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**Abstract:** Maize is one of the most important staple crops in the highlands of the Andean region of Ecuador, Peru, and Bolivia. Most seeds come from landraces, with their own kernel characteristics. The kernels are used for the elaboration of traditional dishes and other elaborates for human consumption. In this region, maize breeding is conducted mainly by public institutions. In this review, we outline the methodology that has been used by the maize breeding programs (MBPs) of the National Institutes for Agricultural Research and other institutions in the highlands of Ecuador, Peru, and Bolivia during the last 20 years. The main objective of MBPs in the region has been to develop more uniform and productive open-pollinated varieties (OPVs) of floury maize (*Zea mays* L. var. Amylacea), which is the most important type of maize in the area. Participatory plant breeding, combined with half-sib, has been used to breed new maize varieties. At least 18 OPVs of floury maize has been very important to conserve diversity and promote consumption in the region, but they have had very little impact on yield. The yield of floury maize is around three times below that of dent or semident maize grown in the region. Therefore, there is a need to apply new breeding techniques in the region to accelerate the development of more productive floury-maize cultivars.

Keywords: floury maize; yield; seed

# 1. Introduction

In the highlands of Ecuador, Peru, and Bolivia, between 1700 and 3500 m above sea level (masl), there are numerous densely inhabited valleys where the cultivation of floury maize (*Zea mays* L. var. Amylacea) is one of the main sources of calories in the diet of the rural population since almost all production is dedicated to human consumption [1–3]. Most of the farmers of these regions are poor owners of small farms (less than 0.5 ha) with very steep slopes and eroded soils [4]. In addition, in southern Peru and Bolivia, maize is commonly affected by drought and cold that occur in areas above 3000 masl [1,5,6], and hail and frost prematurely can end or reduce maize production [7].

Floury corn is mainly intended for human consumption in the form of fresh corn (choclo), dry toasted kernels (tostado, cancha), soups (chuchuca), drinks (chicha, colada, maicena), breads, and other traditional preparations [8]. Additionally, the cane and leaves are used as fodder for guinea pigs, sheep, and cattle. The dry grain is also used to prepare a concentrate for raising poultry, pigs, and guinea pigs. The grain is a source of energy and amino acids due to its starch, protein, and oil content. It also contains nine minerals (K, Fe, Zn, Ca, P, Si, Cu and Mg), nine vitamins (B4, B6, C, E, B2, B3, B1, B9, and A), and phenolic compounds and anthocyanin, in the case of black, red, and blue corn [9,10].

In Ecuador, Peru, and Bolivia, floury maize is grown only in the highlands at around 418,000 ha, which corresponds to approximately 30% of the average maize production of those countries [11–13]. In Ecuador, the area harvested with floury maize was 67,620 ha



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**Copyright:** © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). in 2019, with a yield of 1.36 tha<sup>-1</sup> [11]. In the north of this country (provinces of Carchi, Imbabura, Pichincha, and Cotopaxi), traditional races called chauchos, huandangos, and mishcas are generally sown (soft yellow kernels). In the central zone (provinces of Chimborazo, Tungurahua, and Bolívar), floury white races of maize are preferably cultivated, such as blanco blandito, chazo, and guagal. In the south (provinces of Cañar and Azuay), the type of maize called zhima (white semident kernel) belonging to the cusco ecuatoriano race, is mostly grown [14].

Maize is the most important short-term crop in Peru. Around 280,000 ha are cultivated in the High Andean region, the vast majority corresponding to floury maize [12]. Although floury maize is grown throughout the highlands, the regions with the highest production are Cusco, Apurimac, Cajamarca, Ayacucho, and Huancavelica [15]. The national average floury maize yield is 1.29 tha<sup>-1</sup>, with a maximum yield in the region of Cusco of 2.23 tha<sup>-1</sup> and the lowest yield in Cajamarca with 0.78 tha<sup>-1</sup> [16].

Floury maize is one of the most important crops for the Bolivian food and economy. It is estimated that, in the Andean valleys of Bolivia (regions of La Paz, Cochabamba, Oruro, and Potosí), around 115,000 ha are sown, which represents around 20% of the area sown with maize in the country. The average floury maize yield in Bolivia is 1.2 tha<sup>-1</sup> [13]. In the high valleys, there is a large number of traditional varieties due to the diverse uses for human consumption. The most important native varieties in this region are karapampa, kellu, uchupilla, hualtaco, huillcaparu, pasakalla, chuspillo checchi, and kulli chunkula [17].

