.

In addition to GWAS, genomic information can be used to perform maize hybrids predictions. The genomic prediction (GP) methodology was initially proposed by Meuwissen et al. (2001). Generally, GP predicts the phenotypic behavior of individuals based on genomic information, such as molecular marks (Xu, 2013).

For this purpose, prediction models must be trained from a training population, in which individuals have both phenotypic and genotypic data. Once trained, the model is employed to calculate the genomic estimated breeding values (GEBVs) of only genotyped individuals from a breeding population (Heffner et al., 2009). Then, the candidates’ GEBVs are used to perform selections.

Crossa et al. (2017) present a CIMMYT maize breeding program scheme, in which both phenotypic

![Figure 4. CIMMYT maize breeding program scheme. Adapted from Crossa et al. (2017) •](image)
and genomic selection is performed (Figure 4).

A significant advantage of genomic selection (GS) is increasing the genetic gain rate per time unit since using this tool can significantly reduce the required time to perform a breeding cycle (Dias et al., 2020). In addition, Beyene et al. (2019) report that the inclusion of GS in a tropical maize breeding pipeline reduced the cost by 32% over the phenotypic selection with similar genetic gains. Therefore, it should be incorporated into breeding pipelines to decrease costs and increase breeding program efficiency.

Using genomic prediction tools, Cantelmo et al. (2017b) observed the great utility of the G-BLUP approach in discarding unpromising materials in the initial stages of a maize breeding pipeline. In turn, Santos et al. (2016a) demonstrated the efficiency of adopting this approach in selecting *Stenocarpella maydis* resistant maize lines.

Dias et al. (2020) presented novel strategies for genomic prediction in situations of unbalanced experimental and genetic designs, multiple locations, and years, which are common scenarios in the tropical maize breeding programs. Uniformity of the experimental designs, large and representative training populations, and the inclusion of prior breeding cycles information increase the prediction ability of untested single-cross maize hybrids.

In addition, Atanda et al. (2021) report strategies to maximize genomic selection efficiency in tropical maize breeding programs. In the early stages of GS implementation, a careful selection of a structured training population can supply the lack of consistent historical data at these stages. For populations with close relatives in historical datasets, the selection and advance of maize genotypes into multi-environment trials can be performed using only GEBV. In contrast, for maize populations with a limited family relationship to historical data, it is possible to apply genomic selection to discard unpromising genotypes, reducing the phenotypic evaluation costs in the early stages of the breeding pipeline.

Since its initial proposal, genomic selection has been undergoing constant discoveries and methodological adjustments, such as the study and definition of better prediction models (Desta and Ortiz, 2014), the inclusion of non-additive genetic effects (Melo et al., 2014; Santos et al., 2015; Santos et al., 2016b; Dias et al., 2018a), the inclusion of GxE (Dias et al., 2018a), the optimization of representative training population (Fristche-Neto et al., 2018), the use of deep learning in genomic prediction (Azodi et al., 2019; Montesinos-López et al., 2021), among others.

**Specialty maize breeding**

Due to little interest by the private sector, specialty maize is a niche market that has excellent potential for research and development of cultivars by public sector breeding programs. However, in Brazil, there is a lack of maize breeding programs to supply the specific needs of the white maize market (Paterniani et al., 2020). Currently, works aim to develop high-yielding cultivars, although they do not focus on meeting the specific demands of the canjica industry, such as flint-type grains genotypes, a maize grain trait that improves de-germination process efficiency by the industry.

Although the sweet/green maize market is smaller than the commodity grain market, with a market turnover of around R$ 280 million in 2017 (Paterniani et al., 2020), it is an option for small and medium farmers to reach a higher added value market.

Objectively, sweet/green maize cultivars must
have: i) large and cylindrical ears that present uniform maturation; ii) aligned and dented kernels; iii) cream or yellow-cream color kernels; iv) thin pericarp; and v) slow kernel hardening rate (Paterniani et al., 2020). In addition, it is required that sweet/green maize cultivars present lower ear insertion height to allow manual harvesting, some level of resistance to pests and diseases, lodging tolerance, long harvesting window period, and prolonged postharvest shelf life (Coan et al., 2018).

Sweet/green maize cultivars evaluation (Von Pinho et al., 2001; Albuquerque et al., 2008a, 2008b), combining ability among inbred lines (Rodrigues et al., 2009), and genotypic parameters estimation (Rodrigues et al., 2011) studies, have been performed over the years, but much remains to be elucidated to improve sweet/green maize breeding programs.

Behind only the USA, Brazil is the second-largest popcorn producer. Although the added value of popcorn is higher (Coan et al., 2018), its production costs are twice as high as commodity grain maize, since popcorn seeds used in Brazil are imported from other countries such as the USA and Argentina, due to the higher quality seeds (Paterniani et al., 2020). Popcorn producers are interested in high-yielding cultivars. On the other hand, popcorn consumers are interested in quality traits, such as texture, softness, and popping expansion (Coan et al., 2018). Therefore, for the success of a popcorn breeding program, it is necessary to supply both: producers and consumers.

