EVALUATION OF THE DOMESTICATION SYNDROME IN POPULATIONS OF *ZEA MAYS* TARAPAQUEÑOS FROM THE HYPER-ARID CORE OF THE ATACAMA DESERT, NORTHERN CHILE.

(Evaluación de Síndrome de Domesticación en poblaciones de Z*ea mays* tarapaqueños del núcleo hiperárido del Desierto de Atacama, norte de Chile)

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LIST OF ABBREVIATIONS

Abbreviation	Term
BC/AD	Before Christ/Anno Domini
BP	Before Present
Pdt	Pampa del Tamarugal
PIR	Pircas
PT	Pintados
RAM	Ramaditas
TR	Tarapacá
yr	years

RESUMEN

La evolución del maíz (*Zea mays* L.) es altamente controversial dadas los complejos cambios fenotípicos y genéticos sufridos por la especie, la incidencia de los grupos humanos y los tiempos en que se producen estos cambios. Adicionalmente, las etapas de los procesos de domesticación son difíciles de evaluar frente a la ausencia de registros antiguos y/o fósiles de plantas domésticas, recurriéndose a análogos modernos. La presencia de restos arqueológicos de maíz en aldeas arqueológicas de Tarapacá, Desierto de Atacama, provee de una oportunidad única para estudiar la evolución del cultivo, abarcando una secuencia temporal de al menos 2000 años. Algunos de los cambios reportados para el maíz tarapaqueño incluyen el aumento paulatino de los tamaños de marlos y granos en la secuencia cultural de la zona y una baja diversidad genética asociada a un posible cuello de botella. Sin embargo, queda por establecer si estos cambios responden a una selección artificial, a presiones ambientales, o a la introducción de nuevas variedades e hibridación. Asimismo, se requiere conocer la concomitancia de estos procesos con los desarrollos humanos tarapaqueños prehispánicos Por otro lado, se ha reportado la disminución en la capacidad de los cultivos modernos para fijar nutrientes minerales como Fe, Zn, Mg y Cu, teniendo como resultado una disminución en la calidad de los cereales a nivel mundial. El aspecto más crítico es la disminución de Fe en aproximadamente un 19% en el maíz moderno. Esta modificación, a pesar de su relevancia, no ha sido evaluada en agriculturas preindustriales. Otro aspecto

desconocido son las condiciones ecológicos y uso del agua de estos cultivos. Este elemento es crítico en sistemas áridos y su acceso o limitación nos permite aproximarnos a los complejos cambios sociales y culturales de las sociedades tarapaqueñas. De acuerdo a lo anterior, el objetivo de esta investigación era evaluar el Síndrome de Domesticación en maíces antiguos del Desierto de Atacama, mediante el análisis y comparación de maíces arqueológicos (ca. 2500 a 300 AP). Con ello queríamos responder a las siguientes preguntas: 1) ¿Cuáles son los cambios de fenotipo producidos y/o concomitantes a una manipulación antrópica para el maíz tarapaqueño?, 2) ¿Cuáles fueron los marcos agroecológicos para el establecimiento de una agricultura del desierto?.

Para lo anterior, realizamos análisis cualitativos y cuantitativos sobre ciertos rasgos de muestras arqueológicas de maíces, evaluación del contenido químico e histología de semillas, datación radiocarbónica de maíces arqueológicos y análisis de $\delta^{18}O$ isótopicos de mazorcas y granos.

Los resultados obtenidos establecen la presencia de maíz en la región con fechas que lo retrotraen al 400 AC en la zona sur de Tarapacá, mientras que de la zona norte encuentra un desarrollo más tardío de la agricultura maicera. El set de fechados obtenidos se ancla a una serie de cambios fenotípicos de variedad, tamaño y forma de los maíces que se establecieron en la agricultura tarapaqueña. Respecto a los cambios químicos se estableció una sistemática disminución de Fe a lo largo de las secuencia cultural y agrícola en los maíces, tanto en la distribución de Fe a nivel intercelular y en su cuantificación. Finalmente, se reportan los valores de $\delta\ ^{18}O$ para maíces prehispánicos, los cuales muestran un enriquecimiento de los maíces irrigados por sistemas superficiales en la conducción de aguas, así como también posibles diferencias en la fuentes agua.

ABSTRACT

The evolution of maize (*Zea mays* L.) is highly controversial given the complexity of the phenotypic and genetic changes suffered by the species, the incidence of human groups and the times in which these changes occur. Additionally, the stages of the domestication processes are difficult to evaluate in the absence of ancient records and / or fossils of domestic plants, resorting to modern analogues. The presence of archaeological remains of corn in archaeological villages of Tarapacá, Atacama Desert, provides a unique opportunity to study the evolution of the crop, covering a temporal sequence of at least 2000 years. Some of the changes reported for Tarapaqueño maize include the gradual increase in the sizes of cobs and grains in the cultural sequence of the area and a low genetic diversity associated with a possible bottleneck. However, it remains to be established whether these changes respond to an artificial selection, or to environmental pressures, to the introduction of new varieties and hybridization. Likewise, these processes have to be evaluated according to the pre-Hispanic human developments in Tarapacá. On the other hand, a decrease in the capacity of crops to fix mineral nutrients such as Fe, Zn, Mg and Cu has been reported, resulting in a decrease in the quality of modern cereals worldwide. The most critical aspect is the decrease in Fe by approximately 19% in modern corn. This modification, despite its relevance, has not been evaluated in pre-industrial agriculture. Another unknown aspect is the ecological conditions and water use of these crops. This element is critical in arid systems and its access or

limitation allows us to approach the complex social and cultural changes of Tarapacá societies. According to the above, the objective of this research is to evaluate the stages described for the Domestication Syndrome in ancient maize from the Atacama Desert, through the analysis and comparison of archaeological maize (ca. 2500 to 300 BP). With this we want to answer the following questions: 1) What are the regulated phenotype changes and / or concomitant to anthropic manipulation for Tarapacá corn? 2) What were the agroecological frameworks for the establishment of a desert agriculture? .

We performed qualitative and quantitative analyzes on certain features of archaeological samples of corn, evaluated the chemical content and histology of seeds, radiocarbon dated over maize samples, and analysis of δ^{18}O isotopes of ears and grains.

The results obtained establish the presence of corn in the region with dates that go back to 400 BC in the southern area of Tarapacá, while in the northern area it finds a later development of corn agriculture. The set of dates obtained is anchored to a series of phenotypic changes of variety, size and shape of the maize that was established in Tarapacá agriculture. Regarding the chemical changes, a systematic decrease of Fe was found along the cultural and agricultural sequence in maize, both in the distribution of Fe at the intercellular level and in its quantification. Finally, we report the δ^{18}O values for maize cobs, showing enrichment of $\delta\ ^{18}$O values for maize crops irrigated by shallow water-conducting systems. Likewise, there are some clues on the water sources for different areas.

INTRODUCTION

Maize domestication is the process of interaction between humans and plants, whereby, deliberately or unconsciously, the wild variety (*Zea mays* var. parviglumis/ *Zea mays* var. mexicana, teosinte) was modified in its genetics and phenotype by human action, giving rise to the domestic species *Zea mays var. mays* (Harris, 1989); Preece et al. 2017). This reductionist notion does not describe the complexity of the process, as it postulates a one-way path. Other definitions hold that domestication processes involve at least four stages: a) initiation of domestication including the hybridization between species, b) increase in the frequency of desirable alleles, c) formation of local populations that are adapted to new environments, and d) deliberate cultivation to maximize yield and plant qualities (Meyer and Purugganan 2013). In this sense, domestication represents a continuum of interaction between plants, humans, and their environment, where species are in constant modification (Barker, 2012). Domestication converges into the so called "domestication syndrome" (DS) which highlights the sets of characters that differ from domesticated crops and their wild ancestor (Fuller et al. 2007). There are some general characteristics for DS thar are more or less frequent: elimination or reduction of structures to disperse seeds, increased size of seeds or fruits, loss of germination inhibition, synchronous tillering and ripening. However, DS is species-specific and there is an open debate on the changes involved in DS. In cereals such as sorghum (*Pennisetum glaucum*), the increase in grain size is described as a subsequent stage to the generation of indehiscent fruits, which shows the process's heterogeneity in question (Fuller 2007).

In summary, the characteristics and criteria exposed are challenging to observe, artificially separated, and species-specific. In maize, anthropic modifications are related to selection over the development of the plant structure (Benz 2001), the flavor, color, size and texture of grains (Dinges et al. 2001; Palaisa et al. 2003), and even some chemical changes associated with heavy metal detoxification (Vielle Calzada et al. 2014). These are only a small batch of the many changes of maize.

Given the absence of ancient or fossil plant records, evaluating *Zea mays'* evolutionary history has been a problem for paleo disciplines such as archaeobotany or paleoecology. In most studies related to understanding maize evolution, morphological and genetic studies of current varieties are used. Nevertheless, in Tarapacá region Northen Chile, there are abundant archaeological macro remains of corn which gave us the possibility of evaluating the species' transformations and "domestication syndrome" without resorting to modern analogs.

The Tarapacá region(19°- 21°S), is currently a hyper-arid area (<1 mm of annual rainfall), with no possibilities for agricultural development in the intermediate plateau, the so called Pampa del Tamarugal (PdT). (Figure 1). However, in the past Tarapacá farmers built vast fields of agriculture associated to pre-Hispanic settlements that redundant in abundant archaeological corn remains preserved thanks to the hyperaridity of the Atacama Desert (García et al. 2014, Núñez, 1966). These archaeological settlements are Guatacondo (2290-1890 Cal. years BP), Ramaditas (2340-1870 Cal. years BP), Pircas (2320-1420 Cal. years BP), Pintados 1307 (1427-1568 Cal. years BP), Caserones (1880-1080 Cal. BC), Tarapacá

13 (930-490 Cal. BC.) and Tarapacá Viejo (662- 350 Cal. BC.), among others (Mostny 1971, Núnez 1984, Núñez and Santoro 2011, Uribe and Vidal 2012, Rivera et al. 2005). Except for Pintados 1307, they all correspond to villages with a division of residential, public, and productive spaces, in which the presence of corn is widely registered (Adán et al. 2007, García et al. 2014, Uribe 2006).

Considering our previous studies, we have proposed three agricultural moments (Vidal Elgueta et al. 2019). During a first stage**,** agriculture developed during the Early Formative Period (EFP) (ca. 2500-1450 BP), in which agricultural fields are mainly deployed in Quebrada de Guatacondo situated in the southern area of Pampa del Tamarugal (Rivera et al. 1995-1996, Rivera and Dodd 2013, Vidal et al. 2015). However, corn remains do not exceed 3% of the total plant macros recovered in the period's settlements (García et al. 2014). Concordant, isotope studies ($\delta\ ^{13}C$ and $\delta\ ^{15}N$) on human remains from the Tarapacá 40 cemetery (from the EFP and Late Formative Period, LFP) point to a gradual transition to C^4 plant consumption among Tarapacá groups (Santana-Sagredo et al. 2015). We suggested that maize would not have been the main crop during the EFP, as the silvicultural management of *Prosopis* spp replaced its consumption. (García et al. 2014, McRostie et al. 2017). During the Late Formative period (LFP) (ca.1450- 750 BP), agricultural fields extended towards Pampa Iluga, the northern area of Tarapacá and Quebrada de Maní (QM) the southern area (O'Brian [1765] in Hidalgo 2009, Santoro et al. 1998, Santoro et al.2017). Simultaneously to this expansion, there is a considerable increase of maize in the archaeological record. Especially in Caserones settlement, where maize storage reached a high proportion (Méndez Quirós and Uribe 2010). Most of the debris found in this site corresponded to Algarrobo seed and maize cobs and grains (García et al. 2014: Núñez 1966). Most recently, the evaluation of tumuli of the ceremonial site of Iluga Túmulos shows that maize cobs were used abundantly as material construction for the tumuli structure. Thus, during this second stage, the implementation of stable agriculture was vital for exchanging resources with the Tarapacá coast (Uribe 2006). During a third moment, in the Late Intermediate Period (LIP**)** (ca. 750-500 BP), Tarapacá populations restricted agriculture in the PdT, and moved to the ravines (>2500 masl), implementing a system of terraces that depended almost exclusively on rainfall. A few settlements remain in the PdT, such as Tarapacá Viejo, Pica 8, Iluga Túmulos, and minor sites. The presence of maize in these villages remains steady, and in some cases, there is an intensification of production (Vidal Elgueta et al 2019). The reason for this shift in production is not yet fully known, though environmental fluctuation could be related.

Pre-Hispanic agriculture suggests that environmental conditions must have been different in the recent past. Thus, some wetter moments are corroborated (between 2500-2040, 1615-1350 and 1050-680 years BP) that would have allowed the recharge of aquifers and the presence of permanent water currents (Gayó et al. 2012a, Maldonado and Uribe 2015, Maldonado et al. 2016). In QM the diversity of plant taxa observed in plant macrofossils, suggest riparian vegetation for these moments (Gayó et al. 2012a, Latorre et al. 2013, Nester et al. 2007). In agreement, the vegetal remains of paleo-burrows support the existence of higher humidity in the altiplano (>3000 masl) associated with the Medieval Climate Anomaly (Maldonado and Uribe 2015, Latorre et al. 2013). Besides, remains of corn stalks observed in situ in QM fields, dated between 1050 -730 cal. years BP, corroborate corn agriculture during LFP-LIP for this sector (Gayó et al. 2012b).

The history of Tarapacá agriculture had consequences on crop development, biology and evolution. Unfortunately, because of the Atacama Desert's marginal location, the paradigm that postulated that Atacama Desert was an unproductive space (an "empty space"), it has been assumed that Tarapacá prehispanic corn did not undergo any modifications (Rivera et al. 1995-1996). In contrast, our results indicate a progressive increase in the size of the cobs and grains, a low genetic and allelic diversity (He= 0.479, Ho=0.164, number of alleles 2.3) and the presence of rare alleles in the archaeological maize during the FLP, LIP and Late Period (LP or Late Horizon) (ca.500-450 AP) (Vidal-Elgueta et al. 2016, Vidal Elgueta et al.2019). Other species subjected to artificial selection also present a low allelic diversity: modern Andean maize (Vigouroux et al. 2008), primitive varieties of maize (Grimaldo 2011, Pressoir and Berthaud 2004,), yucca (Contreras 2007) and domestic animals (Paredes et al., 2011). All of which suggests that pre-Hispanic maize from Tarapacá was subject to strong artificial and environmental selection processes**.** Our preliminary observations suggest that only hard endosperm grains (popcorn) are present in the first agriculture stage. This appreciation comes from a limited number of microsatellites (8 SSR) and archaeological specimens and does not include the earliest maize in the region. Further studies are therefore required.

Another phenotypic changess detected in modern maize varieties is the decrease in the capacity to assimilate mineral nutrients such as Fe, Zn, Mg, and Cu (De Fries et al. 2015, Fan et al. 2008). This unanticipated change has been described only for crops after the 1950s onwards (Green Revolution) in association with intensive agriculture (Jobbágy et al. 2014). Since the lower mineral concentration rates are positively related to harvesting rate and not associated with soil nutrient management, it is estimated that the decrease in nutrients would be related to human handling of high-yield grains. It is unknown whether Fe's loss in maize is a current phenomenon only or would also be present in longstanding pre-Hispanic agriculture. Therefore, the evaluation of Fe distribution in maize in pre-Hispanic agriculture opens exciting research lines on the DS of the species.

Considering that maize is one of the most important cereals in food feeding around the world, is the primary source of non-animal Fe for human consumption at present times (Ranum et al. 2014), and still has an essentially social dimension in many cultures, the study of its modifications in the past can give us insight on how to face future challenges for its production.

Considering the above, our research questions are:

1.- What were the main characteristic of archaeological maize in Tarapacá?

2.- Did the Tarapacá farmers prefer some feature of maize over another's? , What were the social and cultural reasons for these choices?

4.-Could the loss of iron could also be observed in archaeological maize, which was under a constant agriculture selection in prehispanic times?

5.-What were the agroecological conditions and water availability for maize production?

We aimed to evaluate the evolutionary changes in pre-Hispanic Tarapacá corn under the Domestication Syndrome and its relationship with the cultural and ecological history of the pre-Hispanic human populations of Tarapacá.

We hypothesize that Tarapacá maize has been subjected to constant artificial selection processes to achieve its adaptation to the Atacama Desert's hyper-arid system. Changes in phenotypes linked to anthropically desirable traits and adapted to arid environments are expected.