Formal maize breeding in the Andean region began in the 1960s, with the establishment of the Public Research Institutes (INIA). In the 1980s, the International Maize and Wheat Improvement Center (CIMMYT), located in Quito (2850 masl), generated eight Andean pools that were populations with a broad genetic base: Pool 1 (early floury white), Pool 2 (late floury white), Pool 3 (early floury yellow), Pool 4 (late floury yellow), Pool 5 (early dent white), Pool 6 (early dent yellow), Pool 7 (late dent white) and Pool 8 (late dent/flint yellow), which subsequently resulted in the release of several open-pollinated varieties (OPVs). Breeding was based mainly on recombination (diallelic crosses), mass selection (SM), half-sib, performance tests, regional adaptation trials, and Andean regional trials [1].

This review describes the main methods used by the maize breeding programs (MBP) to conserve and improve native Andean races in the last 20 years, preserving the tradition and nutritional value of floury corn. It also describes the varieties released in this period and analyzes the future of the genetic improvement of this type of maize. This information is useful to plant breeders in the Andean region and other parts of the world, where social aspects of farmers and breeding strategies used by the MBP are similar.

### 2. Genetic Diversity of Maize in the Region

The geographical, environmental, and social characteristics of the highlands of Ecuador, Peru, and Bolivia created conditions for the development of many races of maize. These traditional races, which have had various names such as creole breeds, farmer varieties, landraces, or local varieties, have been improved by farmers for a long time, and most of them have not been subjected to selection and improvement methods by breeders (Figure S1).

In Latin America, 252 races of maize were described, 132 of which are from the Andean region [18–20]. In Ecuador, 29 races of maize were described, and 17 were found in the highlands, 6 of which are not well defined [14]. In Peru, there are 52 races of maize, and 32 are grown in the highlands; 40 races were described in Bolivia, and 31 are cultivated in the highlands [21].

Andean maize has great variability in grain color, texture, composition, and appearance, and represents an invaluable source of genes for resistance to abiotic stress that is not yet exploited [22]. Maize landraces are a good source to convert native varieties into improved varieties without affecting crop diversity because they have already adapted to

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the particular conditions of the region, they have the characteristics of commercial value, and the population already knows them and has a habit of consuming them [23].

# 3. Breeding Methods Used in the Region

Maize breeding has been very important in the Andean countries to conserve diversity and promote consumption, especially in Bolivia, Ecuador, and Peru, but they have had very little impact on yield (at the national level). One of the reasons for this is that selection has been oriented toward maintaining grain quality for human consumption (texture and color) and introducing foreign germplasms to improve the nutritional quality of the grain (QPM) and disease resistance [24,25]. Another reason is the lack of a certified seed system that distributes seeds of improved OPVs to farmers [26,27].

In general, Andean germplasm is adapted to specific environments; each time this germplasm was crossed with a foreign variety, it lost some of its characteristics, and recovery with backcrosses took many generations to fully recuperate the Andean phenotype, which is absolutely necessary to achieve adoption of the varieties [28].

The development, formation, and maintenance of OPV has been very similar in the three Andean countries due to CIMMYT's influence in training scientists in the region. The most frequently used selection methods in the region are half-sib and MS [29]. Briefly, the breeding process begins by identifying between 8 to 10 of the best accessions or families on the basis of their behavior in trials in several locations and performing diallelic crosses to form the initial OPV (Cycle 0). The F1 seed must be advanced to F2 by manual pollination. Then, 100 to 200 manually fertilized ears are selected, and an equal number of seeds from each ear is mixed to produce the F2 seed. Then, ear families are evaluated and selected on a recurring basis through half-siblings, and after 4 to 6 cycles, evaluations are carried out in experiment stations and in the farmers' fields. Validation tests are carried out before the official release of the new variety [30].

The genetic gain of the OPV obtained by MS and half-sib was evaluated in Peru and Ecuador. In Peru, seven populations were improved by MS and eight through half-sibs. The selection gain for yield using MS, estimated by linear regression, was 3.5% per cycle (year) on average; for the half-sib method, it was 4.6% [31]. In Ecuador, the genetic gain for yield in two Andean pools using half-sib was 1.4% per cycle (year) on average [32]. These pools are the origin of two OPVs sown in the highlands of Ecuador for more than 30 years, with resistance to *Exherohilum turcicum, Cercospora maydis, Puccinia* spp., *Fusarium moniliforme,* and *Maize rayado fino virus* [33–35].

The use of a racial compound (RC) as an alternative population-breeding method was proposed in Peru. This method consists of the development of a cluster of all the native varieties of a race in a region, and letting them naturally recombine before MS. The RC tends to produce more than the average of the varieties that form it since it has more useful variability of adaptive characters, and it could be easily accessible to farmers [36].

The development of hybrids using floury maize germplasm has not prospered in the region. This is due to the high degree of inbreeding that is generated during self-pollination, causing the degeneration and the null formation of grains in the ears. However, trials carried out in Peru showed that it is possible to generate parental lines from populations that were improved by a recurrent selection method [37].

