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RESEARCH PAPER

Status of molecular marker utilization in conventional maize breeding in Ethiopia

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Abstract

The demand for maize (Zea mays L.) has been steadily growing in Ethiopia. It contributes to the greatest share of production and consumption along with other major cereal crops such as tef (Eragrostis tef (Zucc.) Trotter), wheat (Triticum aestivum L.), and sorghum (Sorghum bicolor L.). Three-fourth of the maize produced is consumed at the household level by the small-scale producers themselves. According to recent reports, it was grown by 10.2 million households in the country, which constituted 64.7% of the total cereal producing households. Besides, it contributed to 35.0% of the total cereal production in the country with an average national yield of 4.24 t ha-1, which is among the top three highest national average yield reported in Sub-Saharan Africa (SSA). However, the national average productivity is still low as compared to the world average yield of 5.8 t ha-1, which is attributed to several production constraints. Despite all the efforts and progress made in the development and dissemination of maize technologies for different maize growing agro-ecologies, the biotic and abiotic constraints remained the major limiting factors for maize production and productivity. Genetic improvement provides an option to address some of the constraints facing maize production and productivity in Ethiopia today, but mainly relies on the presence of genetic diversity, systematic characterization, and effective use of available germplasm. To this end, the use of molecular tools in the Ethiopian maize breeding programs has enhanced the breeding selection process; however, a much more effort is need to further consolidate with the conventional schemes. The objective of this manuscript is, therefore, to review the status of molecular markers' contribution to the conventional maize breeding in Ethiopia.

Keywords: Conventional breeding, Maize, Marker assisted breeding, Molecular markers

Introduction

Maize is a widely cultivated crop that is a staple food in many countries of the world, including the United States, Africa, and other areas of the world (Abbas *et al.*, 2022) signifying its global and regional importance to millions of people who rely on the crop in pursuit of food security and livelihoods.

Increased production and consumption trends of maize have been observed in sub-Saharan Africa (SSA) over the past years. In the region, maize is the dominant staple crop grown by the vast majority of rural households (DeVries and Toenniessen, 2001). In SSA, maize is the primary source of calories (466.5kcal/capita/day) and is the second most important source of protein (12g/capita/day) only after wheat (http://faostat.fao.org). Sub-Saharan African countries, however, do not produce enough maize to meet their needs and therefore import more than three million tonnes of maize annually (Pingali and Pandey, 2001). Accordingly, demand for maize in sub-Saharan Africa is projected to increase nearly twofold

by the year 2030 (Bigirwa *et al*., 2003). As Ethiopia is the second highly populated country next to Nigeria in Africa, maize is considered as strategic food security crop to feed the fast-growing population of the country in the short- and long-terms. In addition to strong demand for maize as a staple food, there is also the potential for maize to become an increasingly important non-traditional agricultural export crop.

Similarly, the demand for maize has been steadily growing in Ethiopia. It contributes to the greatest share of production and consumption along with other major cereal crops such as tef (*Eragrostis tef* (Zucc.) Trotter), wheat (*Triticum aestivum* L.), and sorghum (*Sorghum bicolor* L.). Three-fourth of the maize produced is consumed at the household level by the small-scale producers themselves (CSA, 2017). The maize grain is consumed in different forms of food; the stover is used as feed, fuel, and construction material. Besides, it serves as a major source of income and means of employment for tens of millions of farming and business communities. Its production has also been increasing over the years in the major maize producing regions of Ethiopia. In the 1980s, the total production was below 2 million tonnes, and the maize area was slightly more than 1 million hectares (Kebede *et al*., 1993). However, a significant increase in production of 2.34 million tonnes was observed in the 1990s. From 1995-2000, the annual growth rates of yield per hectare, maize area, and total production were 3.10%, 7.10%, and 11.30%, respectively (Mosisa *et al*., 2002). Reports of the Central Statistical Agency (CSA) of Ethiopia showed that maize was produced on about two million hectares with a total production of about 6 million tonnes in 2011/12 main cropping season. During the same year, an average national yield of 2.95 t ha^{-1} was recorded (CSA, 2011). From these data, it could be depicted that the area under maize increased by about 50% and production by 66%, with the national average yield increments from 1.60 to 3.00 t ha^{-1} in 2010 (CSA, 2011). According to recent reports of CSA (2021), maize was grown by 10.20 million households in the country, which constituted 64.70% of the total cereal producing households. In the same year, it occupied 23.97% of the area allocated to cereals and thus contributed to 34.95 % of the total cereal production in the country with an average national yield of 4.24 t ha^{-1}, which is the second highest national average yield reported in Sub-Saharan Africa (SSA), only after South Africa. It was also indicated that maize was produced on about 2.53 million hectares of land and total production of 10.55 metric tons (MT) in the same report. Improved hybrids and open pollinated varieties (OPVs) developed by the national maize breeding program, in conjunction with introduced hybrids by multi-national seed companies, have significantly contributed to such a rapid increase in maize production in the country (Tsedeke *et al*., 2015). However, the national average productivity of maize is still low as compared to the international average yield of 5.75 t ha^{-1} (http://faostat.fao.org), which is attributed to the undermentioned production constraints.

Despite its wide adaptation, maize production in Ethiopia is constrained by several biotic and abiotic constraints. Most of these constraints are common to all maize growing agro-ecologies (e.g., shortage of improved varieties and soil fertility problems), while some of them are particularly important to specific agro-ecologies (e.g., drought). The major abiotic and biotic constraints include factors such as, drought, nutrient deficiencies, diseases, weeds, and insect pests (Ransom *et al*., 1993; Mosisa *et al*., 2012). Among abiotic constraints, drought is the major problem, particularly in areas that receive minimum amounts of annual rainfall as low as 200 mm (Mandefro *et al*., 2002). The second most important abiotic stress is soil nutrient deficiency, and it is a serious problem in most of the potential maize producing areas (Mosisa *et al*., 2002, 2012). This problem is attributed, in part, to the low input purchasing power and lack of cultural practices such as crop rotations and fallows exercised by farmers (Ransom *et al*., 1993). Among the biotic factors, diseases are the principal problems. The most economically significant diseases and their respective causative agents in Ethiopia's maize production system include grey leaf spot (*Cercospora*

zeae-maydis), turcicum leaf blight (*Exserohilum turcicum*), streak disease of maize (Maize streak virus), common leaf rust (*Puccinia sorghi*) (Mosisa *et al*., 2012; Tewabech *et al*., 2012), maize lethal necrotic disease caused by the coinfection of *maize chlorotic mottle virus* (MCMV) and *sugarcane mosaic virus* (SCMV) (Mahuku *et al*., 2015), *maize yellow mosaic virus* (MaYMV), *maize streak dwarfing virus* (MSDV), rotting diseases (ear, kernel, and stalk), maize weevil, stalk borers, fall armyworm, and striga. These diseases are known to cause significant yield losses in cases where environmental conditions are favorable (Demsachew *et al*., 2018, 2019b; Tolera *et al*., 2018).

Apart from the biotic and abiotic factors hindering maize production and productivity, there exist policy and institutional constraints (Alene *et al*., 2000; Tsedeke *et al*., 2015). Among these constraints, the most important ones are limited capacity in research and extension services, insufficient production and distribution of seeds, constrained access to rural credit, and limited competition in input supply markets. Furthermore, the unavailability of improved seed has proved to be a major constraint for the adoption of the newly released improved varieties, a fact that calls for improvements in improved seed delivery to cope effectively with the demands of small farmers.

On the other hand, although biotechnological tools help to solve some of the biotic and abiotic constrains mentioned above, maize biotechnology research activity was lately started (2005) in Ethiopia focusing on the comparison of SSR markers and morphological characters in knowing genetic diversity among maize accessions collected from highland environments of the country (Yoseph *et al*., 2005). Subsequent research also continued with validation of different molecular markers, and it was revealed that molecular markers were more efficient than morphological traits in establishing genetic diversity in maize breeding lines. It was unanimously understood that molecular markers could complement conventional breeding through identification of heterotic germplasm and predicting heterosis (Melchinger, 1999a), genetic finger printing and tracking varietal adoption, and genetic purity and quality control in the development of inbred lines. The objective of this manuscript is, therefore, to review the status of molecular markers' contribution to the conventional maize breeding in Ethiopia.