We present the research in three chapters. The first chapter introduces to phenotypic characteristics observed in maize from different periods of Tarapacá and links various morphological features to precise radiocarbon ages. The second chapter discusses Fe's distribution in intercellular compartments and quantification of Fe during the different maize agriculture stages. This chapter wants to contribute to the still valid controversy over the loss of micronutrients in cereals. Finally, the third chapter explores maize production's ecological and social conditions using an isotopes $\delta^{18}O$ approach. This perspective will let us infer the agroecological conditions and water availability for prehispanic maize.

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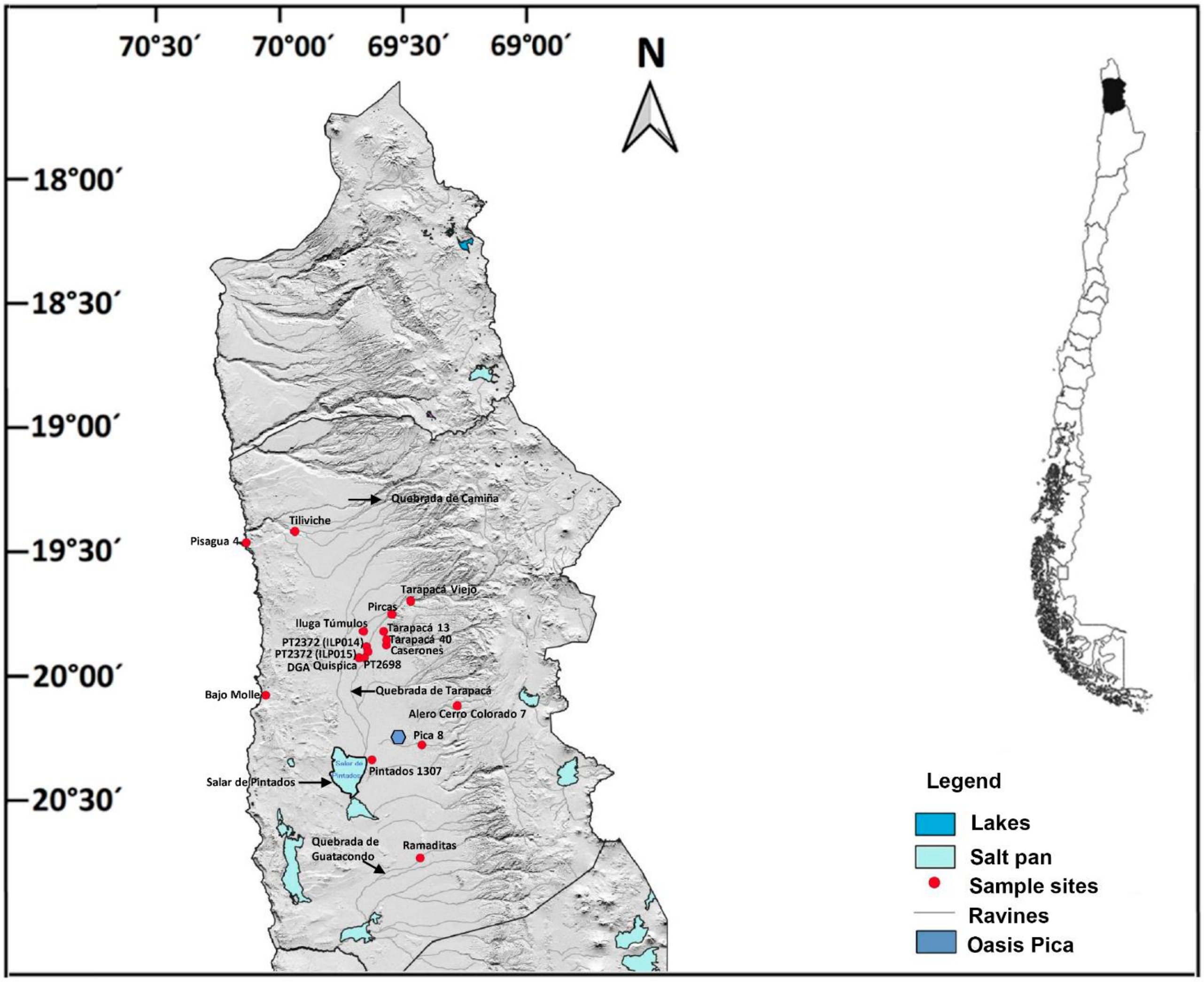
Locations of archaeological sites of this study



Locations of archaeological sites of this study

Maize samples were obtained from the following sites indicated in the map above: Tiliviche (TIL), Pisagua 4 (PSG4), Tarapacá Viejo (TARViejo), Pircas (PIR), Iluga Túmulos (IT), Tarapacá 13(TR-13), Tarapacá 40(TR-40), Caserones(CAS), PT2372, PT2373, PT2698, DGAQuipisca, Bajo Molle (BJM), Alero Cerro Colorado 7 (ACC7), Pica 8 (PIC), Pintados 1307 (PT1307), Ramaditas (RAM).

CHAPTER 1.

RADIOCARBON DATES AND MORPHOLOGICAL CHARACTERISTICS OF TARAPACÁ ARCHAEOLOGICAL MAIZE.

INTRODUCTION

In the XIX century, the Province of Tarapacá (19°-21-30' southern lat.), was the most distant and extensive province of Perú, and the narratives made of this remote landscape highlighted the uninhabited, the lack of cultivation, the sandy and rocky landscapes, the rainless deserts covered with salt flats and no water (Bollaert 1851). This “empty narrative” is still applied today to refer to the Atacama Desert and it is common to read about it "as the driest desert in the world". And in doing so to emphasizes the notion of a space that has always been a blank place. However, this conception is challenged by the archaeobotanical record (Núñez 1966, Castro y Tarragó 1992), the ancient agricultural fields that covered thousands of hectares in the past (O'Brian, 1765 en Hidalgo 1985), and especially the unrefutably evidence that the “desert” was cultivated successfully at some point: the abundance presence of archaeological maize.

In our conception, maize has three conditions that make it a particular material culture. First, as a crop it is halfway between an ecofact and an artifact. In this notion, maize is an organic material found in a human context activity with archaeological meaning. But, it is also the product of these human activities since its origin and biological modification are mediated by human interaction. And so, maize's dual condition transforms it into an excellent indicator of the relationship between human groups and their environments.

Second, maize in Andean communities served as a material culture of identity and status (Bonavia, 2013). And so the esthetic of the varieties (colors of grains, disposition of row, size of the cob, size of plants, etc.) were and are highly valued (Staller, 2010). In 2014 during a collection trip to the agricultural communities of Tarapacá, farmers informed us of how proud they were to produce maize cobs with perfect arranged rows that gave cobs a beautiful appearance. It increased maize's economic value, but it also highlighted the farmers' ability and knowledge to modify the plant as their desires. This task was a challenge accomplished because of the profound knowledge and experience on the plant´s ecology. Another example of this management was when farmers spoke of how they separate maize´s plantation of color cobs from the yellow cobs plants, so they did not mix and lose the color attributes. The examples in which maize has multiples functions way beyond serving just as a food staple are numerous (Staller, 2010).

Finally, during prehispanic times in Tarapacá maize was a polysemic eco-artifact. Thus, we found it in many contexts, such as domestic contexts (Caserones, Pircas, Guatacondo), used as material construction for ceremonial tumuli (Iluga Túmulos), food for human and animals, offered in burials and ceremonial places (pe. Pica 8, Tarapacá 40 and Alero Cerro Colorado) (García et al 2014, Santana Sagredo-2015, Uribe et al. 2020). Then, studying maize as a human nature co-creation can give us an insight into multiple scenarios in Tarapacá prehispanic life.

Because of these three characteristics, maize's modification is an indirect clue of the history of human agricultural communities and, moreover, a glimpse into the modes of production, forms of organization, access or control to water sources, aspects of identity, status, among others social dimensions. In this sense, maize has an agency by itself (*sensu* Ingold, 2006) while it transformed desert landscapes into vibrant agricultural fields.

Considering these conceptions of maize, our questions were a) What were the main characteristic of archaeological maize in Tarapacá? b) Did the Tarapacá farmers prefer some feature of maize over another's? c) What were the social and cultural reasons for these choices? We examine and dated several archaeological maize samples from Tarapacá to evaluate their morphological characterizes and associate the morphological features to a cultural sequence anchored in radiocarbon dates.

Archaeological maize of Tarapacá

In an extraordinary description of geography, flora, fauna, landscapes, and people of the Province of Tarapacá, Williams Bollaert, in 1825, mentions for the first time the presence of maize in the Tarapacá archaeological record. In his observations indicated there were indigenous cobs of maize, a kind of rare "zea rostrata" (sic) in the *huacas* (archaeological burials) of Bajo Molle and Iquique (Bollaert 1851). The cobs were described as elongated, thin, and with big kernels (Latcham & 336, 1936). After that, various mentions for maize have been made in Tarapacá archaeological record. In the littoral maize is present in sites of Cáñamo 1 (Núñez and Moragas 1977), Pisagua (Vidal et al. 2004), Chomache (Vidal and García 2009) in burials and domestic context, in the hinterland La Capilla site, has been proposed as a node of exchange and production of maize (López 1979), Caserones is distinctive for the maize quantities of maize storage (Méndez-Quirós and Uribe 2010). Despite the importance of maize in Tarapacá communities, there is little information on the characteristics of maize. One exception is Tiliviche 1B´s maize. It was considered to be the oldest maize in Tarapacá, around 5000 years old (Núñez, 1986), and so it triggered the attention of the researchers. Back then, it was described with morphological features similar to Chucutuno Chico and Capio Chico Chileno (Núñez, 1986). But these analogies did not contribute to understanding the characteristics of this supposed oldest maize.

A different approach was made by Tartaglia (1980), who analyzed 84 archaeological cobs and 31 kernels from Guatacondo archaeological site. He established two types of maize. These statements were made based on the number of rows for the cobs, where the row number ranged from 14 to 20. Also, 1.2% of the sample reached the high number of 28 rows, which is commonly found in modern varieties of corn. He also examined the width and length of 31 kernels, but no statics differentiation was found. The coat seed characteristics let him postulate that Guatacondo exhibited flour corn (rounded crown enclosed by a thin coat) that represents 67% percent of all kernels. A second type would be a popcorn type characterized as a triangular kernel. Unlike the previous approach that tries to homologate ancient races to modern varieties, Tartaglia approach focused on describing the features that are ductile to manipulation. Thus, we consider this to be a better approach to recognize changes in maize.

METHODS AND SAMPLE

We evaluate 609 archaeological cobs from Tarapacá archaeological sites and three to a modern cultivar (DGA Quipisca), used as a control sample. In total, we included 15 archaeological sites from Tarapacá (Supplementary information figure 1), which included the following sites: Ramaditas, Pircas, Iluga túmulos, PT1307 and PT2698 from the Early Formative Period (ca. 2500-1000 yr BP), Tarapacá 40 and Caserones from the Late Formative Period (ca. 1000-750 yr BP), Tarapacá 13, Pica 8, Bajo Molle, Pisagua 4, PT2372 and PT2373 and Tiliviche 1B maize from the Late Intermediate Period (ca.750-500 yr BP), Tarapacá Viejo from the Late Period (500- 350 yr BP), and Alero Cerro Colorado 7 (samples from 17th century).

For the Caserones site, we divided the sample between cobs from the Formative Period and cobs from the Late Intermediate Period since we have already established a significant difference in size (Vidal Elgueta et al. 2016). Also, for Tarapacá 40 and Alero Cerro Colorado 7, we divided the sample between cobs with kernels and the ones that had lost the grains (bare cobs). We measure 492 archaeological seeds belonging to ten archaeological sites of Tarapacá.

We did a qualitative analysis of cobs and kernels to typify the samples based on observing features distinctive of maize varieties (Paratori et al. 1990). We considered the following characteristics: shape of cobs (elliptic, conic, oval, round, pointed), and kernels (round, square, flat), shape of cupules (round or quadrangular), number of rows, disposition of rows (regular, spiral, irregular) type of kernel (popcorn or floury type) and presence of indentation in kernels. We also performed a quantitative analysis of cobs and kernels. We measured the following features: length, base diameter, tip diameter, middle diameter, number of rows, and numbers of kernels per row for all the complete cobs, and length, width, and thickness of entire kernels. Any incomplete sample was discharged (Figure 1). Then, we performed Principal Component Analysis. Also, we estimated the Coefficient of Variation to analyze the relative variability of the data by sites and periods.

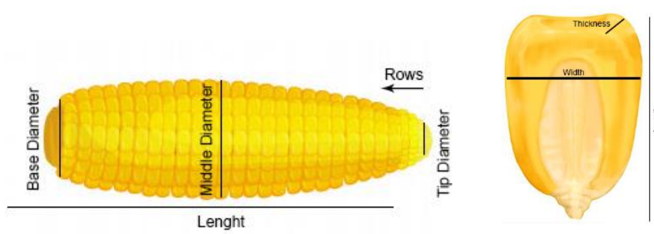


Figure 1 Attributes measured on cobs and kernels.

To assure that the features description of cobs and kernels are attributable to a specific date and period, we performed nineteen archaeological dates directly over kernels and cobs. Radiocarbon dating were performed by DirectAMS radiocarbon dating service laboratory, Seattle, Washington, USA. The samples were calibrated using the OxCal v.4.3.2 online software service, using the SHCal13 atmospheric curve.

RESULTS AND DISCUSSION

Archaeological radiocarbon dates over maize samples

We obtained 19 radiocarbon dates (^{14}C) ranges between 2260 yr BP to 280 yr BP (*ca*. 400 BC to 1750 AD) (Figure 2). Previously, there were only three dates for the Early Formative Period, that reported maize for 2210±55 yr BP in Ramaditas size (Rivera 2006), one maize from Caserones dated on 1750±70 yr BP and Tarapacá 6-7 dated on 1680 ± 60 yr BP (Oakland 2000). Then, two dates from the Late Formative positioned maize in Caserones 1 during 1605 ±18 yr BP and 1470 ± 80 yr BP (Oakland 2000 and Santana-Sagredo et al. 2021). Most maize´s dates assemble into the Late intermediate Period with more than twenty ^{14}C ages (see supplementary information table 1). Finally, four dates ^{14}C ages are related to the Later Period to the Colonial Period in Tarapacá Viejo and Cerro Colorado 7 (De Souza 2017, Uribe et al. 2012, Zori and Urbina 2014).

Therefore, our result corroborates through direct dating, the presence of corn in the southern part of Tarapacá, from at least 2400 years BP. The oldest sample we dated is a popcorn kernel (sample RAM 287) from the Ramaditas site, dated on 2260 ± 28 yr BP (cal. 376 to 206 BC) (95.4% probability). A second date from the same site dated on 2041 ±19 yr BP (cal. 56 BC-45 cal.AD). Our dates are very similar to those reported by Rivera (2006), concluding that Q*uebrada de Guatacondo* is for now, a candidate for the entrance of maize into the region and the first place in Tarapacá where maize agriculture was developed. Other sites such as Pintados 1307, halfway between southern Tarapacá and north part of Tarapacá, have also shown an interesting developed of early maize debris. Our sample from this site dated on 1778± 30 yr BP (224 to 376 cal.AD). However, most of the sites in the Pintados area are domestic sites with no evidence of agriculture *in situ*. Thus, different sites are described as places to rest on the way to the coast of Tarapacá. Early maize in the northern part of Tarapacá is very limited. Our sample from Pircas sites dated on 1897 ±19 yr BP (cal. 114 to 224 AD). Then, a second date from Iluga Túmulos dated on 1730 ±28 yr BP (cal. 250 to 416 AD).

For the Late Formative Period, seven of our dates range from 1638 ±26 yr BP to 1478 ±24 yr BP (cal. 407-652 AD), all maize were obtained from Iluga Túmulos, Caserones and Tarapacá 40. Again, this corroborates the main role of Caserones in the agrarian production during the Late formative. And it suggests there is a switch from south to north in agriculture production. The reasons for these dramatic changes are not yet known.

It is also interesting to note that we have a hiatus of dating between 1350 yr BP to 1050 yr BP approximately. Previously, only one date from this range time was reported by Rivera (2006) over a Ramaditas cob (*ca*. 1065 yr BP). In our case, only one date from Iluga Túmulo, dated in this range span dating on 1109 ± 24 yr BP (cal. 897 to 1021 AD). These absences of dates are quite interesting because they suggest a reduction in agricultural production. In fact, the one cob we dated is a very unusual type of corn in Tarapacá agriculture debris, which could suggest it could be an introduction. Additionally, this moment (Middle Horizon period), coincides with the presence in Chile of the Tiwanaku Estate, which changed in many forms, the organization, including food production for northern Chile prehispanic communities. Although in Tarapacá, there is no clear evidence of the influence or presence of Tiwanaku in material culture, from this negative evidence, we can start to evaluate the relation of Tarapacá farmers with Tiwanaku Estate.