The major challenge faced by popcorn breeders is the negative correlation between popping expansion and several agronomic traits, such as ear weight, ear length, number of rows per ear, and grain yielding (Silva et al., 2010; Parsons et al., 2020). Therefore, public popcorn breeding programs face many challenges in providing popcorn cultivars that associate high-yielding and good grain quality, reducing dependence on imported seeds, and increasing farmers’ profitability.

To supply specific food industry demands for dehydrated, pre-cooked, and frozen meals, maize breeding programs may focus on the development of high amylose content maize (“amylose extender mutant” or ae) and waxy maize (“waxy mutant” or \textit{wx}). The mutant waxy do not possess the Granule-bound starch synthase enzyme (GBSSI), resulting in the formation of a starch composed by amylopectin only (Coan et al., 2018), an interesting trait since amylopectin is more stable at lower temperatures.

Waxy maize plants can be easily identified by evaluating their pollen grains. In dilute iodine-potassium iodide solution, waxy maize pollen grains show a reddish-brown spot, whereas common pollen grains vary, in color, from dark blue to black (Coan et al., 2018). Furthermore, the proportion of pollen grains production can be used to identify heterozygous individuals (Wx\textit{wx}) since equal amounts of \textit{Wx} and \textit{wx} pollen grains are expected (Coan et al., 2018).

**Maize breeding for silage purposes**

According to Daniel et al. (2019), there are 4 million hectares of maize for silage purposes in Brazil. In 2019/20, none of the available cultivars were exclusively for silage production. However, 90 dual-purpose maize cultivars were available, representing 46% of the total (Pereira Filho and Borghi, 2020).

The choice of the right maize hybrid to be cultivated is crucial for producing high-quality maize silage. Therefore, maize breeding programs for silage purposes need to focus on grain yield, biomass production, and silage quality characteristics (Gomes et al. 2006), such as high digestibility, to promote better energy conversion and animal performance.
The agronomic characteristics and bromatological parameters, defined by Neumann (2011), are used as reference values by maize breeders. Wet and dry biomass productions higher than 55 tons ha\(^{-1}\) and 18 tons ha\(^{-1}\), respectively, and at least 35% of grains contained in the dry biomass are desired. In addition, resistance to regional pests and diseases, yield stability, accentuated stay green, and less dry down are desired traits in breeding programs (Marcondes et al., 2012).

Daniel et al. (2019) highlight that high content of starch and greater NDF (neutral detergent fiber) digestibility is nutritionally desirable. In addition, maize breeders can also consider grain texture to perform selections. Due to their higher digestibility, dent genotypes are preferable over flint genotypes (Rossi et al., 2016; Viana et al., 2020), even though it is also possible to obtain high-quality silage from flint genotypes. According to Gomes et al. (2006), the best strategy for obtaining a good breeding population is by performing crossing among high digestibility genotypes that also present good combining ability.

Sattler et al. (2010) highlight the advantages of using *brown midrib* (*bm*) mutant genotypes in maize breeding programs for silage purposes. *Bm* genotypes possess less lignin content, resulting in higher digestibility. However, such materials present 20% less grain yield performance, higher susceptibility to pests and diseases in tropical conditions, being economically unviable in Brazil (Gusmão, 2021).

The genetic variability in tropical maize for silage production is well reported in the literature (Fonseca et al., 2002; Gomes et al., 2004a, 2004b). In addition, effects of sowing date and sowing density (Von Pinho et al., 2007), inter-row spacing (Álvarez et al., 2006), plant cutting height (Von Pinho et al., 2006), GxE (Gomes et al., 2002) interaction on silage quality are also reported.

**Final considerations**

Tropical maize breeding is vital for the development and success of Brazilian agriculture. In a scenario of increasing the world’s population and climate changes, many are the challenges for maize breeding under tropical conditions. Breeding for biotic and abiotic stresses conditions, and a better understanding of genotypes-by-environments interaction, are required to overcome climate changes.

Genomic prediction should be increasingly applied to maize breeding programs, increasing the genetic gain per unit of time. In addition, double haploid technology, genome-wide association, marker-assisted selection should be employed to boost breeding program efficiency. Transgenic technology plays an important role, although new breeding techniques, such as gene editing, will be increasingly employed.

Specialty maize is a high-value niche market and should be better exploited by public sector breeding programs. Furthermore, the development of exclusively silage purposes maize hybrids may improve maize silage production in Brazil. Finally, marginal regions and new agriculture frontiers will increase their importance, representing a new important market for maize breeding programs.
References


Challenges of maize breeding under tropical...


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