Materials and methods

Considering the current average research yield of maize and the actual yield obtained on farmers` fields (Table 1), there is still huge potential to improve maize production and productivity. However, several biotic and abiotic factors are hindering further progress beyond the current levels of productivity. To overcome these constraints, it is mandatory to complement the current breeding methods with modern biotechnology tools such as genotyping/diversity study, marker-assisted selection, genomic selection, and the cutting-edge molecular applications. This review was, therefore, conducted based on secondary data obtained from different sources and document review. The historical data on maize production, area coverage, productivity was collected from the Ethiopian Statistical service (formerly known as Central Statistical Authority) website (https://www.statsethiopia.gov.et) and FAO website (https://www.fao.org/faostat/en/#data/QCL). In addition, the data characterizing the improved maize cultivars over the decades were obtained from the Ministry of Agriculture, Variety Registry Book. The information for the major part of this review work, which are about the status of applications of molecular tools in Ethiopian maize breeding, were compiled using individual papers published in a reputable journal by local and international Ethiopian researchers and scientists.

Results and discussion

Highlights of maize breeding efforts and gaps in Ethiopia

Maize is broadly divided into temperate, subtropical, and tropical germplasm depending on latitudinal variations and environmental characteristics (Paliwal *et al.,* 2000). Tropical maize is further classified into lowland, midaltitude, and highland. The diversified nature of maize agro-ecologies and the environmental variability (both natural and due to management) that prevails within each maize agro-ecology in Ethiopia calls for continuous research aimed at developing high yielding varieties adapted to the different environmental conditions. According to Lynch (1998), there are three approaches of germplasm improvement for grain yield in the farmers' field: (1) improving yield response to high levels of input, (2) improving yield under low input availability, and (3) improving yield under both low and high input availability. Improving crop yield only under high levels of input may result in varieties unsuitable for low input conditions, which occur frequently in resource poor farming conditions. Similarly, improving crop yield when only under low levels of input may result in non-responsive crop types. Generally, the National Maize Research Program has followed the third option for maize improvement (Mosisa *et al*., 2007).

The maize program is the first program of cereals research to start agro-ecology-based research under the Ethiopian Institute of Agricultural Research. It has been undertaking maize research country-wide in four broadly classified major maize agro-ecologies each having specific limitations and potentials, namely: mid-altitude sub-humid (1000-2000 meters above sea level [m.a.s.l.]), highland sub-humid (1800-2600 m.a.s.l.), lowland moisture stress areas (300-1500 m.a.s.l.), and lowland sub-humid (<1000 m.a.s.l.) (Frew and Girma, 2002; Abiy *et al*., 2019). Maize research and development was started in 1950s in the country to enhance its productivity, targeting the needs of small-scale farmers who produce more than 90% of maize (Benti and Ransom, 1993; Mandefro and Tanner, 2002). The subsequent participation of the country in the "East African Cooperative Maize Variety Trial" in the late 1960s and early 1970s enabled the identification of high yielding composites and hybrid varieties that were better adapted to the local growing conditions than those acquired in the 1950s (Benti *et al*.,1993), which was mainly due to agro-ecological similarities. In the 1980's, the national breeding program started to introduce tropical maize germ plasm from CIMMYT, IITA and other national programs in eastern Africa (Benti *et al*., 1993). The introduction and evaluation of a wide range of maize genotypes over the years has enabled the national maize breeding program to develop and release several open pollinated varieties (OPVs) and hybrids for commercial production. In the 1970s and 1980s, locally developed improved OPVs were released for wide area production at different agro-ecologies in Ethiopia. In the late 1980s, the first locally developed non-conventional hybrid was released for the mid-altitude sub-humid maize growing areas. Since then, many improved OPVs and hybrids with pest resistance/tolerance were released (Table 1) for large scale production across different agro-ecologies by the National Maize Research Project of the Ethiopian Institute of Agricultural Research (EIAR). Currently, the National Maize Research Program has three main breeding stations located in the above three major agro-ecologies to address specific demands of variety development for the agro-ecologies.

The mid-altitude sub-humid agro-ecology is a high potential area for maize production in Ethiopia. It is the leading maize growing agro-ecology contributing the largest share of maize produced in the country (Benti and Ransom, 1993; Mandefro and Tanner, 2002; Mosisa *et al*., 2012; Abiy *et al*., 2019). However, production and productivity of maize in this and other agro-ecologies are constrained by several factors. These include unavailability of improved varieties, limited access to improved seeds, diseases such as gray leaf spot caused by *Cercospora zeae-maydis*, Turcicum leaf blight (*Exserohilum turcicum*) and common rust (*Puccinia sorghi*), field and storage insect pests (e.g., maize stalk borers and the maize weevil), low soil fertility and poor market development (Mosisa *et al*., 2002, 2012). Therefore, there is

a need to develop improved maize varieties and their production packages for sustainable maize production in the country.

The lowland moisture stress agro-ecology is the other maize producing agro-ecology of Ethiopia. This agro-ecology encompasses drought affected areas occupying over 40% of the area in the country and contributing 20% of the total maize production (Mandefro *et al*., 2002). However, recent reports indicated that the lowland moisture stress maize agro-ecology occupies up to 20% (Tsedeke *et al.,* 2015; Abiy *et al*., 2019). In addition to the above constraints, recurrent drought is the most important challenge for maize production and productivity in this agro-ecology (Benti and Ransom, 1993; Mandefro and Tanner, 2002).

The high altitude sub-humid agro-ecology, including the highland transition and true highlands, is next to the mid-altitude agro-ecology with greater maize area and production share in Ethiopia. This agro-ecology covers an estimated 20% of the land area devoted to annual maize cultivation and consisting of more than 30% of small-scale farmers who depend on maize production for their livelihoods (Twumasi *et al*., 2002; Abiy *et al*., 2019). The Ethiopian highland maize breeding program is situated at Ambo to coordinate maize research and technology development for the highland agro-ecology. This program was initiated in 1998 in collaboration with the International Maize and Wheat Improvement Center (CIMMYT) and National Agricultural Research Systems (NARS) of east and central African countries including Ethiopia, Kenya, Tanzania, Uganda, Rwanda, and Burundi (http://www.cimmyt.org.com). Research and variety development of highland maize has generally lagged behind other agro-ecologies before the launch of this breeding program (Twumasi *et al*., 2002).

Table 1. Released maize varieties of public research centers and Universities with their agro-ecological adaptations and some agronomic characters (until 2022)

Crop	Variety	Year of release	Altitude (m)	Rainfall (mm)	Plant height (cm)	Ear Placement (cm)	Days to Maturity	Seed Colour	Yield (Qt ha^{-1})		Disease Reaction			
									Research Station	Farmers field	MSV	GLS	TLB	CLR
	BH-140	1988	1000-1700	1000-1200	240-255	105-120	145	White	75-85	47-60	-	MT	MT	MT
Hybrids	BH-660	1993	1600-2200	1000-1500	255-290	145-165	160	White	90-120	60-80	-	T	T	R
	BH-540	1995	1000-2000	1000-1200	240-260	110-120	145	White	80-90	50-65	-	MT	MT	MT
	BH530	1996	1000-1300	1000-1500	200-230	110-120	140	White	80-90	50-60	-	MT	MT	MT
	BH-541	2002	1000-1800	1000-1200	-	-	150	White	65-75	-	-	T	T	MR
	BHQP-542*	2002	1000-1800	1000-1200	220-250	100-120	145	White	80-90	50-60	-	T	MT	MS
	BH-543	2005	1000-2000	1000-1200	250-270	140-150	148	White	85-110	55-65	-	MT	MT	T
	BH-544	2006	1000-2000	1000-1200			147	White	70-80		-	S	S	T
	BHQPY-545*	2008	1000-1800	1000-1200	250-260	120-140	144	Yellow	80-95	55-65	-	T	MT	MT
	BH-670	2002	1700-2400	1000-1500	260-295	150-165	165	White	90-120	60-80	-	T	T	R
	BH546	2013	1000-1750	500-1000	280	140	140	White	85-115	65-75	-	R	R	R
	BH547	2013	1000-1800	500-1000	250	120	140	White	85-115	65-75	-	R	T	R
	BH661	2011	1600–2200	1000-1500	280	170	160	White	95–120	65–85	-	R	R	R
	SPRH1	2015	1000-1800	1000-1200	280	140	145	White	75-85	55-65	-	R	T	R
	SBRH1	2015	1000-1800	1000-1200	280	140	145	White	75-85	50-60	-	T	T	R