For the Intermediate Period we add two dated to the corpus of 25 dates formerly reported. These includes one date of 920 ± 23 yr BP (cal. 1051 -1221 AD) from PT2372 which is domestic site. Likewise, this cob is very unusual in shape. The second maize came from Caserones later occupation dated on 830 ± 24 yr BP (cal. 1214 to 1279 AD). From the concentration of dates, it is evident that the main production of Tarapacá developed during this moment. Whether this production was made in the desert plateau (Pampa del Tamarugal), in the terraced ravines, or in both environments it is something to discuss. But, from the agricultural fields and canals we observe in the desert plateau it seems very probably agriculture was established in the desert.

Finally, for the Late and Colonial Period, we summate four dates: 379 ± 24 yr BP for Tarapacá 13 (cal. 1464 to 1629 AD), 352 ± 25 yr BP and 326 ± 24 yr BP for Tarapacá Viejo (cal. 1496 to 1653 AD) and 280 ± 29 yr BP for Alero Cerro Colorado 7 (cal. 1511 to 1799 AD). We show maize agriculture continue into Colonial time related to specific sites. In this case Tarapacá Viejo was the main center for the Inka occupation. We have already postulate Inka States transformed Tarapacá agriculture (Vidal-Elgueta et al. 2019).

Alero Cerro Colorado 7 corresponds to a ceremonial site occupied mostly during the Early Formative Period (De Souza et al., 2017). However, several cobs of corn were deposited in offering pits of the shelter and dated for a later time: 380 ±30 yr BP (De Souza et al., 2017). Our results are quite similar, with a date of 280 ± 29 yr BP. Because of the sample's homogeneity and the two concordant dates we proposed, this was a unique offering event related to a Colonial occupation of the site.

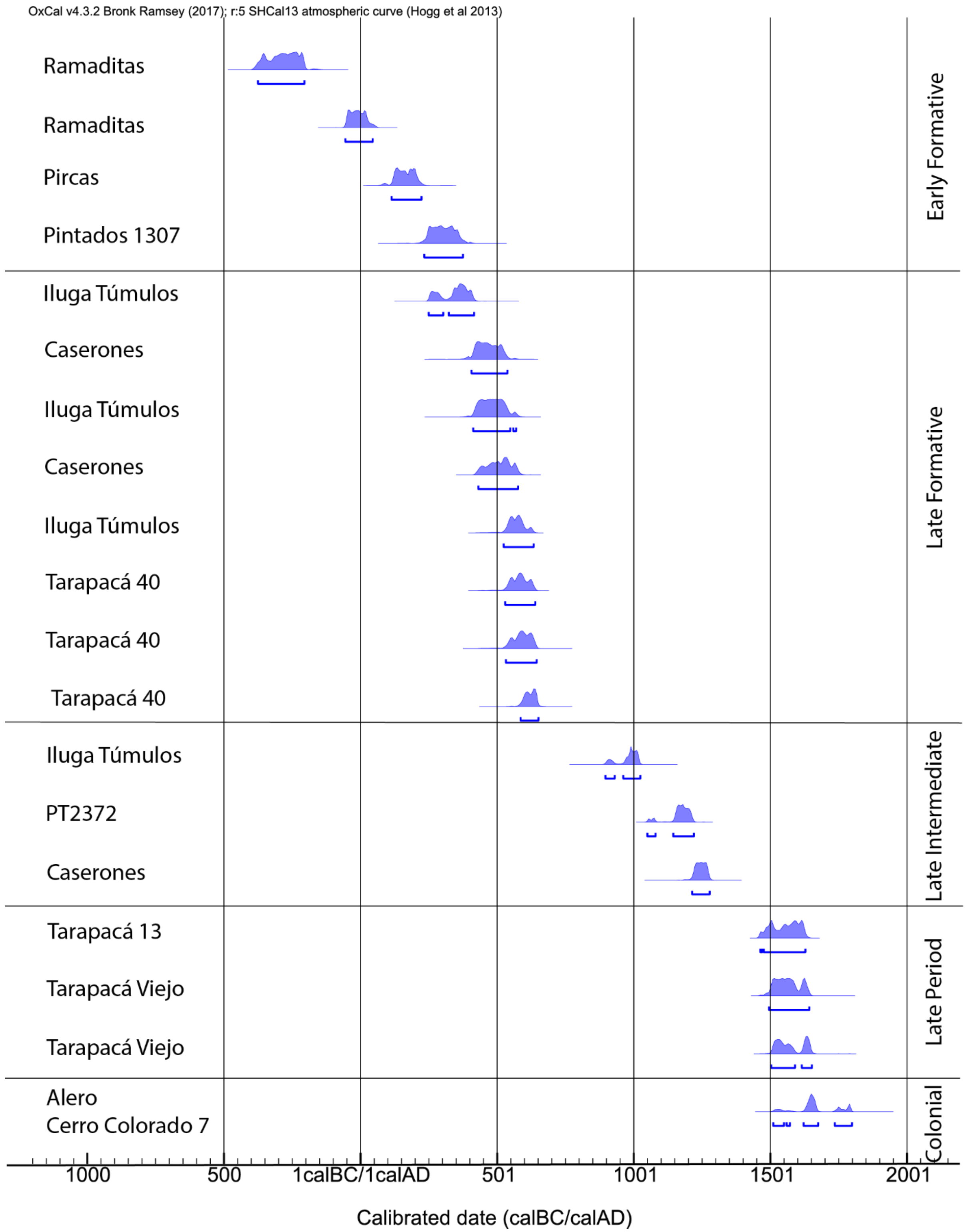


Figure 2: Calibrated radiocarbon dates for 19 archaeological cobs and kernels samples from Tarapacá archaeological sites.

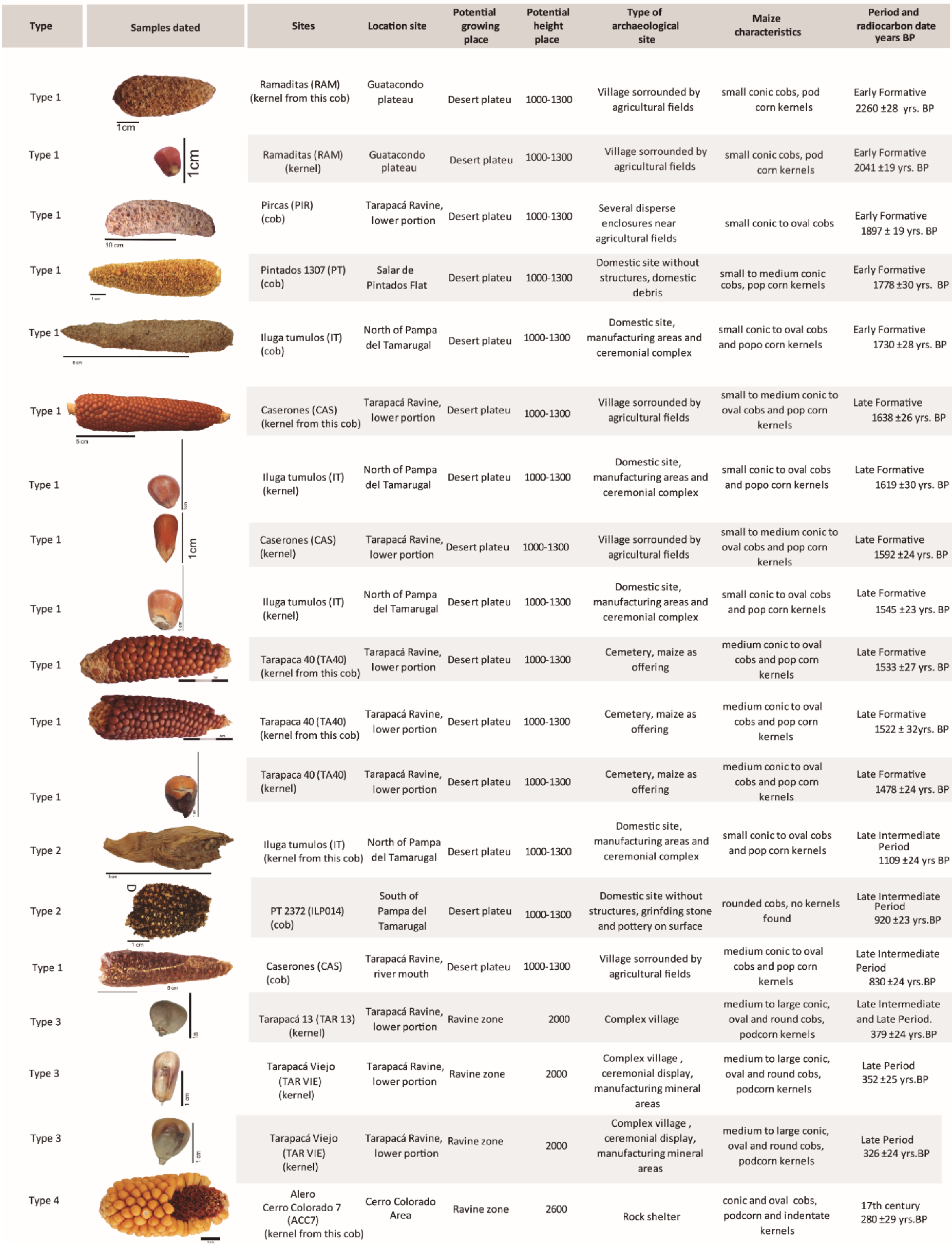
Qualitative analysis results: types of maize.

We found four types of maize for Tarapacá (Figure 3). The early type of maize is commonly present in the Early Formative and Late Formative sites of Tarapacá (Figure 3), including various archaeological sites (domestic, funerary, and ceremonial types). This is the most common type of maize in our sample and was dated from 2260 yr BP to 1545 yr BP. This early maize has oval to conic cob shapes, with round cupules, with length from 55 mm to 90 mm, showing a significant variance in length cobs size. The number of rows is between 11 to 22 (Figure 4). However, there is one standard feature; they present only one type of popcorn kernel, very small with a diameter ranging between 4 to 5.3 mm. These are round to square shape brownish, reddish, and sometimes yellowish hard popcorn kernels. They were described as "*pululos"*, "*curaguas*", or more generally as "r*eventadores*" (Núñez, 2017) because of their capacity to explode when heating.

The second type of maize is present during the Late Formative and Late Intermediate Period. Two samples of this kind were dated in ca. 1100 years BP and ca. 970 years BP. These samples were recovered in Iluga Túmulo and PT2372 site, respectively. The cobs are rounded, pointed, and small (60 mm in length, 20 mm in diameter). The cobs have round cupules, which is an indicator of round kernels. The average number of rows is 18. One of the specimens has kernels of round shape and floury endosperm. They remind us of modern native landrace of Capio Chileno Chico.

The third type is present in Late Intermediate Period sites of Caserones and Tarapacá 13, and Tarapacá Viejo Late Period site. These samples were dated to 830 ± 24 yr BP, 379 ± 24 yr BP, 352± 25 yr BP, 326±24 yr BP. Cobs are conic to oval, with elongated cupule. This type is very similar in shape to the first type, reaching an average length of 62.7 mm, and 16,7 mm in middle diameter. However, the longest cobs measured 149 mm, 106 mm, and 132 mm reaching the average size of some modern landraces. Another interesting feature is that they have a low amount of rows, with an average of 11,3. However the number of kernels per row is higher than any other type, reaching a peak of 32 kernels per row in some specimens. Kernels in this type are floury, square, flat and have an average length of 10 mm, with several specimens of 20 mm in length. The results for coastal sites of Bajo Molle and Pisagua 4 indicates that maize from these sites is also a type 3. Both, Bajo Molle and Pisagua 4 kernels reached important average length of 7.3 mm and 10.54 mm, respectively. These results are expected for Bajo Molle samples, because all kernels are flat and square, the archaeological site is dated for the Late Intermediate Period and it corresponds to a cemetery, where presumably maize is selected as offerings. However, Pisagua 4 belongs preliminary to the Late Formative, thus the length and topology of kernels described for this site are very unusual during Formative Period. Finally, in Tiliviche 1B four cobs were recovered in recent excavations during 2019. They all come from the same unit of excavation. Some of them still have some kernels. The average length of cobs is 72.25 mm, but one of the cobs is 92 mm. They present square and flat seeds. The typology of the cobs matched in terms of size, cupule features, and kernels characteristics with the type 3. The typology is concordant with the direct dates made over three of these cobs (Santana-Sagredo et al. 2021).

The fourth type comes from Alero Cerro Colorado 7, in which one sample was dated to 280 yr BP (17th century). Cobs present an oval shape with a round cupule. The average length 80 mm, and 37 mm in middle diameter with 13 kernels per row. Kernels are oval and round kernels of floury type. Some seeds have pointed tips. The crowns of grains have a small indentation, which is formed when the starch shrinks during drying. Indentation is a feature present only in a few native landraces such as Marcame, Negrito chileno and Harinoso Tarapaqueño (Paratori et al. 1990).



Type	Samples dated	Sites	Location site	Potential growing place	Potential height place	Type of archaeological site	Maize characteristics	Period and radiocarbon date years BP
Type 1		Ramaditas (RAM) (kernel from this cob)	Guatacondo plateau	Desert plateu	1000-1300	Village sorrounded by agricultural fields	small conic cobs, pod corn kernels	Early Formative 2260 ±28 yrs. BP
Type 1		Ramaditas (RAM) (kernel)	Guatacondo plateau	Desert plateu	1000-1300	Village sorrounded by agricultural fields	small conic cobs, pod corn kernels	Early Formative 2041 ±19 yrs. BP
Type 1		Pircas (PIR) (cob)	Tarapacá Ravine, lower portion	Desert plateu	1000-1300	Several disperse enclosures near agricultural fields	small conic to oval cobs	Early Formative 1897 ± 19 yrs. BP
Type 1		Pintados 1307 (PT) (cob)	Salar de Pintados Flat	Desert plateu	1000-1300	Domestic site without structures, domestic debris	small to medium conic cobs, pop corn kernels	Early Formative 1778 ±30 yrs. BP
Type 1		Iluga tumulos (IT) (cob)	North of Pampa del Tamarugal	Desert plateu	1000-1300	Domestic site, manufacturing areas and ceremonial complex	small conic to oval cobs and popo corn kernels	Early Formative 1730 ±28 yrs. BP
Type 1		Caserones (CAS) (kernel from this cob)	Tarapacá Ravine, lower portion	Desert plateu	1000-1300	Village sorrounded by agricultural fields	small to medium conic to oval cobs and pop corn kernels	Late Formative 1638 ±26 yrs. BP
Type 1		Iluga tumulos (IT) (kernel)	North of Pampa del Tamarugal	Desert plateu	1000-1300	Domestic site, manufacturing areas and ceremonial complex	small conic to oval cobs and popo corn kernels	Late Formative 1619 ±30 yrs. BP
Type 1		Caserones (CAS) (kernel)	Tarapacá Ravine, lower portion	Desert plateu	1000-1300	Village sorrounded by agricultural fields	small to medium conic to oval cobs and pop corn kernels	Late Formative 1592 ±24 yrs. BP
Type 1		Iluga tumulos (IT) (kernel)	North of Pampa del Tamarugal	Desert plateu	1000-1300	Domestic site, manufacturing areas and ceremonial complex	small conic to oval cobs and pop corn kernels	Late Formative 1545 ±23 yrs. BP
Type 1		Tarapaca 40 (TA40) (kernel from this cob)	Tarapacá Ravine, lower portion	Desert plateu	1000-1300	Cemetery, maize as offering	medium conic to oval cobs and pop corn kernels	Late Formative 1533 ±27 yrs. BP
Type 1		Tarapaca 40 (TA40) (kernel from this cob)	Tarapacá Ravine, lower portion	Desert plateu	1000-1300	Cemetery, maize as offering	medium conic to oval cobs and pop corn kernels	Late Formative 1522 ± 32yrs. BP
Type 1		Tarapaca 40 (TA40) (kernel)	Tarapacá Ravine, lower portion	Desert plateu	1000-1300	Cemetery, maize as offering	medium conic to oval cobs and pop corn kernels	Late Formative 1478 ±24 yrs. BP
Type 2		Iluga tumulos (IT) (kernel from this cob)	North of Pampa del Tamarugal	Desert plateu	1000-1300	Domestic site, manufacturing areas and ceremonial complex	small conic to oval cobs and pop corn kernels	Late Intermediate Period 1109 ±24 yrs BP
Type 2		PT 2372 (ILP014) (cob)	South of Pampa del Tamarugal	Desert plateu	1000-1300	Domestic site without structures, grinfding stone and pottery on surface	rounded cobs, no kernels found	Late Intermediate Period 920 ±23 yrs.BP
Type 1		Caserones (CAS) (cob)	Tarapacá Ravine, river mouth	Desert plateu	1000-1300	Village sorrounded by agricultural fields	medium conic to oval cobs and pop corn kernels	Late Intermediate Period 830 ±24 yrs.BP
Type 3		Tarapacá 13 (TAR 13) (kernel)	Tarapacá Ravine, lower portion	Ravine zone	2000	Complex village	medium to large conic, oval and round cobs, podcorn kernels	Late Intermediate and Late Period. 379 ±24 yrs.BP
Type 3		Tarapacá Viejo (TAR VIE) (kernel)	Tarapacá Ravine, lower portion	Ravine zone	2000	Complex village , ceremonial display, manufacturing mineral areas	medium to large conic, oval and round cobs, podcorn kernels	Late Period 352 ±25 yrs.BP
Type 3		Tarapacá Viejo (TAR VIE) (kernel)	Tarapacá Ravine, lower portion	Ravine zone	2000	Complex village , ceremonial display, manufacturing mineral areas	medium to large conic, oval and round cobs, podcorn kernels	Late Period 326 ±24 yrs.BP
Type 4		Alero Cerro Colorado 7 (ACC7) (kernel from this cob)	Cerro Colorado Area	Ravine zone	2600	Rock shelter	conic and oval cobs, podcorn and indentate kernels	17th century 280 ±29 yrs.BP

Figure 3: Classification of maize types: maize samples characteristics, radiocarbon dates, and contexts.