# 4. Breeding for Resistance to Ear Root

Ear rot caused by *Fusarium moniliforme* and other species is one of the main limitations for the production of floury corn in the region, reaching losses of up to 40% of the yield [12,27]. The work on the regional level to incorporate genetic resistance to diseases in the highlands began with the Durable Resistance Project for the Andean Zone (PREDUZA) in 1998. The five-year project was based on crossing locally adapted cultivars with resistant genotypes, followed by participatory selection with farmers [38].

With PREDUZA, 41 trials were carried out in Ecuador, Bolivia, and Peru aiming to reduce the incidence of ear rot. The project was oriented toward identifying the components

of durable resistance; for this purpose, in the first two years of the project, work was carried out on methods, times, and concentrations of inoculation with *Fusarium verticillioides*. Simultaneously with these works, genetic improvement activities were carried out in order to obtain varieties with good resistance levels, which were exchanged for evaluation among the countries. Likewise, in each country, extension work began, including field days, guided visits, and participatory research events [39]. These works allowed for the identification of Bolivian maize varieties Aychazara 102 and ODA with high levels of resistance to ear rot, reducing the severity of this disease by 55% in Ecuador [40]. Aychazara 102 was later released in the highlands of Ecuador as INIAP 103 [41].

In the highlands of Peru, work continued to incorporate genetic resistance to ear rot in native floury maize through crosses between Peruvian Complex IV (resistant) and INIA-607 (susceptible), and after six generations, resistance to ear root was increased by 16% [12].

#### 5. Open Pollinated Maize Varieties Generated in the Andean Region

# 5.1. Ecuador

Since 1961, the MBP of the National Institute for Agricultural Research (INIAP), based at the Santa Catalina Experiment Station, has dedicated its efforts to the improvement of native varieties. Since then, the objective of the MBP has been to develop OPVs that satisfy the needs and production systems of farmers in the highlands, on the basis of the local genetic diversity of maize. During the selection process, farmers have actively participated in agronomic evaluations. The selection method used by the MBP was half-sib, following CIMMYT procedures [30]. Since 2000, the MBP has released varieties INIAP-102 Blanco Blandito Mejorado, INIAP-124 Mischa Mejorado, INIAP-103 Misqui Sara, and INIAP-199 Racimo de uva (Table 1).

## 5.2. Peru

The development of maize OPVs began with the Collaborative Program for Maize Research (PCIM), founded in 1953 and based at the La Molina University, with partial funding from the Ministry of Agriculture of Peru. From the beginning, the program was oriented toward genetic improvement for the conservation of the racial diversity of maize, ex situ and in situ. Many local varieties were improved, with selection gains between 5 and 10% per cycle [23].

Later, the MBP of the National Institute of Agrarian Innovation (INIA), founded in 1978, used PCIM germplasm to form the following Peruvian complexes (CP): CP I: Choclero Precoz, CP II: Late Choclero; CP III: Canchero Precoz, CP IV: Canchero Tardío, CP V: Morocho Precoz, and CP VI: Morocho Tardío. At least 28 improved varieties emerged from these genetic pools [15]. In the last 20 years, INIA has released nine floury OPVs on the basis of the selection of native races, mainly through the use of the half-sib breeding method (Table 1).

# 5.3. Bolivia

There are several institutions in charge of the development of maize varieties in Bolivia, including the Pairumani Phytoecogenetic Research Center (CIFP), the National Institute for Agricultural and Forestry Innovation (INIAF), the International Center for Tropical Agriculture (CIAT), and the Institute for Agricultural Research El Vallecito (IIAEV). Additionally, all these institutions have strong involvement in the management and conservation of plant genetic resources. The institutions have given greater importance to the use of the local germplasm for their breeding programs. Between 10 and 60% of native germplasms were used by the MBP. The second source of use has been introductions of improved germplasms through bilateral or multilateral agreements. Crossing exotic and native maize germplasms, followed by MS, is the main breeding method used by the MBP in Bolivia [42].