	BHQP548*	2015	1000-2000	1000-1200	265	150	145	White	75-85	65-75	-	R	T	R
	BH549	2017	1500-1800	1000-1200	220	114	145	White	90-120	65-80	-	R	R	R
	BH520 W1	2020	1000-1800	900-1500	237	134	155	White	90-130	76-100	-	R	R	R
	BH5211	2022	1000 - 1800	900 - 1500	244	118	155	White	90	88	-	T	T	T
	BH5212	2022	1000 - 1800	900-1500	239	116	155	White	91	80	-	T	T	T
	BHA5211	2022	1000-1800	900-1500	240	115	155	Orange	80	75	-	T	T	T
	MH130	2012	1200-1750	500-800	180	85	120	White	65-75	50-60	-	-	MR	MR
	MHQ138*	2012	1000-1800	500-1000	200	100	138	White	85-100	70-80	-	-	MR	MR
	MH140	2013	1000-1800	500-1000	200	100	140	White	90-100	77-80	-	-	T	T
	MH141	2020	1000-1800	500-1000	181	100	141	White	92.5	65	-	-	R	R
	AMH-800	2005	1800-2500	1000-1200	205-225	105-125	175	White	70-80	55-65	-	MR	MR	MR
	AMH-850	2007	1800-2600	1000-1200	220-235	120-130	183	White	80-120	60-80	-	MR	T	R
	AMH-851	2009	1800-2600	1000-1200	220-235	120-130	178	White	80-120	60-80	-	R	R	R
	AMH760Q*	2012	1650-2400	1000-1200	245	143	183	White	90-120	80-100	-	T	MS	T
	AMH852Q*	2016	1800-2600	1000-1200	250	145	182	White	90-100	75-85	-	MR	MR	R
	AMH853	2016	1800-2600	1000-1200	229	168	179	White	90-120	80-90	-	T	MR	R
	AMH854	2022	1800-2600	1000-1200	245	145	190	White	85-125	80-93	-	MR	R	R
	Hora (Amb02syn1)	2005	1800-2400	1000-1200	200-215	100-120	170	White	60-70	40-45	-	MR	MR	MR
OPVs	Kuleni	1995	1700-2200	1000-	240-265	130-145	150	White	60-70	40-45	-	T	T	R

				1200										
	Katumani	1974	1000-1700	450-550			110	White	22.5		-			
	Alemaya Composite	1973	1600-2200	1000-1200	280-300	160-190	163	White	50-70	38-42	-	T	T	T
	A-511 (AwARC)	1973	500-1800	800-1200	230-250	130-145	150	White	50-60	30-40	-	MT	MT	MT
	Gibe-1	2001	1000-1700	1000-1200	240-260	130-140	145	White	60-70	40-45	-	MT	MT	T
	Gibe-2	2011	1000-1800	1000-1200	224	122	143	White	65-70	45-50	-	T	T	T
	Gibe-3	2015	1000-1700	1000-1200	238	123	145	White	65-75	45-55	-	T	T	T
	Gutto	1988	1000-1700	800-1200	165-190	90-110	126	White	30-50	25-30	-	MT	MT	MT
	Morka (improved UCB)	2008	1600-1800	1200-2000	270-300	145-180	180	White	70-90	40-60	-	T	T	T
	Rare-1	1997/98	1600-2200	900-1200	250-270	130-150	163	White	60-70	40-45	-	T	T	T
	ACV3 (Fetene)	1996	-	-	-	-	100	-	-	-	-	-	-	-
	ACV6 (Tesfa)	1996	-	-	-	-	110	-	-	-	-	-	-	-
	Melkasa-1	2001	1000-1700	600-1000	140-160	65-70	90	Yellow	35-45	25-35	-	-	T	T
	Melkasa-1Q*	2013	1000-1800	700-1200	190	103	130	Yellow	35-45	25-35	-	-	T	T
	Melkasa-2	2004	1000-1700	600-1000	170-190	80-90	130	White	55-65	45-55	-	-	T	T
	Melkasa-3	2004	1200-1700	600-1000	170-175	75-80	125	White	50-60	45-50	-	-	T	T
	Melkasa-4	2006	1200-1700	600-1000	160-170	70-80	105	White	35-45	30-35	-	-	T	T
	Melkasa-5	2008	1200-1700	600-1000	180-190	80-90	125	White	40-50	35-40	-	-	T	T
	Melkasa-6Q*	2008	1200-1700	600-1000	165-175	70-75	120	White	45-55	30-40	-	-	T	T

	Melkasa-7	2008	1200-1700	600-1000	170-182	80-90	115	Yellow	45-55	30-40	-	-	T	T
	Abo-Bako	1986	300-1000	900-1200	220-230	120-130	112	White	50-60	35-45	T	T	T	T
	Gambella Comp-1	2002	300-1000	900-1200	200-220	105-115	116	White	60-70	40-50	R	T	T	T
	HrU22 (Bate)	2017	1300-2000	650-1200	180	95	145	White	45-60	35-45	-	-	T	T
	HrU28(Afran Qallo)	2017	1300-2000	650-1200	180-220	95-120	148	Yellow	45-60	35-50	-	-	T	T
	BOS20W1 (sweet)	2020	1000-1800	900-1500	220	112	140	White	51.1	-	-	R	R	R
	BOS20Y1 (sweet)	2020	1000-1800	900-1500	212	104	140	Yellow	57.4	-	-	R	R	R
	Gibe-awash Fendisha	2012	1000-1800	700-1200	190	103	130	Yellow	36	30	-	T	MS	T

Note: * = QPM, T= tolerant, R= resistant, MT= moderately tolerant, MS= moderately susceptible, S= susceptible, MR= moderately resistant, MSV= maize streak virus, GLS=gray leaf spot, TLB=turcicum leaf blight, CLR=common leaf rust. Source: Ministry of Agriculture- variety release registry books of different years.

Some limitations of the conventional breeding approaches and the need for molecular marker application in the Ethiopian maize breeding programs

Despite all the efforts to develop maize germplasm for the various agro-ecologies of Ethiopia by the National Maize Breeding Programs, maize productivity remains still far below the potential due to several factors responsible for the yield gap, some of which were mentioned above as constraints. Though initial adoption of hybrids by resource poor farmers was very slow, the demand for hybrid seeds has gradually increased in Ethiopia as a result of changes in government policy including the establishment of several local seed companies and the launching of a national extension program by government and non-governmental organizations (NGOs), such as Sasakawa Global 2000 (Tsedeke *et al.,* 2015). The rapid adoption of some of the hybrids, however, brought a major concern on the quality of hybrid seed sold to resource poor farmers. Farmers reported a high level of mixture of plants in their fields and low yield in a given area. Despite increased number of actors in the seed production and marketing venture, a vibrant national seed regulatory body to undertake effective seed quality assurance, including seed inspection and certification has been creating a huge gap in the sector (Berhanu *et al*., 2015). Routine inspection of the initial parental seed (breeder, pre-basic and basic seeds) produced by different actors in the seed value chain is critical and often done by inspecting production fields at vegetative and flowering stages. However, inspection of seed production fields based on a limited number of morphological and agronomic traits is time consuming, laborious, expensive, and at times can lead to inaccurate conclusions. The verification of seed lots and seed production fields could have been effectively improved through the use of quality control (QC) genotyping using molecular markers (Kassa *et al*., 2012a; Berhanu *et al*., 2015).

The other gap filling advantages of molecular tools is that maize breeders often use a number of phenotypic traits and combining ability studies for evaluating maize germplasm as well as assigning inbred lines into distinct heterotic groups. Expression of phenotypic traits, however, are often influenced by environmental factors, which may affect the consistency and reliability of combing ability-based classification. Therefore, use of molecular markers to characterize locally available inbred lines can complement and fine-tune the combining ability based heterotic grouping of inbred lines (Berhanu *et al*., 2017; Demissew *et al*., 2018; Dagne *et al*., 2019). In the work of Demissew *et al*., (2015), conversions of non-quality protein maize (non-QPM) into QPM had been done using phenotypic selections without monitoring the genetic backgrounds. Consequently, recombinants were selected and a very small portion of the genome of the recurrent parents was recovered, and hence suggested the use of marker-assisted backcross or marker-assisted selection (MAS) in the future. Because marker-assisted breeding and/or MAS would be used to facilitate background selection and avoid disruption of the newly established heterotic groups.