Quantitative analysis results

Results on cobs

The length of cobs and the number of rows per cob vary widely among sites (Figure 4, Supplementary information Table 2). The PCA over cobs shows that PC1 and PC2 explain 92% of the variance. The length of cobs is the most important variable in PC1 (Figure 5), whereas the number and length of rows are the most important variables in PC2. PC2 separates Tarapacá 40 maize (Late Formative Period, yellow dots below and right of de biplot) from samples of the same period (yellow dots of Caserones) and different periods. The other maize samples are overlapped. It is interesting to note that samples from the Early Formative Period, including Ramaditas, Pircas and PT1307 cobs (red dots) are mixed with samples from Tarapacá 13 and Tarapacá Viejo (Late Intermediate Period and Late Period, blue dots and light blue dots, respectively).

The length of cobs also varies widely among sites. The Coefficient of Variation (CV) ranges between 20% to 47% (Table 1). Caserones early cobs are distinctively more variable in length than the rest of the formative sites (CV 40%). A similar case is shown for PT2373 (CV 47%) and Tarapacá Viejo site (CV 42%) that highlight among the later sites. The CV for the number of kernels per row is particularly interested in Ramaditas showing a lower variability (10%) than the rest of the formative sites. Conversely, Tarapacá 13 and DGA Quipisca present the most variability on the number of kernels per row (CV 46% and 42%). When we compared the Coefficient of variation of cobs between periods (Table 2), we observe that some variables such as length, size of base, tip and number of rows are more variable during the Formative Period than during the Late Intermediate, the Late Period and the Colonial period.

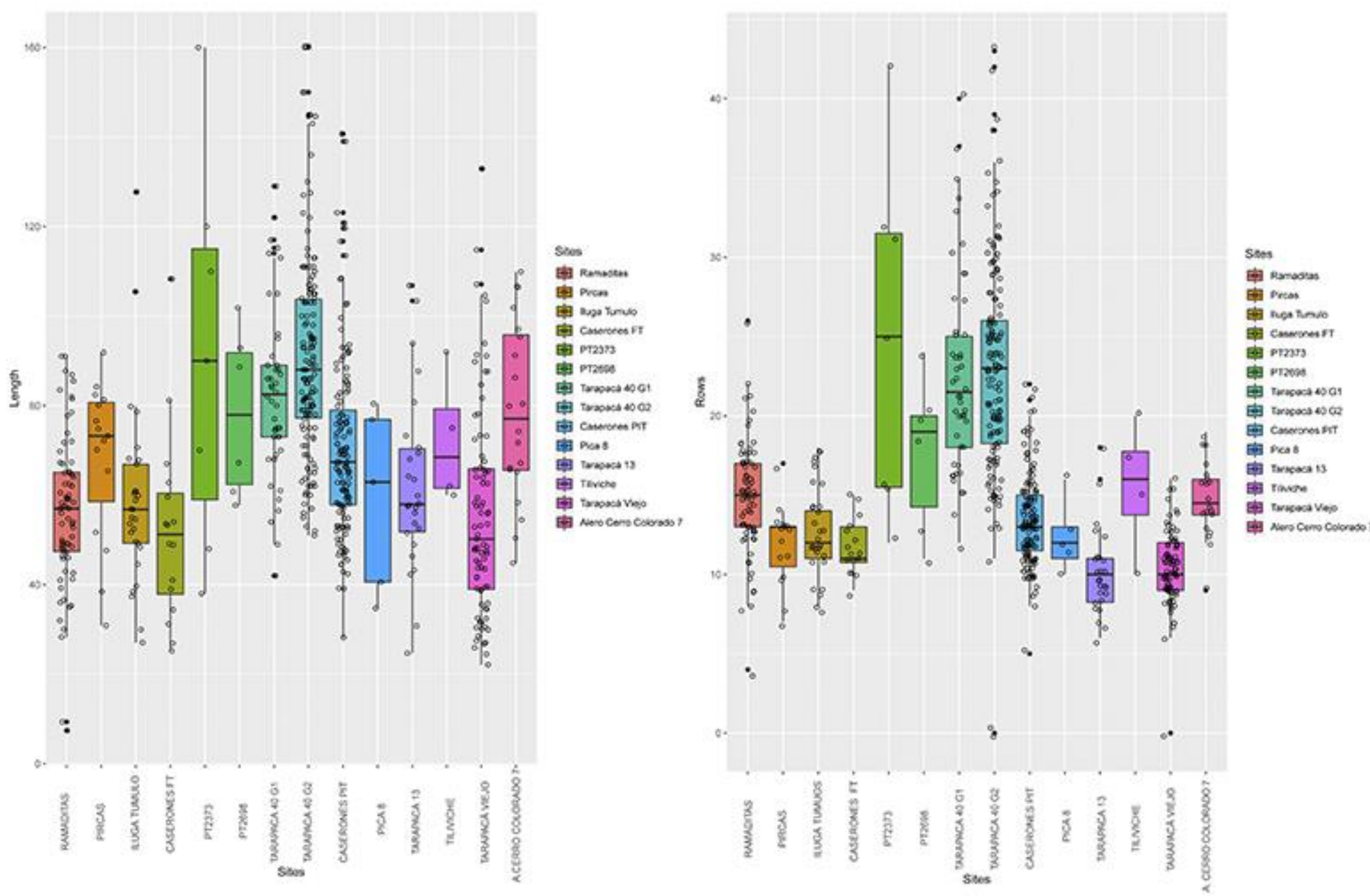


Figure 4. Boxplot of values for length and number of row per site.

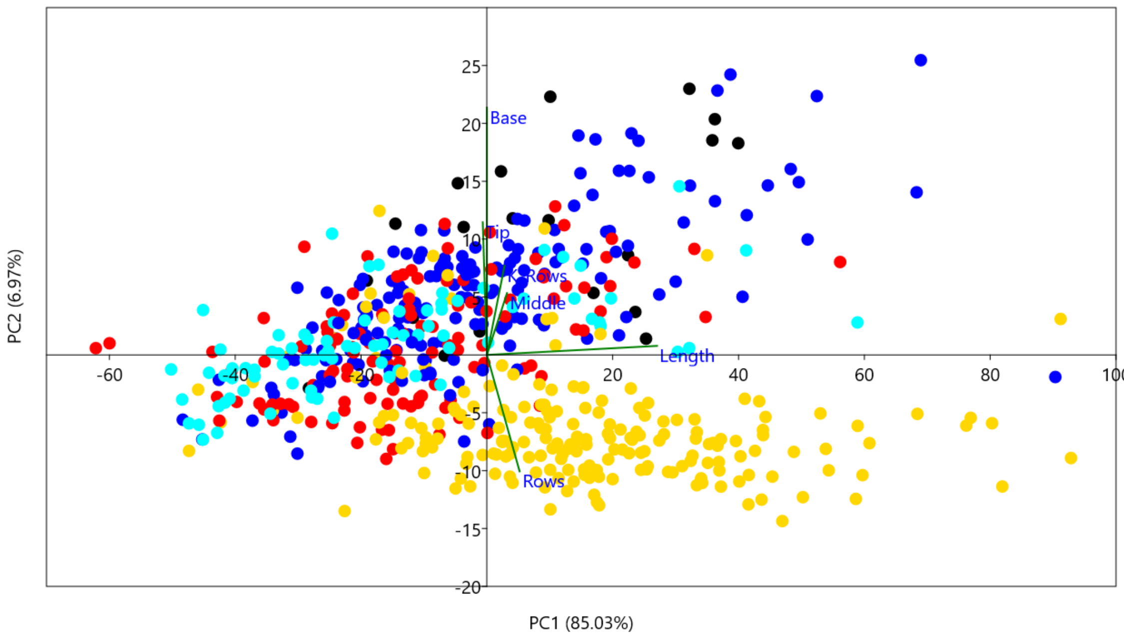


Figure 5. PCA over maize cobs grouped by Periods. Red dots= Early Formative (Ramaditas, Pircas, Iluga Tumulos, PT2373, PT2698), Yellow dots= Late Formative Period (Caserones LF , Tarapacá 40), Blue dots = Late Intermediate Period (Caserones LIP, Pica 8, Tarapacá 13, Tiliviche), Light blue dots= Late Period (Tarapacá Viejo), Black dots=17th century(Alero Cerro Colorado 7).

Coefficient of Variation							
Site	Features (Cobs)						
	Lenght	Base Diamater	Middle Diameter	Tip Diameter	Number of Rows	Average number kernels per Rows	Period
Ramaditas	0.33	0.30	0.23	0.44	0.30	0.10	EFP
Pircas	0.26	0.26	0.16	0.33	0.21	0.36	
Iluga Túmulos	0.35	0.33	0.46	0.39	0.23	0.34	
Caserones FT	0.40	0.28	0.24	0.27	0.15	0.33	LFP
Tarapacá 40 G1	0.23	0.25	0.21	0.44	0.27	0.23	
Tarapacá 40 G2	0.25	0.43	0.19	0.40	0.30	0.23	
PT2698	0.22	0.18	0.13	0.38	0.34	0.31	
Caserones PIT	0.28	0.28	0.25	0.30	0.22	0.32	LIP
PT2373	0.47	0.26	0.18	0.36	0.44	0.38	
Pica 8	0.35	0.26	0.18	0.43	0.21	0.27	
Tarapaca 13	0.32	0.28	0.18	0.32	0.27	0.46	
Tiliviche	0.20	0.07	0.08	na	0.27	0.17	
Tarapacá Viejo	0.42	0.21	0.17	0.31	0.23	0.38	LP
A. Cerro Colorado 7	0.25	0.35	0.29	0.42	0.16	0.25	Colonial
DGA Quipisca	0.25	0.27	0.27	0.11	0.12	0.42	Modern

Table 1. Coefficient of variation of cob traits grouped by site.

	Coefficient of variation					
Period	Length	Base Diameter	Middle Diameter	Tip Diameter	Number of Rows	Average number kernels per rows
Early Formative	0.32	0.33	0.26	0.42	0.26	0.30
Late Formative	0.25	0.42	0.25	0.42	0.27	0.20
Formative Period	0.33	0.48	0.28	0.55	0.36	0.25
Late intermediate	0.31	0.30	0.24	0.35	0.32	0.35
Late Period	0.42	0.20	0.17	0.31	0.19	0.38
Colonial	0.25	0.35	0.29	0.42	0.16	0.25

Table 2. Coefficient of Variation of cobs grouped by periods. Formative period includes Early and Late formative.

Results on kernels

The quantitative features of kernels in length and width show two groups (Figure 6) (Supplementary information Table 3). The first group integrates all the samples from the Early Formative Period and Late Formative Period including Tarapacá 40. The second group is formed by the Late Intermediate Period and Late Period. The same two groups are recovered by PCA analysis on kernels (Figure 7). PC1 and PC2 explain 96% of variance, being the length of kernels the most important variable in PC1 (loading=0.867).

The variability obtained by CV indicates that in the formative period, Iluga Túmulo presents the highest variability in size (length and width 24%), while for the Late Intermediate and Late Period Tarapacá Viejo shows the most variable values (length 25%, width 24% and thickness 30%) (Table 3 and 4).

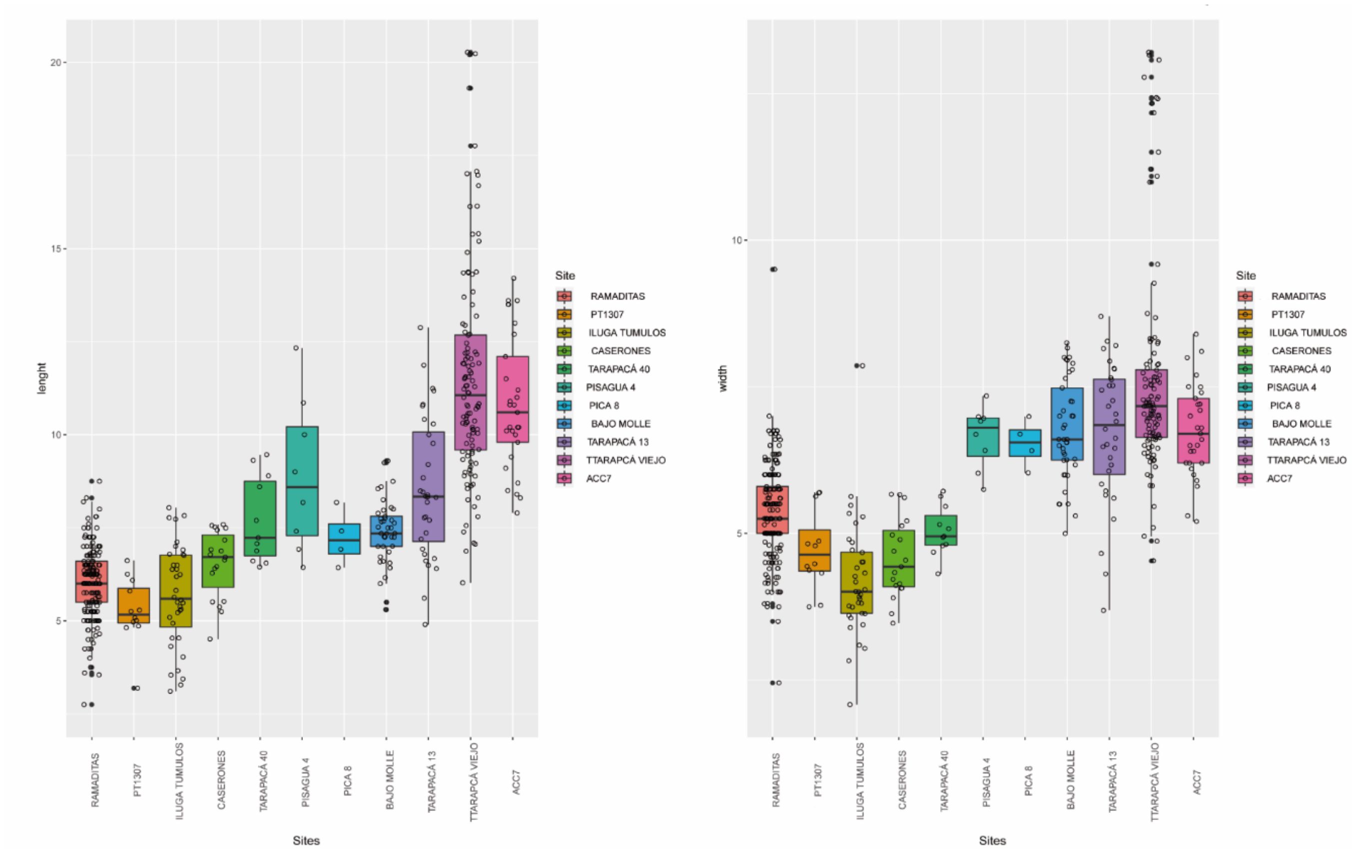


Figure 6. Comparative box plots length of kernels and width of kernels. Corn type 1 present in Ramaditas, PT1307, Iluga Túmulos, Caserones, Tarapacá 40 and Pica 8. Type 3 present in Bajo Molle, Tarapacá 13 and Tarapacá Viejo. Type 4 in Alero Cerro Colorado 7.