Country	Year of Release or Registration	Name	Color *	Breeding Method	Range of Altitude (masl)	Reference
Ecuador	2000	INIAP-102 "Blanco blandito mejorado"	White	Half-Sib	2200–2800	[43]
	2002	INÍAP-124 "Mishca mejorado"	Yellow	Half-Sib	2200–2900	[44]
	2013	INIAP-103 "Mishqui Sara"	White	Half-Sib	1700–2650	[41]
	2017	INIAP-199 "Racimo de Uva"	Black	Half-Sib	2400-3000	[45]
Perú	2004	INIA 603 Choclero	White	Half-Sib Full-Sib	2600–3000	[46]
	2004	INIA 606 Choclero Prolífico	White	Half-Sib	2200–2900	[47]
	2005	INIA 607 Ch'ecche Andenes	Mottled gray and purple	Half-Sib	2900–3500	[48]
	2007	INIA 615 Negro Canaán	Black	Half-Sib	2000–3000	[49]
	2012	INIA 618 Blanco Quispicanchi	White	Half-Sib	3100-3350	[50]
	2013	INIA 620 Wari	White	Half-Sib	2800-3400	[51]
	2013	INIA 621 Pillpe	Mottled gray and white	Half-Sib	2800-3400	[52]
	2014	INIA 601 Maíz Morado	Black	Half-Sib	2600–2900	[53]
	2020	INIA 623 Cumbemaino	Yellow	Half-Sib	2500-3000	[54]
Bolivia	2009	Pairumani Choclero 2	White	Mass Selection	2000-2800	[55,56]
	2009	Pairumani Choclero 3	White	Mass Selection	1800-2600	[56,57]
		Aychasara 102	White	Mass Selection	1800-2800	[24,58]
		Áychasara 7	Black	Mass Selection	2600-2800	[24,58]
	2019	Morado Criollo	Black	Mass Selection	2600-2800	[58]

Table 1. Floury open pollinated maize varieties generated in Ecuador, Peru and Bolivia in the last 20 years.

\* Figure S2.

CIFP has produced the largest number of varieties of floury maize in the highlands of Bolivia, generating varieties with kernels that look the same as those of native or traditional varieties, but with better nutritional quality (QPM), higher adaptability to various environments, and better performance. The opaque-2 gene donor was developed by CIMMYT (Tuxpeño Opaque-2). The crosses were followed by several cycles of MS [24]. Although several institutions are breeding maize in Bolivia, there is little available information about floury maize varieties. CIFP released four floury maize OPV in the last 20 years: Aychazara 7, Aychazara 102, Pairumani Choclero 2, and Pairumani Cholcero 3 (Table 1).

The MBP of INIAF has bred using native and improved maize germplasms, with the aim of improving quantitative and qualitative characteristics but maintaining local characteristics, such as earliness, disease tolerance, and organoleptic quality [28].

# 6. Breeding Prospective

Despite the fact that several institutions have been working with floury maize in the last 20 years, there is still little recognition of the role of native maize in the daily diet of people. It is necessary to increase research and disseminate the nutritional quality of native corn, such as black maize due to its high anthocyanin content, and to incorporate other nutritional characteristics such as Zn and pro vitamin A content in the grain.

Modern tools such as double haploids, genomic selection, and gene editing must be incorporated into the MBP of the region in order to increase yield and resistance to diseases. The yield of floury maize in the region is similar to the yield that existed a hundred years ago in the United States, when OPVs were used [59]. Of course, the increase in yield must come with the improvement of agronomic practices since agronomy and genetics contribute in equal parts to the production of grain, although yield potential is driven by genetics [60].

Seed dissemination of the new varieties is a bottleneck for all plant breeding institutions in the Andean region of Ecuador, Peru, and Bolivia. These institutions make great efforts through communication networks and field days to have their improved OPVs reach farmers and consumers, but with disappointing results. This is reflected in the small area with improved varieties planted by farmers. The development of high-yield maize cultivars facilitates the development of seed companies, such as what happened in the lowlands of Ecuador, Peru, and Bolivia with the release of hybrids.

#### 7. Conclusions

At least 18 OPVs of floury maize have been released in the highlands of Ecuador, Peru, and Bolivia in the last 20 years. These varieties were developed mainly using the half-sib breeding method in order to conserve native genetic diversity and grain quality (texture, color). Additionally, crossing between exotic and native germplasms was performed to incorporate the opaque-2 gen and resistance to ear rot, which is the main disease that affects this kind of maize.

Despite the achievements shown in terms of the number of released varieties, Andean maize in these three countries faces serious challenges: (a) breeding has not been able to significantly increase the yield of floury maize in the region, (b) floury maize is very susceptible to ear rot, and (c) there is no formal certified seed system that distributes improved seeds to farmers on a large scale.

In the future, it is necessary to implement in the MBP new biotechnological tools available for maize breeding in developed countries, such as double haploids and genomic editing. Additionally, it is crucial to implement a seed production and commercialization system between public and private institutions to promote the use of certified seeds. These innovations contribute to solving the above-mentioned challenges and reducing the time to obtain a new variety. This allows for the development of more productive and resistant maize cultivars.

**Supplementary Materials:** The following are available online at https://www.mdpi.com/2073-439 5/11/2/212/s1, Figure S1: Samples of maize landraces found in the highlands of Ecuador, Peru, and Bolivia. It is shown the diversity of shapes, colors, and textures of ears and kernels which represents a great genetic diversity of maize found in the region. Figure S2: Ears of floury open pollinated maize varieties developed in the highlands of Ecuador, Peru, and Bolivia. Colors white, yellow, black, and mottled are shown.

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