Molecular marker applications in maize breeding

Genetic improvement provides an option to address some of the constraints facing maize production and productivity in Ethiopia today, but mainly relies on the presence of genetic diversity/variability, characterization and systematic classifications, and effective use of available germplasm. Table 2 shows the status of molecular work done using introduced and locally developed maize germplasm of Ethiopia. Each of the published molecular works listed in this table is further narrated one by one after the table in different headings based on the sequence of the studies.

Table 2. Summary of studies conducted in and out of Ethiopia on molecular marker applications for maize germplasm developed and/or used in Ethiopia

No.	Maize type	Molecular marker type and application	Reference
1	Tropical highland adapted maize accessions	AFLP and SSR (comparison of the two marker systems for diversity study)	Yoseph *et al.*, (2005)
2	Tropical highland adapted maize accessions	AFLP (genetic diversity study)	Yoseph *et al.*, (2006)
3	Tropical highland adapted maize inbred lines	SSR (genetic diversity study)	Legesse *et al.*, (2006)
4	Tropical highland adapted maize inbred lines	AFLP (genetic diversity study)	Legesse *et al.*, (2008)
5	Tropical mid-altitude QPM maize inbred lines	SSR and RAPD (comparison of the two marker systems in the power of detection of polymorphism)	Demissew *et al.,* (2012)
6	Tropical mid-altitude maize inbred lines	SSR (genetic variability study)	Wende *et al.,* (2013)
7	Tropical highlands adapted QPM and conventional maize inbred lines	SSR (genetic variability study and population structure)	Demissew *et al.*, (2015)
8	Tropical mid-altitude maize hybrids and inbred lines	SNP (genetic purity and identity study using GBS markers)	Berhanu *et al.*, (2015)
9	Tropical mid-altitude maize inbred lines	SNP (genetic variation and population structure study using GBS markers)	Berhanu *et al.*, (2017)
10	Tropical mid-altitude conventional & QPM maize inbred lines	SSR and phenotypic traits association study	Demissew *et al.,* (2018)
11	African highland adapted maize inbred lines	SNP (genetic purity, genetic variability, and population structure study using GBS selected markers)	Dagne *et al.,* (2019)
12	Tropical yellow maize inbred lines	SNP (Association study between functional DNA markers and quality trait- using GBS selected markers)	Girum *et al.,* (2013)
13	Tropical mid-altitude DH lines	SNP (GWAS & genomic prediction to identifying QTLs regions associated with agronomic traits under optimum & Low-Nitrogen)	Berhanu *et al.,* (2020)
14	Tropical drought tolerant maize inbred line and OPV	Maize genetic transformation study to transfer genes for marker assisted breeding	Bedada *et al.,* (2016, 2018)

Genetic diversity studies

Maize and wheat have been extensively exploited in genetic and cytogenetic studies compared to other cereal crops. Maize is one of the domesticated crop species with the highest level of molecular polymorphism. Nucleotide diversity of more than 5% has been reported at some loci of the maize genome (Henry and Damerval, 1997), and has been verified by high genetic variability both within and among maize populations as revealed by several genetic diversity studies. The molecular diversity of maize is approximately three to tenfold higher than that of other domesticated grass species (Buckler *et al*., 2001).

Molecular markers such as RAPD, AFLPs, SSRs, and SNPs are proposed to be an appropriate tool not only for breeding lines and hybrids (Bastia *et al*., 2001) and cultivars (Mohanty *et al*., 2001) but also facilitate the monitoring of introgression, mapping of QTLs (Paterson *et al*., 2003) and the assessment of genetic diversity (Warburton *et al*., 2002; Kassahun and Prasanna, 2003; Legesse *et al*., 2007; Yoseph *et al*., 2006; Pooja and Singh, 2011; Demissew *et al*., 2015; Berhanu *et al*., 2017; Dagne *et al*., 2019) in different crops

including maize. Several DNA marker technologies have been developed and are available to study genetic diversity. The genetic diversity/variability studies on maize in Ethiopia using the different markers are summarized as follow:

Yoseph *et al*., (2006) analysed 62 traditional Ethiopian highland maize accessions collected from different parts of Ethiopia using 20 simple sequence repeat (SSR) markers and 15 morphological traits with the objectives to assess genetic diversity and relationships among the accessions and to assess the level of correlation between phenotypic and genetic distances. Their finding showed that the average number of alleles per locus was 4.9. Pair-wise genetic dissimilarity coefficients ranged from 0.27 to 0.63 with a mean of 0.49. Ward minimum variance cluster analysis showed that accessions collected from the Northern part of the country were distinct from the Western and Southern parts. However, there was no differentiation between the Western and Southern accessions. This suggested gene flow between these regions. The relationship between morphological and SSR-based distances was significant and positive ($r = 0.43$, $p = 0.001$). The high genetic diversity observed among these set of accessions suggests ample opportunity for the development of improved varieties for different agro-ecologies of Ethiopia. From a conservation perspective, sampling many accessions from all agro-ecologies would be an effective way of capturing genetic variation for future collections and conservation.

Yoseph *et al.,* (2006) also did the same work on the 62 Ethiopian highland maize accessions but using a different marker platform known as amplified fragment length polymorphism (AFLP) markers and morphological traits. Eight *EcoRI/MseI* primer combinations and 15 morphological traits were used. Of a total of 650 AFLP markers scored, 89.5% were polymorphic. The authors found out that the relationship between morphological and AFLP-based distances were significantly positive and concluded as saying that regardless of the large variation in environmental conditions between agro-ecologies where the accessions were collected, only 9% of the total genetic variation was found between agro-ecologies, while 91% was found within the maize agro-ecologies in Ethiopia. The authors further suggested implications for this finding could probably be explained by long distance seed exchange, continuous seed introduction and gene flow between agro-ecologies. A similar work was done on genetic diversity of 56 highland and mid-altitude maize inbred lines obtained from CIMMYT and EIAR breeding programs in Ethiopia and Zimbabwe by Legesse *et al*., (2006). The inbred lines were genotyped using 27 SSR loci. In total, 104 SSR alleles were identified with a mean of 3.85 alleles per locus from the work. The average polymorphism information content (PIC) was 0.58. Genetic distance expressed as Euclidean distance varied from 0.28 to 0.73 with an average of 0.59. From the results obtained, the authors concluded that the variability detected using SSR markers could potentially contribute towards effective utilization of the inbred lines for the exploitation of heterosis and formation of genetically diverse source populations in Ethiopian maize improvement programs.

On the other hand, Legesse *et al.,* (2008) also conducted a study on the relationship between hybrid performance and AFLP-based genetic distance in highland maize inbred lines to estimate genetic distance (GD) among the inbred lines and tester parents and to investigate the relationship of GD with hybrid performance and mid-parent heterosis. From the AFLP analysis it was depicted that 32 parental genotypes produced a total of 601 bands, of which 80.5% were polymorphic. Polymorphism ranging from 42 (AGG/CGA) to 66 (ACA/CCC) bands with a mean of 50 was detected across nine primer combinations. Polymorphic information content values ranged from 0.25 to 0.40. Genetic distance calculated in terms of dissimilarity for all possible combinations among 32 genotypes ranged from 0.40 to 0.72 with an average of 0.59 units. Genetic distance estimates for the 26 female and six male parent combinations varied from 0.63 to 0.72 with a mean of 0.67. With further sub-groupings of the pairwise combinations into population testers and line testers, mean GD values for population tester and line tester combinations were 0.68 and 0.66, respectively.

Finally, the authors concluded the effectiveness of AFLP markers for diversity analysis in that the relationships between GDs of population tester combinations with their corresponding F1 grain yield, plant height, and mid-parent heterosis were negatively correlated. On the contrary, GDs of inbred line tester combinations showed positive and significant correlation coefficients with F1 performances and mid-parent heterosis for most traits but with low magnitude to warrant prediction of hybrid performance.