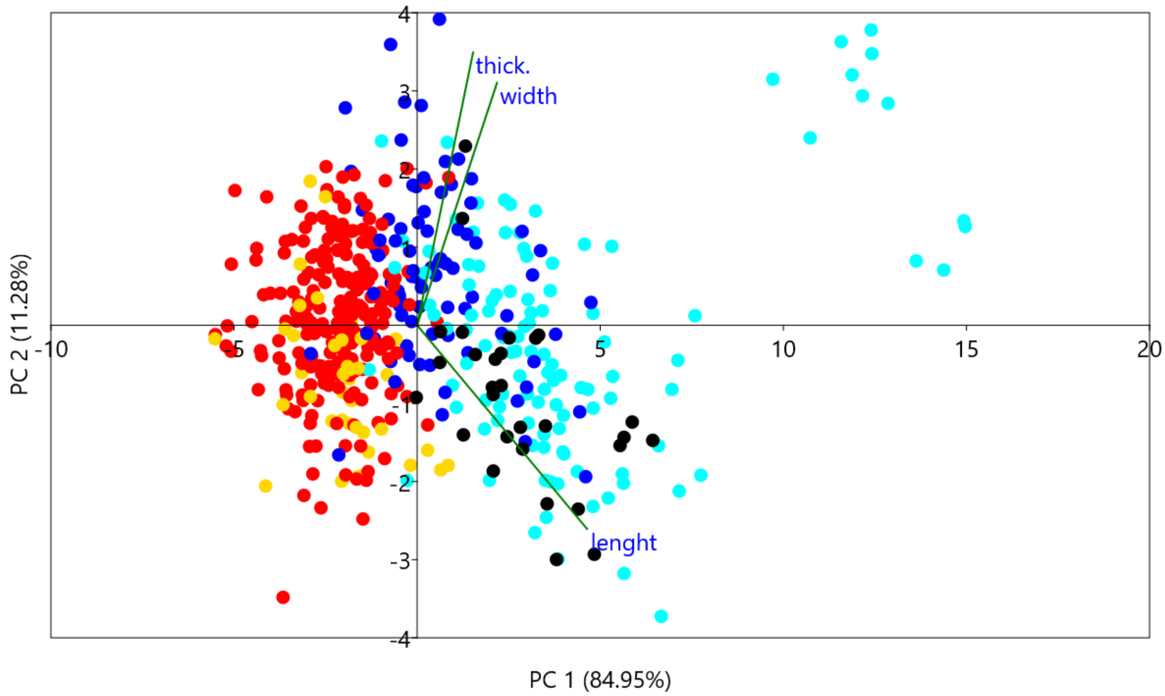


Figure 7. PCA over maize kernels grouped by Periods. Red dots= Early Formative (Ramaditas, Iluga Tumulos), Yellow dots= Late Formative Period (Caserones LF , Tarapacá 40, PT1307), Blue dots = Late Intermediate Period (Caserones LIP, Pica 8, Tarapacá 13, Bajo Molle), Light blue dots=Late Period (Tarapacá Viejo), Black dots= 17th century maize.

	Coefficient of variation			
Site	Features (Kernels)			
	Lenght	Widht	Thickness	Period
Ramaditas	0.15	0.15	0.15	EFP
PT1307	0.17	0.14	0.17	
Iluga Túmulos	0.24	0.24	0.16	
Caserones	0.14	0.15	0.21	LFP
Tarapacá 40	0.14	0.07	0.11	
Pisagua 4	0.23	0.08	0.10	
Pica 8	0.10	0.06	0.12	LIP
Bajo Molle	0.12	0.13	0.18	
Tarapacá 13	0.22	0.18	0.16	
Tarapacá Viejo	0.25	0.24	0.30	LP
A.Cerro Colorado 7	0.17	0.12	0.12	Colonial

Table 3. Coefficient of variation on features for kernels.

	Coefficient of Variation		
Site	Lenght	Width	Thickness
Early Formative	0.17	0.18	0.17
Late Formative	0.21	0.19	0.21
Formative Period	0.20	0.18	0.17
Late Intemediate	0.19	0.15	0.17
Late Period	0.25	0.24	0.30
Colonial	0.17	0.12	0.12

Table 4. Coefficient of Variation of kernels grouped by periods. Formative period includes Early and Late formative.

CONCLUSION

We conclude that Tarapacá maize has low variability in morphological features. Thus, we found only four types of maize for the whole agricultural sequence. During a 1000 years the popcorn type (type 1) predominated in the archaeological record. It is present at the beginning of the cultural Formative Period, and it tends to diminish during the Late Intermediate Period. Whether due to cultural or agroecological reasons, this one type was chosen by tarapacá farmers as the main crop. We postulate that the presence of this unique kind and its perseverance throughout time reflects the adaptation of this particular variety to salty soils and challenged irrigation conditions of the Atacama Desert. Similar phenotypical types are described for analogous environments of Paracas and Northwest Argentina´s agriculture (Bonavia 2013; Lema 2014). In the case of Northwest of Argentina, a pop corn type is dated ca. 3000 yr BP. On the other hand, Paracas cultures dates from ca. 2750 yr BP. We believe the popcorn type adaptation was not a particular event only for Tarapacá and it was an early introduction from neighbors' areas. However, this affirmation needs to be further corroborated. Type 2 and Type 3, the rounded cob and the floury type with large flat seeds, respectively, are present during the Late Intermediate Period mostly. So far, we have not seen these varieties for early moments. Type 2, however, is for now scarce in the archaeological record, which suggests that it was a later introduction that was not successful. Type 3, on the contrary, seems to be well represented in sites such as Tarapacá Viejo and Tarapacá 13, and also at the coastal sites, being a late introduction in the region, before the presence of the Inca state, but enhanced by the Inca culture.

When we compared the Coefficient of variation of cobs (Table 2), we observe that some variables such as length, size of base, tip and number of rows are more variable during the Formative Period, than the Late Intermediate, the Late Period and the Colonial periods. The exceptions are the length of cobs for Tarapacá Viejo (42%) and tip size (42%) and middle diameter (29%) in colonial samples. But, in general terms, and considering all features for the Formative samples' cobs, these are more variable (Table 2).

When comparing sites, Caserones and Ramaditas reach more than 30% of the variability in length size. Tarapacá Viejo also shows 42% of the variation in the same feature but a small variation in the rest of the characters.

An overall impression is that cob size is a feature that was not under total control by farmers of the Formative period, hence the variability of size when comparing with LIP, LP and colonial time. Even in Caserones, which is postulated as a center of the domestic and ritual life of Tarapacá (Uribe and Vidal 2012), there is no clear evidence farmers are selecting the maize size they are using.

A second feature is the number of rows. We observe that between Formative and LIP there is no significant difference in the number of rows (15.9 and 15.6 average, respectively) and the variation (36% and 32%, respectively). On the contrary, Tarapacá Viejo only site for the Late Period is 10.42 rows per kernel ant the internal variation of this character is 19%. During Colonial times the variation is even smaller at 16%. In the case of Formative cobs the smaller amount of rows is related to the small width of cobs. In the Late period site, the reduced number of rows is due to the augmentation of kernels's sizes, which is not proportional to the width of cobs, thus reducing the space for more rows. Once more, these values support the idea that the selection specific features in cobs was accomplished during later times of tarapacá agriculture.

For kernels length coefficient of variation (Table 4) there is no significant difference between Formative (20%) and LIP samples (19%). But in opposition to cobs, kernels during the Late Period are more heterogeneous in size (25%). However, when compared to specific sites such as Iluga Túmulos, Pisagua 4, and Tarapacá 13, they present similar coefficient variation values. So we have not a reliable interpretation for this variation. One alternative is that internal dispersion in Tarapacá Viejo could be to the incorporation of seabird fertilizer (Santana-Sagredo et al. 2021), that overall augmented size of kernels rapidly and at the same time affected the management of this character. Also, changes in water conditions could have influence kernels size variation.

In general terms, we do observe a progressive augmentation of the size of kernels from the Formative to the Late Intermediate and to Late Period (Figure 6 and Figure 7). In traditional agriculture, small cobs are due to the early harvest of plants, poor soil nutrition, and stress water conditions. On the other hand, big cobs can be produced when water's optimal conditions, soil nutrition, and temperatures are managed. Then, we suggest that small sizes of cobs and kernels and the variation in size during Formative Period reflect the initial circumstances of desert agricultures for Tarapacá.

One interesting exception is Tarapacá 40 that highlights having the highest number of rows (average 22) and the largest cobs of Formative Period samples, regardless of whether they kept the kernels attached to the cobs (Figure 4, Supplementary information Table 2). We believe that Tarapacá 40 maize was selected due to the special conditions of serving as burial offerings. We think that cobs were chosen carefully to fulfill the living community's expectations and their ancestors. In contrast, other offerings found in Tarapacá 40 burials, specifically the miniatures pots, baskets, and textiles, were made expeditiously for this only purpose (Catalán, 2006). Thus maize behaves as an important eco-artifact where this materiality marks the inscription of individualities for the formative populations. The selection of maize in Tarapacá 40 emphasizes the importance of agriculture products for the community in terms of symbolic communications and corn's polysemic conditions.

Additionally, maize features' variability in size characters shows that maize production for a long time in Tarapacá was not under a central production. We could expect that under central production, cobs' aesthetics and desired features (for instance, colors, distribution of row, and size.) are homogenized. But this is not the case of Tarapacá maize in which size, the number of rows and number of kernels per row differ between periods and sites (Figure 4, Supplementary information Table 2). Instead, we postulate that family organization levels produced maize. Moreover, if maize behaves as an identity firm for farmers, showing the abilities and knowledge to produce particular corn characteristics, we expect a well deal of differentiation between maize at a family or clan organization level. We believe this is the case for Tarapacá at least during the Formative and Late Intermediate Period. Besides, there is no replacement of maize varieties for hundreds of years in Tarapacá. Farmers could have access to other races of maize from Valles Occidentales (Arica), Northwest of Argentina, or

San Pedro de Atacama, with which they exchanged other resources. Instead, they had a conservative strategy, supporting the idea that Tarapacá popcorn maize played a role in identity terms.

These family or clan production conditions shift to a more controlled circumstance when Inca State is present through Tarapacá Viejo settlement. Thus, kernel size augmented notoriously, and certain characters of cobs were less variable. We do not know that sea bird guano was applied to this sample, explaining the growing of seed (Santana- Sagredo 2021). Nevertheless, this does not explicate the low variabilities in diameter and number of rows in cobs.

Finally, according to this study, we postulate that farmers of Tarapacá preferred for cultural and environmental reasons popcorn type 1 for a long time. As stated before, popcorn type adapted to Pampa del Tamarugal agriculture and endured storage conditions. At some point, popcorn type was abandoned as the primary production leading the way to floury types. This shift was before the Inca state; thus, it can not be explained as recurring to a state imposition. Instead, during the Late Intermediate period, Tarapacá communities established new social relationships with Altiplano groups and partially abandoned Pampa del Tamarugal to higher lands. The floury type 3 of corn was probably introduced from this area, providing new flavors, new colors, and larger kernels that made them more desirable for Tarapacá cultural preferences. Finally, farmers also focused their efforts to improve the size of kernels and progressively reduced the variation in size´s character cobs, making clear the complex relationship between human groups and maize.

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SUPPLEMENTARY INFORMATION

Site	Location	Material	Laboratory ID	14C yrBP	From	to	Reference
Ramaditas	*Quebrada Guatacondo*	Maize cob	TO-4810	2210±55	-260	na	Rivera 2006
Caserones 1	*Quebrada de Tarapacá*	Maize	Beta 61806	1750 ±70	140	520	Oakland 2000
Tarapacá 6-7	*Quebrada de Tarapacá*	Maize cobs	Beta 65304	1680± 60	249	567	Oakland 2000
Caserones 1	*Quebrada de Tarapacá*	Maize cob	OxA 39453 / CSR-A13	1605± 18	425	568	Santana-Sagredo et al. 2021
Caserones 1	*Quebrada de Tarapacá*	Maize	Beta 61805	1470± 80	436	766	Oakland 2000
Ramaditas	*Quebrada de Guatacondo*	Maize cob	IVIC-168	1175± 90	885	na	Rivera 2006
Tana Norte	*Quebrada de Tiliviche/Tana*	Maize cob	OxA 39455/ TAN-A1	1030± 17	1016	1145	Santana-Sagredo et al. 2021
QM-22E	*Quebrada de Maní*	Corn cob	UGAMS-84336	985± 15	1021	1152	Gayó et al. 2012
Cerro Pintados 3	*Pampa del Tamarugal*	Maize cob	Beta 150710	970± 50	1024	1210	Briones et al. 2005 en Núñez and Briones 2017
Tiliviche 1B	*Quebrada de Tiliviche/Tana*	Maize	AA 56416	920 ±32	1045	1227	Rivera 2006
QM-2A	*Quebrada de Maní*	Maize canes	UGAMS129007	870 ±25	1050	1255	Gayó et al. 2012
Tiliviche 1B	*Quebrada de Tiliviche/Tana*	Maize cob	OxA 39542/ TIL-A3	925± 18	1053	1215	Santana-Sagredo et al. 2021
Pica 8	*Pica*	Maize grains	OxA 39874/ PI8-A1a	903± 17	1157	1219	Santana-Sagredo et al. 2021
Tana Sur	*Quebrada de Tiliviche/Tana*	Maize cob	OxA 39456/ TAS-A1	898± 18	1159	1221	Santana-Sagredo et al. 2021
Tiliviche 1B	*Quebrada de Tiliviche/Tana*	Maize cob	OxA 39877/ TIL-A1	894 17	1160	1223	Santana-Sagredo et al. 2021

Site	Location	Material	Laboratory ID	14C yrBP	From	to	Reference
Tiliviche 1B	*Quebrada de Tiliviche/Tana*	Maize cob	OxA 39457/ TIL-A2	860± 17	1189	1269	Santana-Sagredo et al. 2021
Tarapacá 13	*Quebrada de Tarapacá*	Corn cob	58346	775 ±15	1223	1274	Boytner and Uribe 2009* Ms
Tarapacá 13	*Quebrada de Tarapacá*	Corn cob	58345	770 ±15	1225	1275	Boytner and Uribe 2009 Ms
Pica 8	*Pica*	Maize grains	OxA 39875/ PI8-A34a	731± 17	1279	1381	Santana-Sagredo et al. 2021
Tarapacá 13	*Quebrada de Tarapacá*	Corn cob	58350	645± 15	1287	1390	Boytner and Uribe 2009 Ms
Tarapacá 13	*Quebrada de Tarapacá*	Corn cob	UCIAMS58355	705± 15	1288	1386	Boytner and Uribe 2009 Ms
Tarapacá Viejo	*Quebrada de Tarapacá*	Corn cob	AMSAA82248	618± 39	1289	1405	Zori And Urbina 2014
Pabellón de Pica	*Costa de Tarapacá*	Maize	Beta 256617	600± 50	1301	1445	Uribe-Fondecyt
Tarapacá Viejo	*Quebrada de Tarapacá*	Corn cob	Irvine AMS 58816	495± 15	1413	1440	Zori and Urbina 2014
Tarapacá Viejo	*Quebrada de Tarapacá*	Kernel maize	Beta 269052	350± 40	1450	1650	Zori and Urbina 2014
Alero Cerro Colorado 7	*Área deCerro Colorado*	Maize	Beta 412958	380± 30	1463	1629	De Souza et al. 2017
Tarapacá Viejo	*Quebrada de Tarapacá*	Maize	Beta 269052	310± 40	1493	1797	Uribe et al. 2012
Tarapacá Viejo	*Quebrada de Tarapacá*	Maize	Beta 269052	310± 40	1493	1797	Uribe et al. 2012

Sup. Table 1. Radiocarbon dates of maize from Tarapacá previously reported. Dates reported as the original articles by the authors. No additional calibration was made. Also, the material samples are noted as the original information given in the reports. * Authors gave their authorization to use ^{14}C dates indicated.

Sup.Table 2 Average measures on archaeological cobs from Tarapacá

Site	Number sample of cobs	Archaeological Period	Average Length	Average Base Diam.	Average Tip Diam.	Average Middle Diam.	Average Number of rows	Average of kernel per Row	Location
Ramaditas	73	Early Formative	56.9	14.4	8.68	19.12	14.5	13.4	Quebrada de Guatacondo
Pircas	15	Early Formative	68.05	16.83	9.7	18.67	11.8	14.3	Quebrada de Tarapacá
Iluga Túmulos	29	Early Formative	58.99	9.12	5	14.4	12.8	16.1	Pampa del Tamarugal
Caserones	17	Late Formative	53.8	14.12	9.53	14.72	11.76	13.47	Quebrada de Tarapacá
Tarapacá 40 (G1)	46	Late Formative	82.8	6.42	4.22	15.22	22.6	13.8	Quebrada de Tarapacá
Tarapacá 40 (G2)	142	Late Formative	90.49	7.93	4.4	24.82	22.85	14.2	Quebrada de Tarapacá
Caserones	135	Late Intermediate	70.2	18	10.84	18.3	13.4	16.4	Quebrada de Tarapacá
PT2372	1	Late Intermediate	60	20.17	10	20.17	18	14	Pampa del Tamarugal
Tarapacá 13	26	Late Intermediate	62.3	13.2	9.6	16.14	10.11	12	Quebrada de Tarapacá
Tarapacá Viejo	83	Late Period	55.8	13.4	9.83	15.5	10.42	11.04	Quebrada de Tarapacá
Alero Cerro Colorado 7 (G1)	5	Colonial	72.25	13.22	6.16	17.65	13.4	18.04	Cerro Colorado
Alero Cerro Colorado 7 (G2)	15	Colonial	80.7	23.7	9.19	36.9	15.2	17.1	Cerro Colorado
DGA Quipisca	3	Modern cultivar	59.08	9.21	2.5	9.21	9	17.3	Pampa del Tamarugal
TOTAL	590								
Measures on cobs without radiocarbon direct dates over maize									
Site	*Number sample of cobs*	*Archaeological Period*	*Average Length*	*Average Base Diam.*	*Average Tip Diam.*	*Average Middle Diam.*	*Average Number of rows*	*Average of kernel per Row*	*Location*
Pica 8	5	Late Intermediate	39.1	13.9	5.88	17.2	12.4	13.6	Pica
PT2373	7	ca. Late Intermediate	90.8	16	10	16.9	24.7	21.8	Pampa del Tamarugal
PT2698	6	ca. Early Formative	78.17	17.08	6.46	20.57	17.6	25.5	Pampa del Tamarugal
Tiliviche	4	Late Intermediate	72.25	14.5	2	18.75	15.5	19.75	Quebrada de Tiliviche/Tana
Huaylacan W	120	Late Intermediate	73.1	12.75	7.96	17.5	14.5	19.55	Arica
TOTAL	142								
Total	**732**								

Table 2. Average measures on archaeological cobs from Tarapacá. Archaeological sites, number sample, an archaeological period associated, features, and location of samples cobs. G1 = cobs with lost of kernels, G2= include cobs with kernels.

Sup Table3 Average measures on archaeological kernels from Tarapacá

Archaeological kernels with direct radiocarbon dates						
Site	***Number sample of kernels***	***Archaeological Period***	***Average Length***	***Average Widht***	***Average Thickness***	***Localidad***
Ramaditas	192	Early Formative	6.03	5.34	4.64	Quebrada Guatacondo
Pt1307	12	Formative/Late Forma	5.26	4.71	4.25	Pampa del Tamarugal
Iluga Túmulos	36	Early Formative	5.68	4.2	3.69	Quebrada de Tarapacá
Caserones	19	Late Formative	6.5	4.56	4.14	Quebrada de Tarapacá
Tarapacá 40	11	Late Formative	7.7	5.04	3.91	Quebrada de Tarapacá
Tarapacá 13	32	Late Intermediate	8.56	6.84	5.7	Quebrada de Tarapacá
Tarapacá Viejo	112	Late Period	11.55	7.59	6.12	Quebrada de Tarapacá
A. Cerro Colorado 7	29	Colonial	10.82	6.75	5.29	Área Cerro Colorado
Total	**443**					
Archaeological kernels witouh direct radiocarbon dates.						
Site	***Number sample of***	***Archaeological Period***	***Average Length***	***Average Widht***	***Average Thickness***	***Localidad***
Pica 8	4	Late Intermediate	7.23	6.53	5.27	Pica
Bajo Molle	41	Late Intermediate	7.39	6.75	5.36	Costa Tarapacá
Pisagua 4	4	Late Intermediate	10.54	6.74	5.49	Costa Tarapacá
Total	49					
TOTAL	**492**					

Table 3. Average measures on archaeological kernels from Tarapacá. Archaeological sites, number sample, archaeological period associated, features and location of samples kernels.

4. Maize characteristics and archaeological contexts.

Ramaditas maize

Ramaditas is situated at the north ridge of the Guatacondo basin, described as complex site with various conglomerated compounds (Mostny, 1971; Rivera and Dodd,2013).

The corn cobs length from 28.3 mm to 91 mm with mean size of 57.8 mm. Middle diameter range from 8.5 mm to 29.5 mm. The mean number of rows are 15 (Sd 3.57), but some reached 26 rows similar as described previously (Tartaglia, 1980). The average kernel per row is 13 (Sd). Most cobs present conic shapes.

Kernels size range from 2.7 mm to 8.75 mm with a mean value of 6 mm. They present to shapes: rounded and square. They characteristics of the coat is thin, and some pop up kernels found among the sample indicates they are the popcorn type.

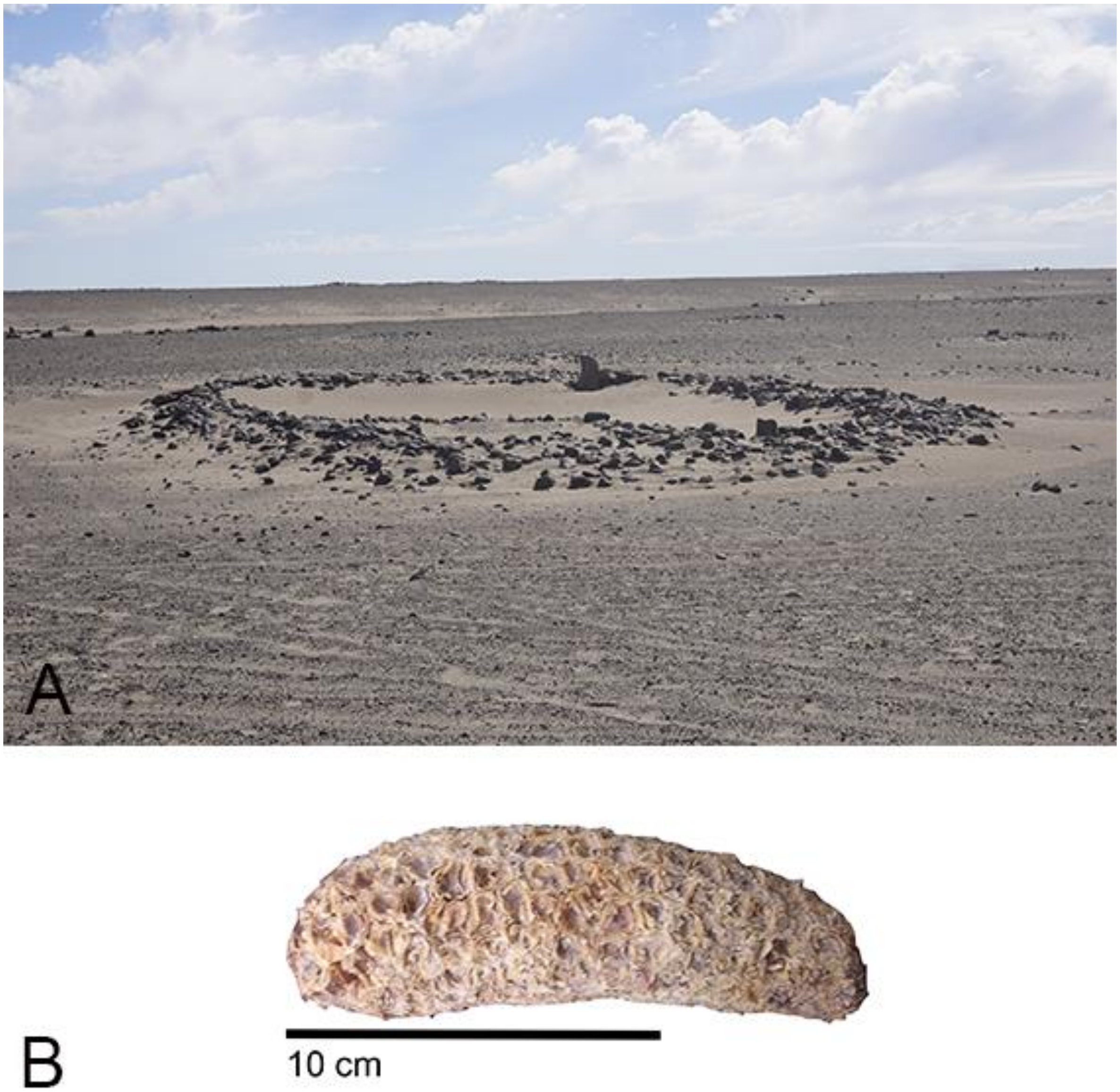
Overall, these values indicate small and thin cobs. The reduced size of these cobs question if they were mature cobs or still in developed. However, the kernels associated are mature grains. There, we can presume that the small size is due to ecological conditions and properties of the variety. All cobs have rounded cupules which match the form of kernels.

Two kernels samples were dated ranging from 2260 ± 28 yr BP to 2041 ± 18 yr BP (cal. 376- 206 BC and cal. 56 BC-45 AD) (Figure 1).

Sup. Figure 1. A and B Cobs and kernels from Ramaditas, C: general view of the main compound of the site. Large bar =8 cm, small bar= 1cm. The author took all the pictures in 2018.

Pircas maize

Pircas cobs length range from 30.8 mm to 91.85 mm with a mean size of 68 mm. Middle diameter range from 12.6 mm to 23.8 mm. The mean number of row are 11 (Sd 2.47). The average kernel per row is 14. Most cobs present conic and oval shapes. We have no kernels samples from this site. One cob dated to 1897 ± 19 yr BP (cal. 114- 224 AD) (Figure 3B).

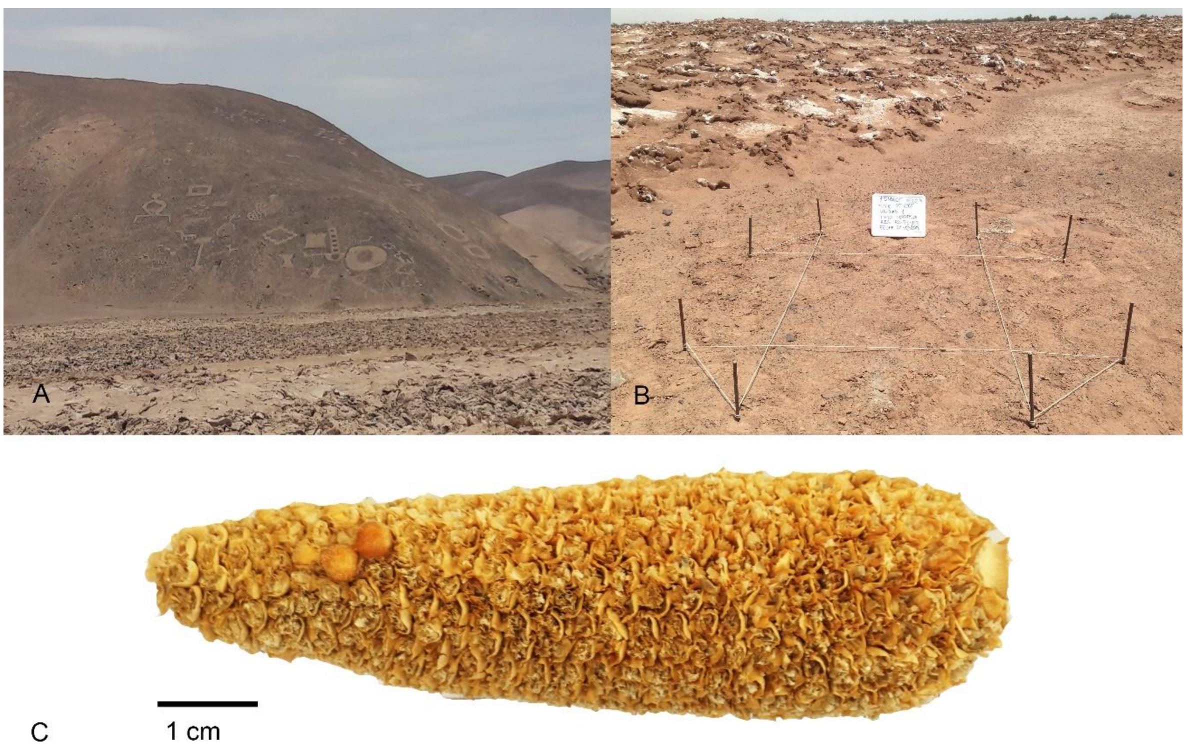


Sup. Figure 2 A: General view one of the enclosure of Pircas, B: Cob dated from Cal. 114- 224 AD. Bar = 10 cm. The author took all the pictures in 2017.

Pintados 1307 maize

Near the Pintados Salt flat, a discrete domestic occupation was excavated during 2016 by Mauricio Uribe and collaborators. It was described as a domestic site with various cultural materials like Loa Café Alisado pottery, malacological and archaeobotanical elements, copper ore, and lithics debris. This site is dated from cal. 390-475 AD and cal. 382-523 AD. Additionally, we dated directly one cob for 1778 ±30 yr BP (Cal. 234-376 AD). This cob has a conic shape, 88.62 mm length, 22.41 mm diameter, 20 rows, and an average number of 28 kernels and it still had some kernels (Figure 4C).

Kernels size ranged from 3.19 mm to 6.62 mm in length, with a mean value of 5.2 mm. They present just rounded shape and the and are probably the popcorn type.



Sup. Figure 3. A: Salar de Pintados and Geoglyph, B:Pintados 1307, at the back of image is the Salar de Pintados, C: cob dated from Cal. 234-376 AD. Bar = 1 cm. The author took all the pictures in 2016.

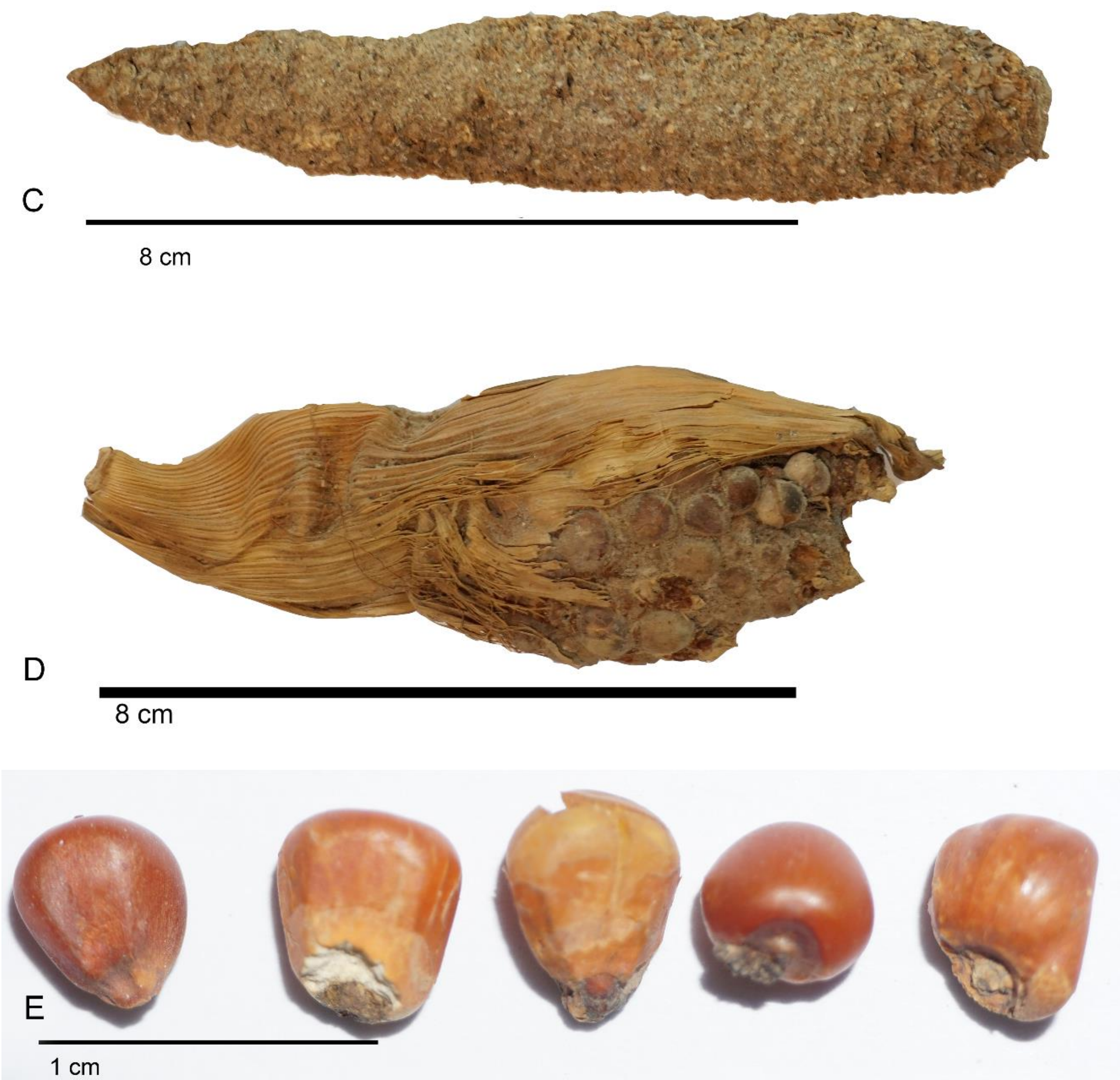
Iluga Túmulos maize

Iluga Túmulos is a heterogeneous site located north of PdT, with domestic occupations, agricultural production, diverse manufacturing areas, and a ceremonial display characterized by more than 100 artificial tumuli (Uribe et al. 2020) (Figure 5A, 5B). Preliminary radiocarbon dates and ceramic analyzes indicated that it was occupied from the Early Formative Period to Colonial times (Uribe et al. 2020). During 2016 we excavated four units distributed in different functional areas of the site. We obtained two radiocarbon dates from the bottom of these excavations (terminal levels), indicating that debris dated from Cal. 132-215 AD and Cal. 120-237 AD (date of Units 1 and 2). To have some stratigraphic control, we decided to evaluate the cobs and kernels from only these units (units 1, 2, and 3) and run three additional radiocarbon dates over two cobs and one kernel. However, one of the cobs did not come from the excavation units but a surface collection. We analyze this cob (id. 1000IT) because of its particular morphology that reminds us of the Capio Chileno Chico. Dates over maize are as follow: 1730 ±28 yr BP (cal. 250-416 AD, cob), 1619 ± 30 yr BP (cal.525-634 AD, kernel), 1545 ± 23 yr BP (cal. 525-634 AD), and 1109 ± 24 yr BP (cal. 897-1025 AD, 1000IT). The first date corresponds to a cob characterized as conic, with 14 number row and small round cupule (Figure 5C). The kernel is rounded with 4 mm of diameter (Figure 5E), and the third date corresponds to a cob of round shape, 60 mm length and 12 rows (Figure 5D).