Additional studies of genetic variability using molecular markers had also been conducted by Wende *et al*., (2013) and Demissew *et al*., (2015). Wende *et al*., (2013), in their study of genetic interrelationships among 20 elite intermediates to late maturing tropical maize inbred lines, used 20 selected SSR markers. The 20 SSR primers identified 108 alleles among the 20 maize inbred lines. The number of alleles scored across SSR loci ranged from 1 to 11, with a mean of 5.4 alleles. The two loci (*Phi 037, Umc1296*) revealed one allele, and the maximum numbers of alleles were detected at the *Bnlg 2190, Umc2214* and *Umc1153* loci. The PIC estimated for all loci ranged from 0.0000 to 0.8028 with a mean of 0.54. Expected heterozygosity (He) values, as a measure of allelic diversity at a locus, varied from 0.0000 to 0.8395 with an average of 0.5774. These values were well-correlated with the number of alleles. Ten SSR loci (*Umc1568, Nc003, Umc2214, Umc2038, Phi085, Umc1153, Bnlg238, Phi054, Bnlg2190, and Bnlg240*) manifested a PIC value of more than 0.6, reflecting their potential to detect differences between the inbred lines. From the results, the authors found out that the genetic diversity existing in the study materials was the most important factor limiting the number of alleles identified per microsatellite locus during screening. However, other factors such as, the number of SSR loci and repeat types, and the methodologies employed for the detection of polymorphic markers, have been reported to influence allelic differences.

Similarly, in their study of genetic purity and patterns of relationships among tropical highland adapted 36 quality protein and normal maize inbred lines (30 QPM and 6 non-QPM), Demissew *et al*., (2015) used 25 microsatellite markers. A summary of the 25 SSR markers used in the study is given in Table 3. There were two to four pairs of markers for each chromo-

Table 3. Summary of the 25 SSR markers used in the Demissew et al., (2015) study

Marker	Chromosome	Bin number	Repeat length	Repeat motif	Annealing temperature (°C)	Minor allele frequency	Number of alleles	Observed heterozygosity	PIC
nc130	5	5.0	3	AGC	54	0.056	3	0.000	0.404
nc133	2	2.1	5	GTGTC	54	0.143	3	0.000	0.454
phi029	3	3.0	4	AGCG	56	0.029	3	0.029	0.410
phi046	3	3.1	4	ACGC	60	0.028	3	0.000	0.412
phi056	1	1.0	3	CCG	56	0.121	4	0.030	0.633
phi065	9	9.0	5	CACTT	54	0.028	4	0.056	0.604
phi072	4	4.0	4	AAAC	56	0.014	4	0.056	0.401
phi075	6	6.0	2	CT	54	0.097	3	0.028	0.354
phi076	4	4.1	6	GAGCGC	60	0.029	6	0.143	0.663
phi079	4	4.1	5	AGATG	60	0.056	5	0.028	0.690
phi084	10	10.0	3	GAA	54	0.333	2	0.056	0.346
phi102228	3	3.1	4	AAGC	54	0.083	3	0.000	0.337
phi114	7	7.0	4	GCCT	60	0.061	4	0.000	0.524
phi123	6	6.1	4	AAAG	54	0.167	3	0.000	0.505
phi299852	6	6.1	3	AGC	58	0.028	7	0.028	0.735
phi308707	1	1.0	3	AGC	56	0.167	3	0.000	0.541
phi331888	5	5.0	3	AAG	58	0.028	4	0.028	0.512
phi374118	3	3.0	3	ACC	54	0.083	4	0.000	0.542
phi96100	2	2.1	4	ACCT	56	0.125	4	0.083	0.659
umc1161	8	8.1	6	GCTGGC	56	0.015	8	0.091	0.577
umc1304	8	8.0	4	TCGA	54	0.014	3	0.143	0.380
umc1367	10	10.0	3	CGA	62	0.028	4	0.000	0.303
umc1545	7	7.0	4	AAGA	54	0.029	5	0.000	0.423
umc1917	1	1.0	3	CTG	52	0.057	4	0.029	0.497
umc2250	2	2.0	3	ACG	58	0.500	2	1.000	0.375
Mean						0.093	3.92	0.073	0.491

some except chromosome 9 that had only a single marker. The number of alleles scored for each marker varied from 2 in *phi084* and *umc2250* to 8 in *umc1161*. The 25 markers amplified a total of 98 alleles, with an average of 3.9 alleles per marker. Minor allele frequency (MAF) was the lowest (0.014) in *umc1367* and *phi072* and the highest (0.500) in *umc2250*, and the overall average was 0.093. The polymorphism information content (PIC) ranged from 0.303 (*umc1367*) to 0.735 (*phi299852*), and the overall average was 0.491. The authors also described the importance of PIC in that it provides an estimate of how informative a particular marker is by considering both the number of alleles that are expressed and the relative frequencies of those alleles (Smith *et al*., 1997). For example, in the present study, PIC values ranged from 0.303 (less discriminative marker– *umc1367*) to 0.735 (highly discriminative marker– *phi299852*) with a mean of 0.491. According to Botstein *et al*., (1980) PIC guideline, 14 markers were reasonably informative (0.30< PIC <0.50) and the remaining 11 markers were highly informative (PIC > 0.50). It was noted that the relatively smaller PIC values in the study could be due to the presence of only a single di-nucleotide repeat SSR as opposed to more di-nucleotides used or lower genetic variability among the germplasm used for the study.

Comparison of two marker systems (SSRs and RAPDs) for determining the power of detection of polymorphism was also studied by Demissew *et al*., (2012). The study revealed that the RAPDs produced several polymorphic bands although the resolution power of the agarose gel electrophoresis was not good enough to allow the bands of both marker systems to be seen clearly. In this study, a total of 31 alleles were detected for the 25 polymorphic RAPD loci, at an average of 1.24 alleles per locus, which is also equivalent to 80.7% polymorphic loci. Thirty-seven out of 40 RAPD primers showed a monomorphic banding pattern, while three RAPD primers exhibited polymorphic bands. The results were consistent with the findings of Asif *et al*., (2006). However, the PIC value was greater for the SSR marker, suggesting better discriminating power of SSR markers over RAPDs that makes them ideal for use in fingerprinting of maize lines, as was reported by Smith *et al*., (1997) and Liu *et al*., (2003).

Association of phenotypic and genotypic data

Morpho-agronomic characters of crop plants have traditionally been used for germplasm identification. However, identification based on these characters is not efficient and reliable as they are highly affected by environmental factors. Despite the limitations, morphological traits are useful for preliminary evaluation because they are fast, simple, and can be used as a general approach for assessing genetic diversity among morphologically distinguishable accessions. Since the late 1980s, different electrophoretic (Zillman and Bushuk, 1979; Tkachuk and Mellish, 1980) and reversed-phase high performance liquid chromatography (RP-HPLC) (Marchylo *et al*., 1988; Scanlon *et al*., 1989) of seed storage proteins have been developed and are considered effective methods for cultivar identification. But the ability of the techniques to discriminate among cultivars is limited. On the other hand, DNA-based molecular markers are breeding tools, which are capable of providing high discrimination power (Perry, 2004). They are used in the identification of specific sequence variation between two or more genotypes and, in many cases, are more effective than biochemical assays (Lorz and Wenzel, 2008). Molecular markers are not influenced by environmental factors and are also fast, efficient, and more sensitive than field evaluation for the detection of large numbers of distinct differences between genotypes at the DNA level (Melchinger, 1999a).

Several DNA marker technologies have been developed and are available for studying genetic variability. The choice of the most appropriate marker system greatly depends on the species, the objective of the marker analysis, and the available resources (Lorz and Wenzel, 2008). PCR-based markers are widely preferred for genotype characterization in diverse crop species, including maize, as they are relatively simpler to use, non-distructible,

and require a smaller amount of DNA, thus permitting many reactions from a single sample (Powell *et al*., 1996; Soleimani *et al*., 2002). In addition, genetic distance estimates using molecular markers are reportedly helpful to identify the best parent combinations for new pedigree starts and to assign lines into heterotic groups (Melchinger *et al*., 1990; Benchimol *et al*., 2000; Reif *et al*., 2003a; Reif *et al*., 2003b; Bertan *et al*., 2007; Flint-Garcia *et al*., 2009; Lu *et al*., 2009; Demissew *et al*., 2015).