The cobs length ranged from mm to 27.08 to 127.7 mm, with a mean size of 58 mm (Sd 20.58 mm). Most of the large cobs are from unit 3, associated with Inca ceramic type (Late Period). Middle diameter ranged from 6.27 mm to 33.1 mm. The mean row number is 12 (Sd 2.92), but some reached 18 rows. The average kernel per row is 16. All cobs have conic shapes, except for the dated cob, which presents a round form.

Kernel's size ranged from 3.11 mm to 8.04 mm with a mean value of 5.68 mm. They present rounded and square shapes. The thin coat and some popup kernels found among the samples indicate they are the popcorn type.

Sup. Figure 4A: Tumuli of Iluga Túmulos site, B: Agricultural fields toward NE of Iluga Tumulos site. The author took all the pictures in 2016.



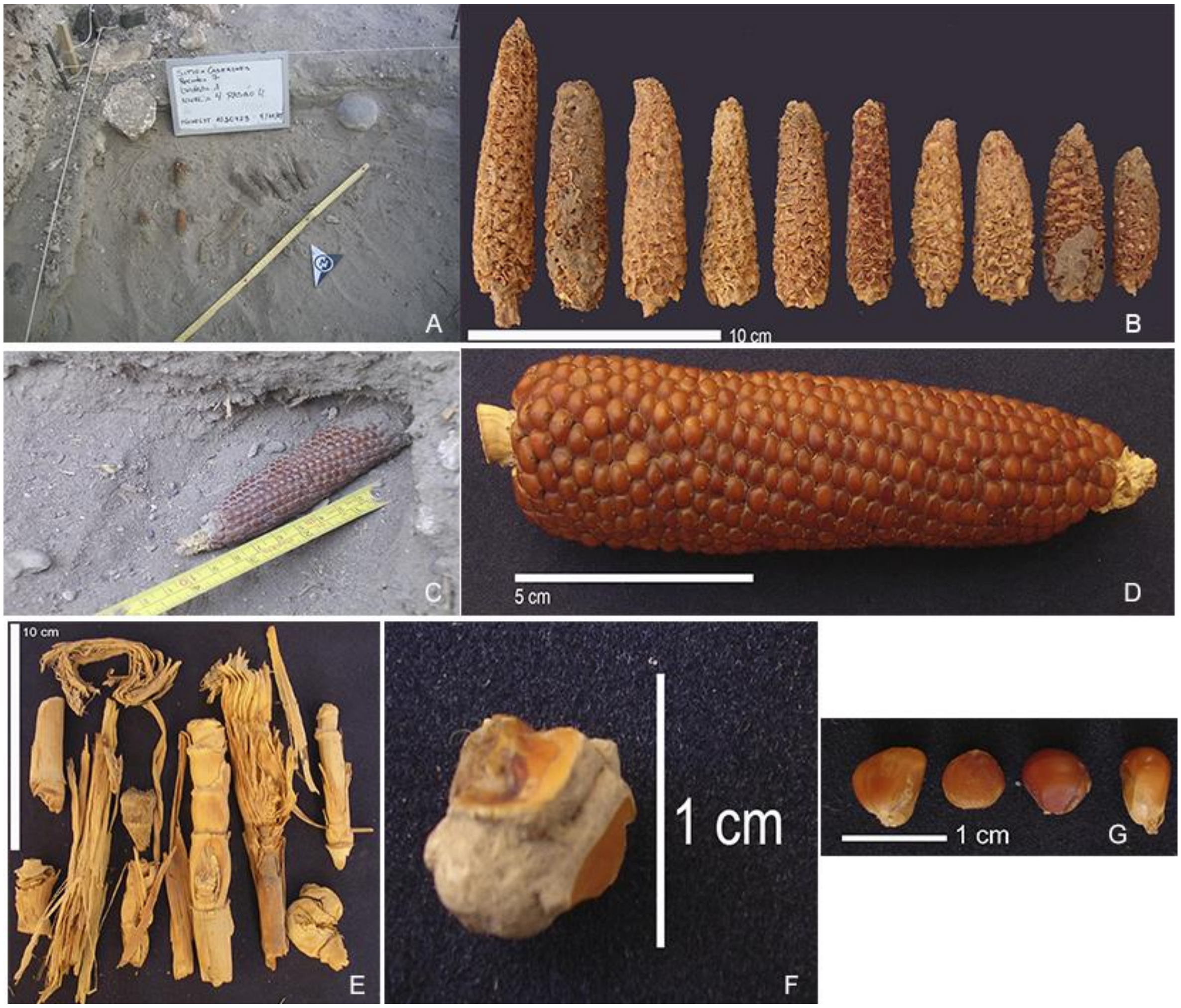
Sup. Figure 4. Maize dated from Iluga Túmulo. C: cob dated Cal. 250-416 AD (cob), D: cob dated Cal. 897-1025 AD (1000IT), E: kernel dated Cal.525-634 AD. The author took all the pictures.

Caserones maize

Caserones village highlights due to the storage units containing a large number of vegetables remain (Figure 6A to 6E). Among these remains, we analyzed a total of 152 cob corns from two periods: Late Formative Period (n=17) and Late Intermediate Period (=135). Additionally, we measured 19 kernels. We dated two kernels that ranged from 1638 ± 26 (cal. 407-538 AD) 1592 ± 24 (cal. 432-577 AD) and (Figure 6C and 6D), and one cob dated on 830 ± 24 yr BP (cal. 1214-1279 AD) (Figure 6A and 6B).

The total length of cob corn ranged between 25.2 to 149 mm, with a mean value of 68.8 mm. The middle size range is from 4.5 mm to 34.6. The variance is due to the time distance between these two groups. We have previously approached this difference (Vidal et al. 2016), showing the statistical variance of size cobs in Caserones. The mean row number is 13, and the average number of kernels is 16. But some specimens reach 32 kernels per row. Kernels have a length between 4.51 mm to 7.59 mm. The mean value in length is 6.5 mm. The kernels observed have rounded and square shapes.

Sup. Figure 5 Caserones surround and site. A: View of Caserones site from the opposite ridge, B: Tarapacá Ravin during 2005, C: Compounds inside Caserones, D: Archaeological agricultural fields at the bottom of Caserones. The author took all pictures in 2005.



Sup.Figure 6 Maize from Caserones 1. A: Archaeological maize in situ (B) during the excavation of compound 7 of Caserones, B: maize cobs of enclosure 7 dated from Cal. 1214-1279 AD., C: Archaeological cob in situ (D) during excavation of compound 526, D: cob from enclosure 526 level 3B dated from Cal. 407-538 AD, E: maize stalks from enclosure 298, level 7B, F: popcorn from enclosure 298, level 7c. G: kernels popcorn type from enclosure 298. The author took all pictures in 2005 for the project FONDECYT 1030923.

Tarapacá 40 maize

Because Tarapacá 40 is a cemetery, the archaeological cobs were disposed of as offerings, showing excellent preservation conditions. Thus, 46 cobs without kernels and 138 with kernels were measured.

Cobs without kernels measured from 42 mm to 129 mm in length with a mean size of 82 mm. The middle diameter ranged from 8.6 mm to 24 mm. The mean number of row are 22 (Sd 3.3). The average kernel per row is 13.8. All cobs presented oval shapes.

Cobs with kernels measured from 51 mm to 160.3 mm in length with a mean size of 90.1 mm. The middle diameter ranged from 11 3m to 36 mm. The mean number of rows are 23 (Sd 6.2). The average kernel per row is 14.4. All cobs presented oval shapes.

Kernels show a range size in length from 6.45mm to 9.46 mm, with a mean value of 7.7 mm. They present round shape and square shapes and correspond to popcorn type.

We dated two cobs from Tarapacá, resulting in: 1522 ±32 yr BP (cal. 530-640 AD) and 1533 ± 27 yr BP (cal. 533-645 AD) (figure 7 cobs A and B, respectively) and one kernel from sector L tomb 9: 1478 ± 24 yr BP (cal. 586-652 AD). The contemporary of dates indicates that maize from TR40 are of the same population corn, though not necessarily from the same harvest, since a 100 years implicates at least around 100 harvest.

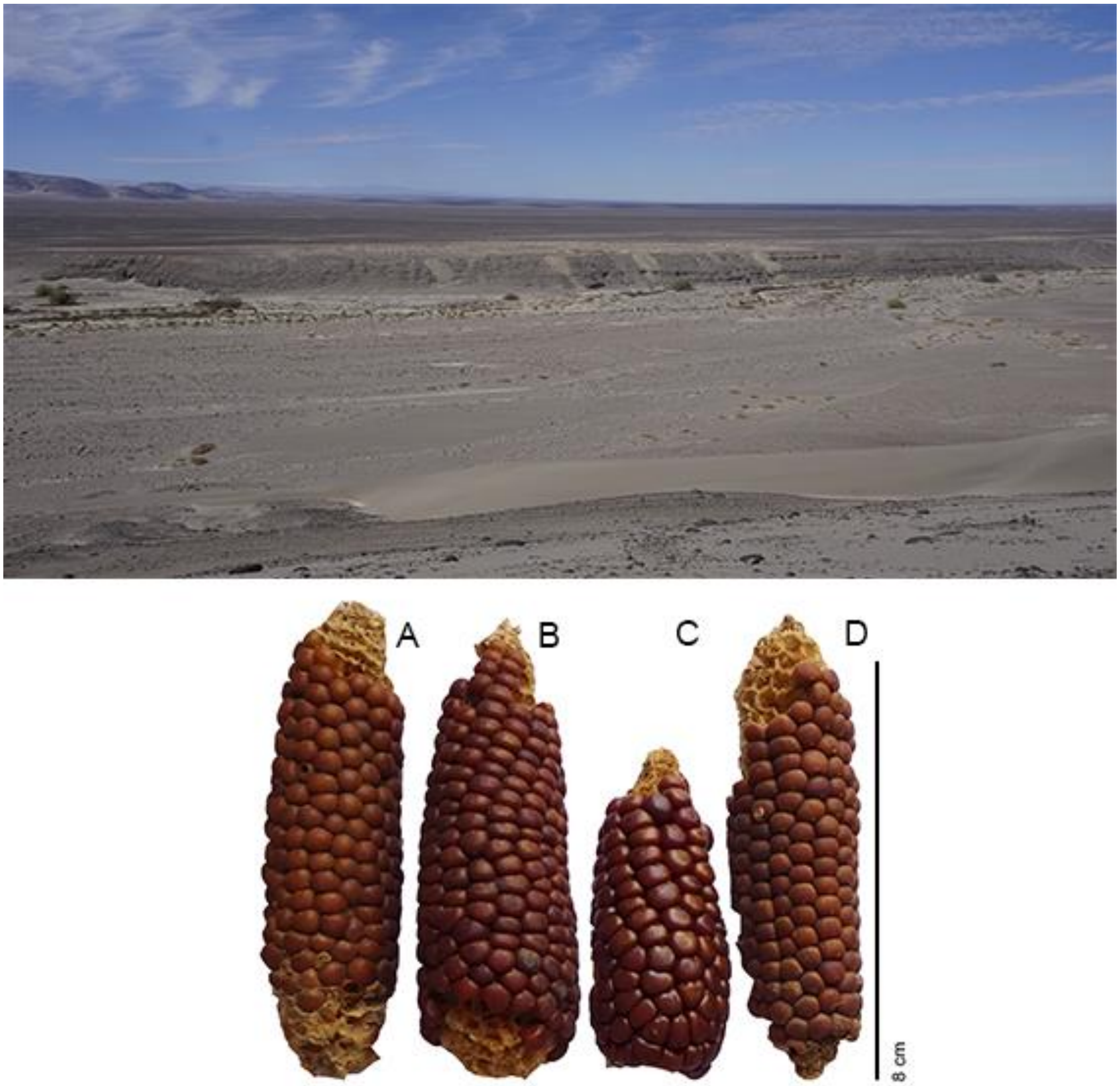
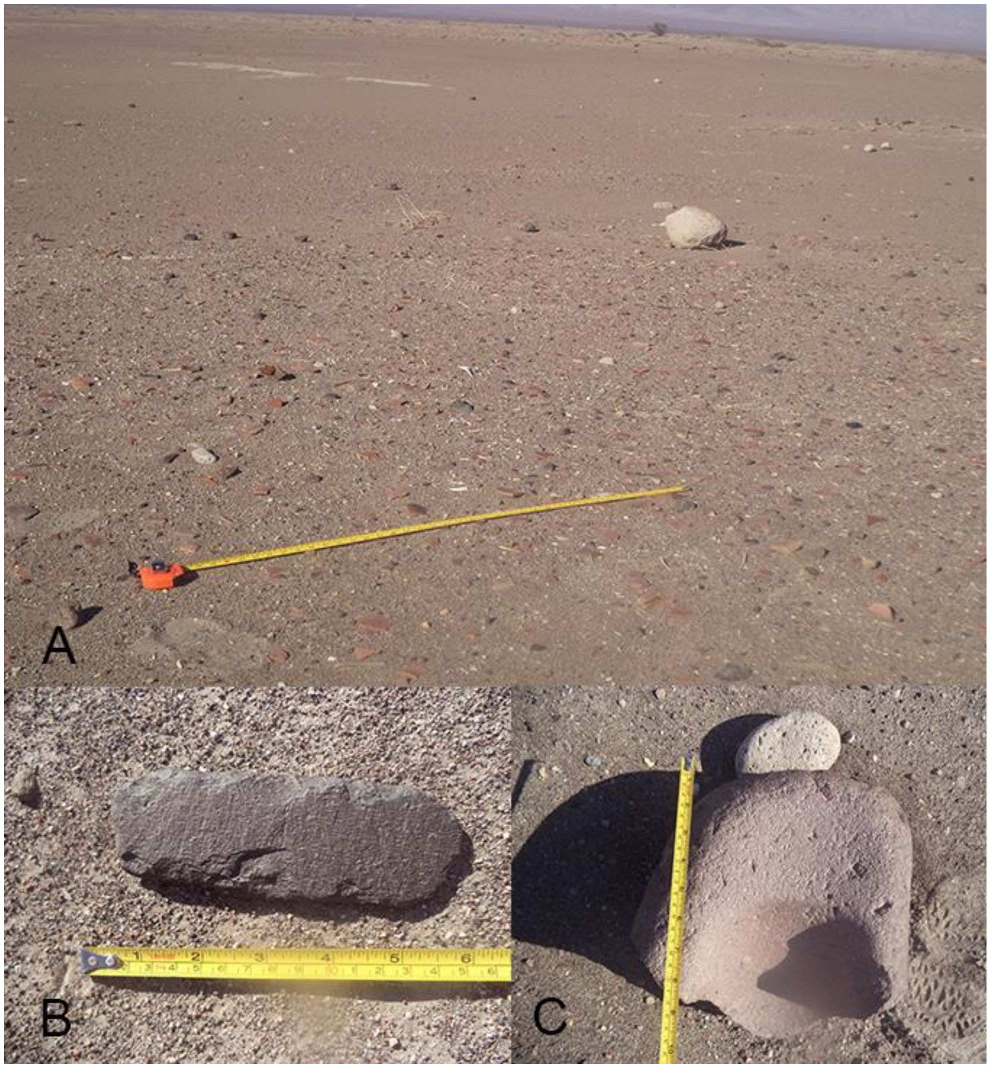


Figure 7 Tarapacá 40 site: View from Tarapacá 40 to the southern ridge of Tarapacá Ravine, A: cob and Cal. 530-640 AD, B: cob dated Cal. 533-645 AD. The author took all pictures in 2016 and 2018.