A comparative study of molecular and morphological methods for describing genetic relationships in traditional Ethiopian highland maize was conducted using a total of 15 morphological traits, eight AFLP primer combinations, and 20 simple sequence repeat (SSR) loci by Yoseph *et al*., (2005) to: (i) study the morphological and genetic diversity among 62 selected highland maize accessions, and (ii) assess the level of correlation between phenotypic and genetic distances. Summary of results from the study exhibited that the mean morphological dissimilarity (0.3 with a range of 0.1-0.68) was low in comparison to mean dissimilarity calculated using SSR markers (0.49 with a range 0.27-0.63) and AFLP markers (0.57 with a range 0.32- 0.69). Mantel's, (1967) test of correlation between the morphological dissimilarity matrix and the matrices of genetic dissimilarity based on SSR and AFLP markers was 0.43 and 0.39, respectively (p = 0.001). Whereas the correlation between SSRs and AFLPs dissimilarity matrices was 0.67 (p = 0.001). Therefore, the authors concluded that the correlation between SSR and morphological data analysis was higher than between AFLP and morphological data analysis, indicating that SSR markers may be a better choice for marker-trait association genetic studies in open pollinated maize accessions than AFLP. Moreover, Ethiopian highland maize accessions appear to be environmentally more stable, as observed by the good agreement between phenotypic and molecular distances suggesting that the observed phenotypic variation was at least partly caused by genetic factors. The correlation between the two molecular markers was also higher than the correlation with morphological traits depicting that when compared with DNA fingerprinting techniques, morphological traits are relatively less reliable and efficient for precise discrimination of closely related accessions and analysis of their genetic relationships.

Another similar work on the phenotypic characterization of elite QPM inbred lines adapted to tropical highlands and the association studies using SSR markers was reported by Demissew *et al*., (2018). The objectives of the study were to characterize newly developed QPM inbred lines adapted to tropical highlands using phenotypic traits and to determine the association with SSR markers. Accordingly, thirty-six maize inbred lines (30 QPM and six non-QPM) adapted to tropical highlands of Ethiopia were evaluated using 18 phenotypic traits and 25 selected SSR markers. The results of the study showed that significant phenotypic variations were observed among inbred lines for all measured traits from both phenotypic and molecular marker analyses. Dendrograms constructed using the phenotypic traits and the SSR markers classified the test inbred lines into four genetic groups for systematic selection (Fig. 1). The findings of the study further revealed that although the diversity analysis based on phenotypic or molecular markers resulted in a similar number of distinct groups and a similar concentration of genotypes in each group, the correlation between the two markers system was low. According to Demissew *et al*., (2018), the suggested reasons for the lack of significant association between the phenotypic and SSR data could, in part, be attributed to the relatively small number of SSRs used in this study, and the molecular markers did not adequately sample the genomic regions that were responsible for the phenotypic variation among the inbred lines (Alves *et al*., 2013). The authors further added that several factors such as the distribution of markers in the genome, the number of markers used, and the nature of the evolutionary mechanism underlying the variation measured can affect the genetic distance estimates (Powell *et al*., 1996).

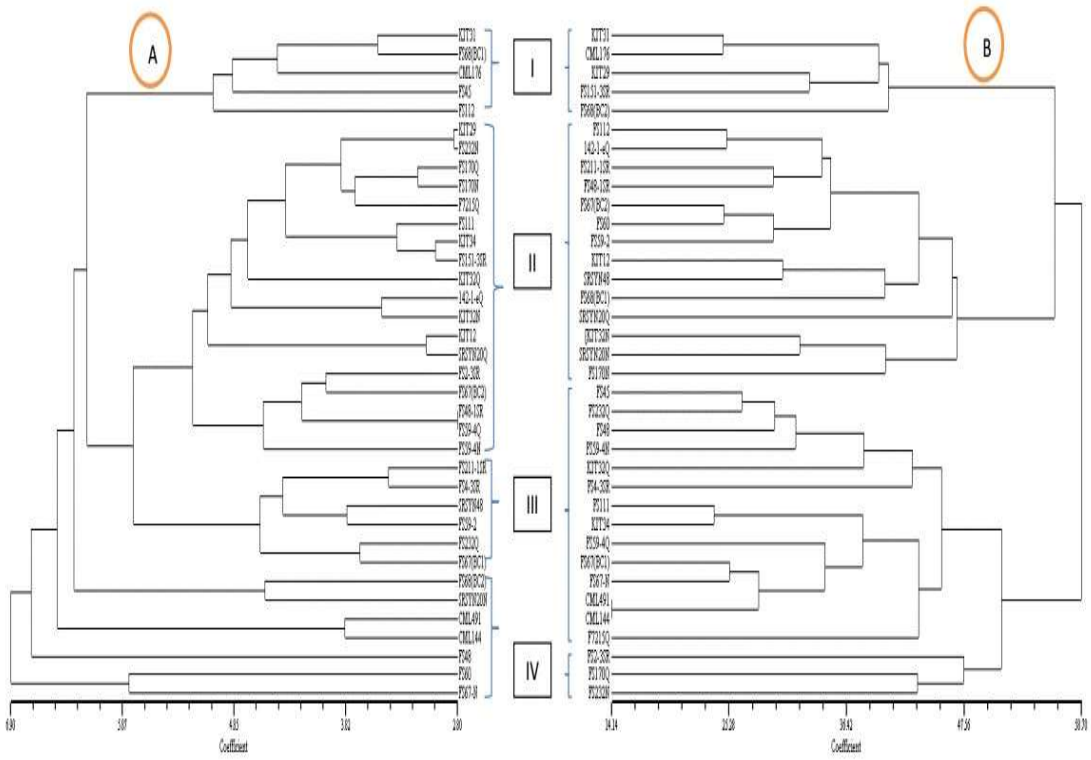

Fig 1. Dendrogram of 36 (30 QPM and six non-QPM) maize inbred lines constructed using UPGMA cluster analysis based on Euclidean genetic distances of phenotypic data combined across two locations (A) and SSR markers (B). Source: Demissew et al., 2018.

Population structure and heterotic grouping

Estimates of genetic distances are indicators for the presence or absence of relationships among genotypes. The estimates can be made using different types of molecular markers, including restriction fragment length polymorphism (RFLP), AFLP, SSR, and SNPs). As heterotic group assignment is made based on combining ability from combining ability experiments, several authors suggested the use of molecular markers in heterotic grouping (Melchinger *et al.*, 1990; Benchimol et al., 2000; Reif *et al*., 2003a; Reif *et al*., 2003b; Yoseph *et al*., 2005; Flint-Garcia *et al*., 2009; Lu *et al*., 2009; Demissew *et al*., 2018).

For example, Yoseph *et al*., (2005) conducted a comparative study of molecular and morphological methods of describing genetic relationships in Ethiopian highland maize accessions. They analysed a representative sample of 62 Ethiopian highland maize accessions using a total of 15 morphological traits, eight AFLP primer combinations and 20 simple sequence repeat (SSR) loci to classify the accessions into groups based on molecular profiles and morphological traits. The study allowed the identification of three groups of maize accessions with distinctive genetic profiles and morphological traits. The first group constitutes the early maturing, short-statured accessions (cluster I), which were collected from the northern agroecology from which they probably acquired earliness. The second group includes the tall, high yielding varieties (cluster II), which are currently the most important landraces grown in the southern and western parts of Ethiopia. The third group includes tall, late maturing and low yielding accessions (cluster III), which are being cultivated in some parts of the northern,

western and southern highlands of Ethiopia. The authors suggested that accessions from the northern agro-ecology may be used as base materials for the development of improved varieties for the drier parts in the highlands of Ethiopia, as the accessions were able to grow and produce seed under very harsh environmental conditions (drought, poor soils, excessive radiation, etc) and had adaptation traits (e.g., short flowering, short ear, and plant height narrow leaf), while accessions from the western and southern agro-ecologies can be used for the development of high yielding varieties suitable for high potential maize growing regions of Ethiopia.

The study by Demissew *et al.*, (2015) also investigated the extent of genetic differentiation, population structure, and patterns of relationship among 36 maize inbred lines developed from CIMMYT source germplasm with 25 SSRs using model-based population structure analysis, neighbour-joining cluster analysis, and principal coordinate analysis. All these different multivariate methods revealed the presence of two to three primary cluster groups, which was in general agreement with pedigree information and partly with the putative heterotic groups. The model-based population structure analysis in the same study assigned about half of the inbred lines into their putative heterotic group defined by breeders. There were 17, 14 and 5 inbred lines in cluster groups I, II and III, respectively. Cluster Group I was dominated by six lines from Ecuador heterotic group, four from Kitale group, two from Pool 9A group, and three from previously uncategorized lines. Cluster Group II was dominated by five lines extracted from Kitale heterotic group, four from Ecuador, four Pool9A, and one previously uncategorized line. In cluster Group III, two previously uncategorized lines, one from Kitale and one from Pool9A were all included in this group. However, the authors further explained that genotypes having the same name may be grouped differently in other studies at times. Such incongruities in assigning inbred lines into heterotic groups may occur due to seed handling or pollination errors (Rajab et al., 2006). It may also be caused by differential selection of the different lines in different environments, genetic drift, and mutation (Senior *et al.*, 1998). Legesse *et al.*, (2006), in their study entitled 'Genetic diversity of maize inbred lines revealed by SSR markers', managed to group 56 highland and mid-altitude adapted tropical maize inbred lines derived from local sources and CIMMYT origin using 27 SSR loci. Accordingly, cluster analysis using average linkage method (UPGMA) suggested five groups among the inbred lines. Most of the inbred lines adapted to the highlands and the mid-altitudes were positioned in different clusters with a few discrepancies. The pattern of groupings of the inbred lines was mostly consistent with available pedigree information.

Dagne *et al.*, (2019) analysed high-density genotyping by sequencing data from 298 African highland maize inbred lines, assessing genetic purity, relatedness, and population structure using 955,690 SNPs from Cornell University. The study selected 237,018 SNPs with a minor allele frequency (MAF) of ≥0.05 and a maximum missing data of 20%. The results showed that the log probability of the data (LnP(D)) and ad hoc statistics ΔK obtained from the model-based population structure analysis suggested that the 298 lines could be divided into two or three possible groups or sub-populations. However, when the results at various K values were compared with their pedigree information and breeding history, the groups obtained at K=3 were considered as the best possible number of groups. The proportions of inbred lines assigned to Group-1, Group-2, and Group-3 were 64%, 23%, and 12%, respectively, with only two lines belonging to a mixed group (Fig 2). According to the model-based structure, the neighbour-joining (NJ) tree constructed from the genetic distance matrix grouped 296 of the 298 inbred lines into three major groups and five sub-groups (Fig 3).

Another study by Berhanu *et al.*, (2017) on genetic variation and population structure of 265 maize inbred lines adapted to the mid-altitude sub-humid maize agro-ecology of Ethiopia used 220,878 SNP markers obtained through GBS. In this study, the population structure of the inbred lines was assessed using

Principal Component Analysis (PCA), discriminant analysis of principal components (DAPC), and the model-based structure. All the three methods revealed the presence of three distinct groups, with 94% agreement on group membership predicted by the different methods. Using DAPC, the first group was composed of 175 quality protein maize (QPM) and non-QPM inbred lines that were mainly extracted from broad-based pools and populations, such as PooL9A for non-QPM lines and Pop 62 and Pop 63 for QPM inbred lines. The authors finally concluded their work by suggesting the incorporation of high-density molecular marker information in future heterotic group assignments.

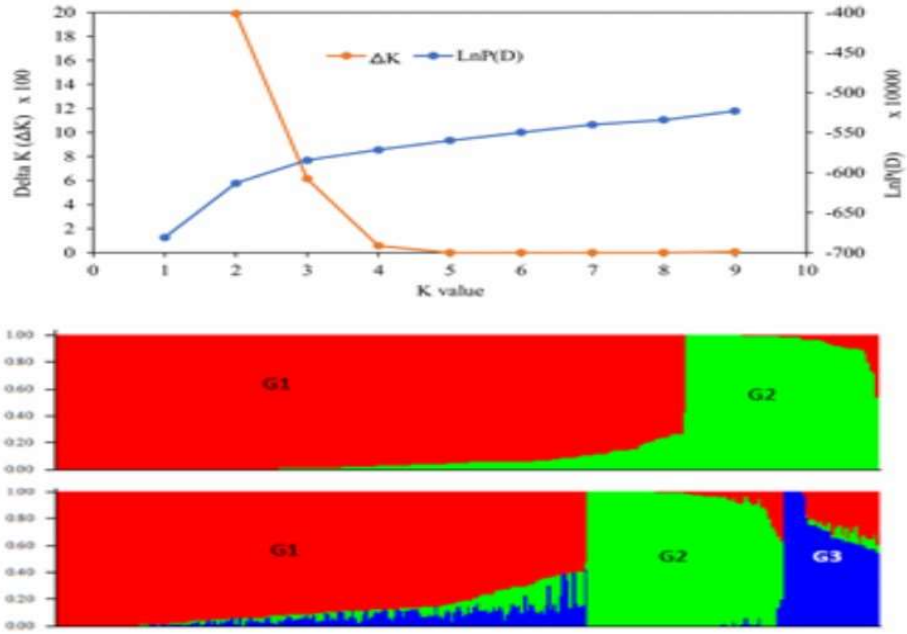

Fig 2. Population structure of 298 maize inbred lines based on 22,500 SNPs in Dataset-3: (a) plot of LnP(D) and a ΔK calculated for K ranging from 1 to 10, with each K repeated thrice; (b) population structure of the 298 inbred lines at K = 2 and K = 3. Every line is represented by a single vertical line that is partitioned into K colored segments on the x-axis, with lengths proportional to the estimated probability membership (y-axis) to each of the K inferred clusters. Source: Dagne et al. (2019).

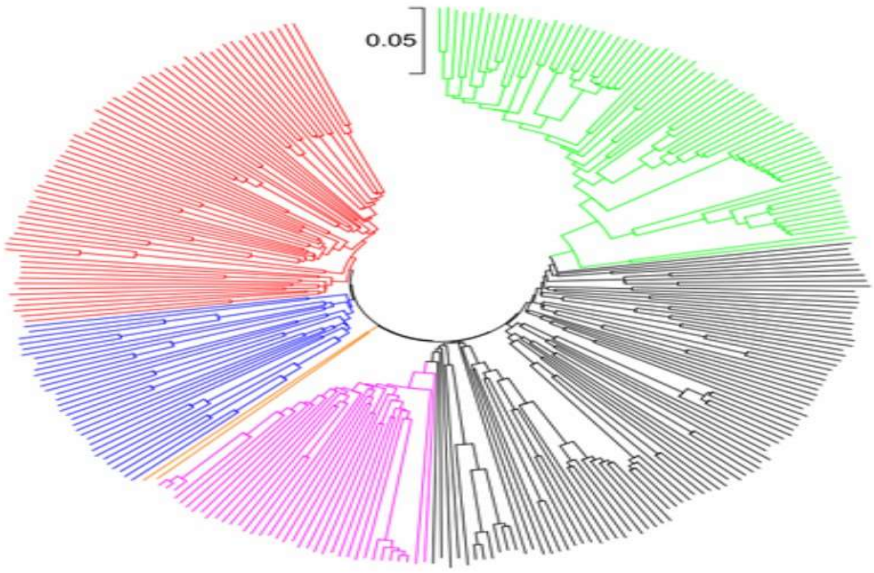

Fig 3. Neighbour-joining tree of 298 inbred lines based on identity-by-state genetic distance matrix computed from 235,019 SNPs, each with minor allele frequency >0.05. Line colors are as follows: Group-1A (black); Group-1B (red), Group-1C (blue), Group-2 (green), Group-3 (pink) and ungrouped (orange). Group-1, Group-2, and Group-3 were obtained based on the model-based STRUCTURE. Source: Dagne *et al*. (2019)

Genetic purity and quality control

Marker-based quality control (QC) is essential for ensuring purity and true-to-type maize genetic material within maize breeding programs. Mainstreaming QC using DNA markers provides breeders with rapid and cost-effective tests of the homozygosity of inbred lines, the homogeneity of populations, and the fidelity of crosses (Melaku and Abebe, 2019). Attempts to utilize markers for QC have been initiated in the era of low-throughput SSR marker assays (Semagn et al., 2012a). The rapidly declining cost of SNP based genotyping has opened up an opportunity for the routine use of SNP markers for quality control (QC) analysis, which is an important component in maize breeding and seed systems (Kassa et al., 2012a). Demissew et al., (2015) conducted a study to: i) understand the genetic purity existing in the maize inbred lines, ii) determine the effect of conversion of normal maize lines to QPM, and iii) patterns of relationships among 36 white maize inbred lines (30 QPM and 6 non-QPM) using 25 SSR markers. The study revealed a heterozygosity range of 4 to 16.7 % in the inbred lines with an average 7.9 %. More than half of the tested inbred lines had higher than the expected (6.25 %) mean residual heterozygosity for inbred lines developed after four generations of selfing.

Genotyping by next-generation sequencing (GBS) is an emerging method of SNP genotyping, which is being increasingly adopted for discovery applications, but exploration of its suitability for QC analysis has been limited (Brehanu et al., 2015). The same authors evaluated the magnitude of genetic purity and identity among two to nine seed sources of 16 inbred lines (including parental lines of eight popular Ethiopian hybrids (BH140, BH540, BHQP542, BH543, BHQPY545, BH660, BH670, and BH661) and different sources collected from the maize breeding program of the EIAR, seed companies, the Ethiopian Institute of Bio-diversity Conservation (IBC), and CIMMYT (Berhanu *et al*., 2015). The study used 191 Kompetitive Allele Specific PCR (KASP) and 257,268 GBS markers, compared the correlation between the KASP-based low and

the GBS-based high marker density on QC analysis. The authors revealed that the genetic purity and identity among two to nine seed sources of 16 inbred lines using 191 KASP and 257, 268 GBS markers varied from 49 to 100% for KASP and from 74 to 100 % for GBS. Almost all the inbred lines obtained from CIMMYT showed 98 to 100 % homogeneity irrespective of the marker type. In contrast, only 16 and 21 % of the samples obtained from EIAR and partners showed ≥95 % purity for KASP and GBS, respectively. The genetic distance among multiple sources of the same line designation varied from 0.000 to 0.295 for KASP and from 0.004 to 0.230 for GBS. The correlation between the 191 KASP and 257,268 GBS markers was 0.88 for purity and 0.93 for identity. A reduction in the number of GBS markers to 1, 343 decreased the correlation coefficient only by 0.03. Their results revealed high discrepancy both in genetic purity and identity by the origin of the seed sources irrespective of the type of genotyping platform and number of markers used for analyses. The conclusion from both methods was basically similar, which clearly suggested that smaller subsets of preselected and high-quality markers are sufficient for QC analysis that can easily be done using low marker density genotyping platforms, such as KASP.

Genome-Wide Association Study (GWAS)

Genome-wide association study is becoming a powerful tool to address interspecies relationships based on genotype by sequencing and phenotype data association study (Huang and Han, 2014). Genotyping by sequencing (GBS) is a next-generation sequencing (NGS) based genotyping approach that has dramatically facilitated large-scale genome-wide marker development and GWAS in crop species (Varshney et al., 2014). Several loci associated with agronomic traits such as plant height, yield and yield components, flowering time and plant architecture in a range of crops, including maize (Wang et al., 2012). Girum et al., (2013) also identified functional DNA markers such as crtRB1-5′TE and crtRB1-3′TE associated with provitamin A content across the tropical maize inbred lines. Berhanu et al., (2020) studied the nitrogen use efficiency in tropical adapted maize germplasm under optimum and low-nitrogen stress environments using GWAS and genetic prediction. Their study helped to identify most QTLs conferring tolerance to nitrogen stress were on a different chromosome position under optimum conditions. Such types of studies indicate the importance and wider application of GWAS in maize. However, application of GWAS and genetic prediction on complex traits in Ethiopia is limited. Combining GWAS and GS (genomic selection) with marker-assisted selection (MAS) accelerates maize breeding to develop improved cultivars with better performance for grain yield and other complex traits under diverse management conditions.

Genetic transformation attempts in maize

Over the last few decades, considerable research progress in plant biotechnology has allowed the development and formation of genetically modified maize varieties that have shown a significant yield improvement worldwide. In 2019, research reports indicated that 30% of the maize growing areas were covered by genetically modified maize varieties (ISAAA database, 2022), which contained transgenes associated with biotech traits such as herbicide, insect, disease resistance, abiotic stress tolerance, yield, improved nutritional quality, and were traits expected to be introduced into the market soon (Simmons et al., 2021; ISAAA database, 2022). The summary of research progress in maize genetic transformation protocols, applications, status, and regulatory issues in Ethiopia are briefly discussed below.

In Ethiopia, before starting genetic transformation techniques to develop and adopt any genetically modified organisms (GMOs), approval and written consent must be issued from the Ethiopian Environmental Protection Authority (EPA), according to the Biosafety Proclamation No. 896/2015, which is ratified by the Ethiopian House of Peoples Representatives. Legal permission and opinions could be granted by the EPA based on data provided by the applicant, inspection of

laboratories, and field trial sites. Additionally, approval should also be admitted from the Ethiopian National Biosafety Advisory Committee (NBAC) following the Council of Ministers of FDRE under Council of Ministers Regulation No. 411/2017.

Bedada et al., (2016) conducted a genetic transformation study on locally adapted African tropical maize genotypes by transferring the isopentenyl transferase gene to develop drought-tolerant tropical maize. The transferred (IPT) gene codes for the isopentenyl transferase enzyme, which catalyzes the rate-limiting step in the biosynthesis of cytokinin and has the function of delaying drought-induced leaf senescence. This study has the objective to investigate if the IPT gene can be useful in enhancing drought tolerance in locally adapted African tropical maize genotypes. The tropical maize inbred line CML216 was transformed with the IPT gene using the Agrobacterium-mediated transformation method. The study revealed that five transgenic lines were stably transformed through Southern blot analysis with copy numbers of two to four per event. Also, the drought assay carried out in the glasshouse, showed transgenic lines expressing the IPT gene are tolerant to drought as revealed by delayed leaf senescence compared to the wild-type plants. In addition, the study indicated that transgenic plants maintained higher relative water content and total chlorophyll during the drought period and produced significantly higher mean grain yield of 44.3 g/plant than the wild type (1.43 g/plant). This study suggested the transgenic lines developed need to be further tested for tolerance to drought under contained field trials to be used in maize breeding programs.

In another maize transformation study, Bedada et al., (2018) were able to evaluate the genetic transformability of regenerable tropical maize genotypes using the Agrobacterium-mediated transformation method and identify genotype(s), which can be used as better transgene recipients for future research. In this study, Agrobacterium strain EHA 101 was used to infect immature zygotic embryos using the phosphomannose-isomerase gene as a selectable marker. The transgenic plants were analyzed using PCR, Southern blot, and semi-quantitative RT-PCR and the result revealed the presence, stable integration, and expression of the transgene. Also, in this study, the author showed the genotype-dependent response of African tropical maize to Agrobacterium-mediated genetic transformation. Among the tested six maize genotypes, the CML216 (CIMMYT inbred line) and Melkassa-2 (Ethiopian open-pollinated variety) produced normal and fertile transgenic plants and were suggested for future use in genetic transformation research.

Conclusion and recommendations

In spite of all the efforts and progress made in the development and dissemination of maize technologies for different agro-ecologies in Ethiopia, the biotic and abiotic constraints remained the major limiting factors for maize production and productivity. The use of molecular tools in the Ethiopian maize breeding programs at small-scale has so far contributed to the enhancement of the breeding selection processes to some extent. Particularly, the relationships between molecular markers and phenotypic traits could be a significant diagnostic tool in marker assisted maize selection/breeding. The efficiency of the markers in different genetic backgrounds as well as their usefulness in breeding programs for the development of inbred lines and hybrid maize cultivars with different features need to be further demonstrated for wide applications of marker-assisted breeding techniques to enhance the breeding efficiency of maize improvement in Ethiopia.

Some recommended applicable areas, but not limited to, where molecular tools such as marker assisted breeding (MAB) are useful to maize breeding in Ethiopia can also be mentioned as future research directions. For example, several desirable traits in maize start to express only when the crop has reached flowering or get matured. But understanding a plant's genetic make-up before flowering or at seedling stage using MAB could be useful to make crossing plans between selected parents faster than the conventional approach. The other important area of application is that

environmental variations in the field reduce a trait's heritability, especially the low heritable traits, because complications in phenotypic selections of the traits are compounded by environmental variation, experimental error, or genotype x environment interaction, whereby MAB could be an effective method to make progress in phenotypic selections under various stresses and environmental conditions. Besides, there can be some desirable agronomic or quality traits in maize that may be governed by recessive genes and are of interest for use through conventional breeding. In conventional backcross breeding, plants with recessive genes are identified by progeny testing after inbreeding or testcrossing to a recessive tester. However, this process can be done within a short period of time by applying MAB to detect recessive genes linked to specific markers. Genetic purity and quality assurance tests using molecular techniques for commercial cultivars have been an untapped potential in seed business in Ethiopia, and hence need to be widely adopted.

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