PT2372 maize

This archaeological site is situated in the southern area of Pampa del Tamarugal. It corresponds to a great dispersion of materials, associated to grinding instruments (conanas and mortars) and lithic tools. There are vegetables, malacological materials and abundant Pica Charcollo pottery of the LIP on the surface. We chose to date one archaeological cob recovered on the surface because of its peculiar shape. The cupules' shape and disposition remind us of the Capio Chileno Chico landrace (Paratori et al., 1990).

The cob is rounded, with a length of 60 mm, 20.17 base diameter, a 10 mm tip, a 20.17 mm middle diameter, 18 rows, and 14 kernels per row (Figure 8D). Interestingly, this cob dated on 920 ±23 yr BP (Cal. 1051-1221 AD). We did not recover any grains from this site.



Sup. Figure 8 PT2372 site. A: general view of the site, B: lithic agricultural shovel, C; grinding stone. The author took all the pictures in 2016.

Sup.Figure 8D: cob maize dated between Cal. 1051-1221 AD. The author took the picture in 2019.

Tarapacá 13 maize

Tarapacá 13 is a small village surrounded by a wall in the southern area of Tarapacá Ravin (Núñez 1983).

Previously, Boytner and Uribe (2009) reported five dates over corn cobs from Tarapacá 13. These dates indicated that maize ranged as follows: 835 ± 20 yr BP, 775 ± 15 yr BP, 770 ±15 yr BP, 645 ±15 yr BP, 705 ±15 yr BP (Boytner and Uribe 2009). All of them belonging to the Late Intermediate Period. In our case, we date one kernel from this site, which surprisingly dated on 379 ±24 yr BP (Cal. 1464-1629 AD), belonging to the latest and final occupation of the site. However, our samples belong to the same Areas, Units, and Locus of the Late Intermediate Period dated maize. Overall, we consider the maize sample to be mostly from LIP. The dated kernel was square to flat form, and measured 7.79 in length, 6.19 in width and 7.33 in thickness. The endosperm was a floury type.

The total length of cob corn ranged between 24.68 to 106.8 mm, with a mean value of 62.3 mm. The middle size range is from 7.69 mm to 23.14 mm. The mean row number is 10, and the average number of kernels is 12. But some specimens reach 27 kernels per row. Cobs show different types of forms such as elongated (Fig. 9A), conic (Fig.9B, 9C) and round (Figure 9D and 9E). This last shape also has a pointed tip and, again, remind us of Capio Chileno Chico native landrace. Also, shape of the cupules are elongated according to the new kernels that appears, more flat and square.

Kernels have a length between 4.9 mm to 12.88 mm. The mean value in length is 8.5 mm. In this site, there is a change in kernels shapes. We observed round, square, plain, and elongated forms.

Figure 9 Maize cobs from Tarapacá 13. A: elongated cob, B and C: conic cobs, D and E: round cobs. The author took all the pictures.

Tarapacá Viejo (Tarapacá 49) maize

Tarapacá Viejo is situated at the south margin of Tarapacá river in front of the modern town of San Lorenzo de Tarapacá. It belongs principally to the Late Period (Late Horizon), has a short occupation during the Late Intermediate Period and the last occupation during the 16th and 17th century (Núñez, 1984; Uribe and Zori 2012) (Figure 10A,10B, 10C). Before, Zori and Urbina (2014) reported two dates over maize: a) over a corn cob dated on 495 ±15 yr BP b) and over a kernel dated on 350 ±40 yr BP . Our own date over two kernels indicate a range from 352 ±25 yr BP to 326 ± 24 yr BP (Cal.1496-1643 AD and Cal. 1504-1653 AD, respectively) These results coincide with preceding reports. The kernels dated are floury types, flat and oval shape. The first one measured 20.24 mm in length, 13.07mm width and 9.93 mm in thickness. The second one measured 11.55, 7.74 and 5.6 mm.

The cobs length ranged from mm to 22.11 to 132.9 mm, with a mean size of 55.8 mm (Figure 10 D). Most of the large cobs are from unit 3, associated with Inca ceramic type (Late Period). Middle diameter ranged from 9.23 mm to 23.5 mm. The mean row number is 10, but (Sd 2.3), but some reached 16 rows. The average kernel per row is 10.73, but some cobs reached 22 kernels per row. The shapes of cobs are rounded, elongated, conical and oval.

Kernel's size ranged from 6.02 mm to 20.27 mm length with a mean value of 11.55 mm (Sd=2.9 mm) Figure 10 E). They present flat and rectangular shapes. The endosperm is flour type.

Sup Figure 10 Tarapacá Viejo site. A: General view of Tarapacá Viejo, B: Detail of the Inka road (QapacÑan) in Tarapacá Viejo, C: Petroglyph of Tarapacá Viejo. The author took all the pictures in 2016.

Sup. Figure 10 cobs Samples from Tarapacá Viejo. D: Diverse cobs from Tarapacá Viejo. The author took all the pictures.

Sup. Figure 10E: kernels from Tarapacá Viejo. The author took all the pictures.

Alero Cerro Colorado 7 maize

Alero Cerro Colorado is a rock shelter situated over 2600 m above sea level around 30 km east of Pozo Almonte town. It corresponds to a ceremonial site occupied mostly during the Early Formative Period, dated from ca. 390 BC-250 AD. (De Souza et al., 2017). Several cobs of corn were deposited in offering pits into the shelter (Figure 11A). Despite the early dates, the cobs dated for 380 ± 30 yr BP (De Souza et al., 2017). The authors explain the divergence in dates as constant reoccupation of the place due to its ceremonial character for the Tarapacá communities. We dated one kernel from cob corn to be sure of the previously reported date. Our results are quite similar, with a date of 280 ±29 yr BP (Cal. 1511-1799 AD). Because of the sample's homogeneity and the two concordant dates we proposed, this was a unique offering event. Additionally, it is probably that all cobs belonged to the same populations (same harvest).

In particular, this cob (Figure 11B) measured 66.14 mm length, 34 mm middle diameter and presents 14 rows with an average kernel of 17.

We measured the cobs with kernels on them and five cobs without seeds (Figure 11C) for our purpose of comparison. The length range for cobs without kernels is 44.82 mm to 97.05 mm in length, with a mean value of 72.25 mm. The middle diameter ranges from 12.44 to 24.7 mm, with a mean value of 17.65 mm. The row number goes from 9 to 19. The kernels per row are 9 to 24. The color of kernels are yellow, but the bare cobs are deep red. Cobs have two main shapes: conic and oval and some pointed tips. One attractive characteristic is that seeds cover the whole cob. Kernels are average 10.82 of length, 8.4 width and 4.2 of thickness. These kernels differ from the rest of the sample because of their round and pointed shapes. Additionally, all cobs show some grains with indentation.

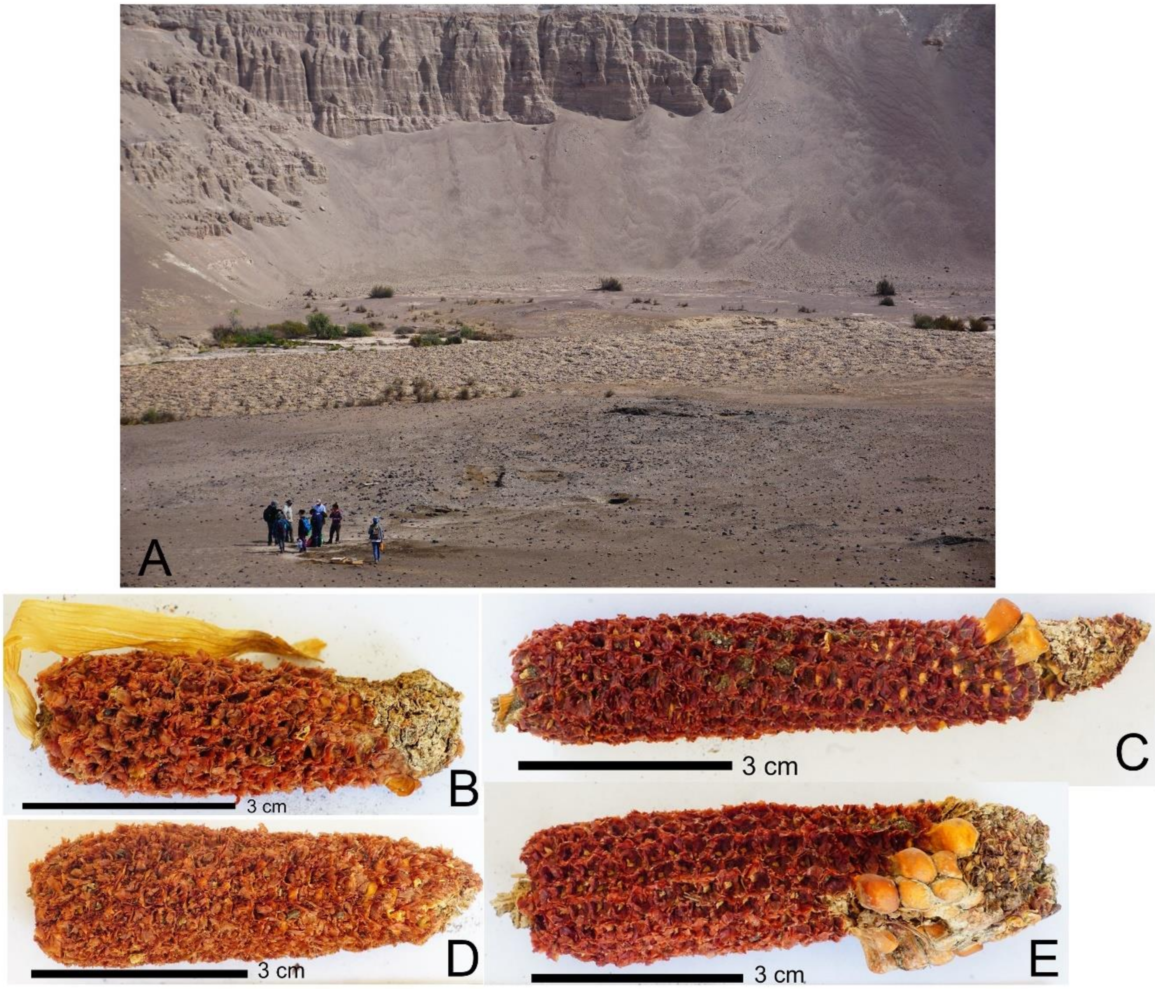
Sup. Figure 11. A: Cerro Colorado. Cobs corn during the process of excavation. Image published in De Souza et al. 2017.

Figure 11 B: Cerro Colorado cob dated for Cal. 1511-1799 AD, C: bare cobs from Alero Cerro Colorado 7. The author took all the pictures.

Tiliviche 1B maize

Tiliviche is situated 25 km east to the coast of Pisagua. Is in the second terrace of Tiliviche ravine, where today there is no water. However, we can still see patches of vegetation that support some aquatic plants such as *Cortaderia* sp. and *Phragmites* sp. that suggest that in the past water was available. Tiliviche 1B was dated from the Archaic Period from 7810-4100 BC and 3235-2720 BC (Núñez 1986). The stratigraphic location of maize cobs let Núñez (1986) to postulate that maize of Tiliviche 1B was from the Archaic Period near the 5900 BC. However, posterior radiocarbon dating made directed over the cobs indicates they belong to the Late Intermediate Period, with a date of 920 ±32 yr BP (Rivera, 2006). Concordantly, Santana-Sagredo et al. 2021 dated three new cobs as follows: 925 ±18 yr BP, 894 ±17 yr BP, 860 ±17 yr BP (Figure 12 B, C and E).

These three cobs have an average length of 72.5 mm and average row of 15.5 per cobs. They present conic and oval shape and one of them has a pointed tip (Fig. B). Cupules are rounded in concordance with the kernels of shape. Two of them (Fig C and E) present square elongated kernels, floury type.



Sup. Figure 12 Excavations of Tiliviche 1B during 2019 and maize. A: The cobs were recovered during 2019 excavation (FONDECYT 1130279), B= cob dated 1160-1223 AD, C=cob dated 1189-1269, D= cob recovered in the same units as the other dated cobs, E= cob dated 1053-1215 AD. The author took all the pictures.

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CHAPTER 2

2000 YEARS OF AGRICULTURE IN THE ATACAMA DESERT LEAD TO CHANGES IN THE DISTRIBUTION AND CONCENTRATION OF IRON IN MAIZE

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Keywords: archaeological maize, iron, histology, Perls/DAB

ABSTRACT

We performed a histological and quantitative study of iron in archaeological maize seeds from prehispanic times recovered from Tarapacá, Atacama Desert. Also, we examined iron distribution changes at the cell level in embryos from ancient versus new varieties of maize. Our results show a progressive decrease in iron concentration from the oldest maize to modern specimens. We interpret the results as an effect of prehispanic agriculture over the micronutrient composition of maize.

INTRODUCTION

There is no consensus among agronomic, nutritional, and chemical sciences on the changes suffered by micronutrients such as iron, zinc, copper, and manganese in cereal crops (1). The unequal conditions of the experiment that difficult comparisons (2), discrepancies in statistical methods (3), and the lack of historical data (4) causes these contradictory positions. The controversy deepens when it comes to the iron accumulation in maize because iron is quite variable (9.6 to 63.2 mg kg-1) in modern maize cultivars (5). Moreover, despite the nutritional importance of maize today, with more than 713 million tons of annual production (6), there are limited studies on the physiological changes associated with the absence or presence of micronutrients throughout the history of maize´s agronomic production.

Intensive agriculture for thousands of years among prehispanic agricultural communities in Tarapacá, Atacama Desert (lat. 19°00´ 21°00´S), led to changes in maize morphology. Thus, our previous studies (7) indicated that Tarapacá prehispanic farmers modified phenotypically their ancient maize. These modifications included the significant augmentation of cobs and kernels size from a mean length of 5 cm to more than 15 cm length for cobs. Kernels size augmentation ranges from 5 mm to more than 10 mm in length. Accordingly, Tarapacá maize is clearly differentiated between two phenotypic groups: a first group dated ca. 400 BC-500 AD and a later group dated ca. 500 AD to modern times (Supplementary Figure 1). Tarapacá maize also shows a low genetic diversity caused by human selection and genetic similarities between the prehispanic and modern maize (7).

A more recent process of plant selection has been studied in terms of nutritional quality. The Green Revolution had a negative impact in the total among micronutrients in cereals (8, 9, 10). Until date, the nature of these changes is still unknown. Then, the opportunity to evaluate the micronutrient distributions and accumulation directed over ancient maize could determine if all agricultural selection, indistinctive of time and techniques, results in the loss of micronutrients in cereals.

Additionally, different methods and plant models have been used to determine where micronutrients accumulate in seeds. Focusing on iron as a model, it has been shown, by a histological approach, that this essential micronutrient accumulates in vacuoles of a specific cell layer in the *Arabidopsis thaliana* mature dry embryos (11). Iron distribution in maize has not been studied extensively. Recently, using Perls staining on maize showed that iron accumulates principally in the embryo close to the endosperm face (12).

In this article, we used histological and quantitative methods to observe changes over iron distribution and iron concentration in archaeological and modern maize in continuous agricultural production in Tarapacá, Atacama Desert. Thus, we asked whether the loss of iron in crops could also be observed in archaeological maize, which was under a constant agriculture selection in prehispanic times.

MATERIAL AND METHODS

Plant samples and radiocarbon dating

Tarapacá region is situated in the northern part of Chile, and the hinterland is part of the Atacama Desert characterized as a hyper arid environment crossed by several basins. Today only two basins have permanent runoff (Tarapacá basin and Camiña basin). The archaeological sites selected for this study are all situated in the same cultural area. Thus, representing different moments of the material, social and cultural life of prehispanic communities of Tarapacá.

A total of 65 samples were analyzed. Fifty-nine archaeological maize samples were used as follows: 19 kernels were dated, 18 kernels were used for histological analysis, and 22 kernels were used for iron quantification. Additionally, 3 modern kernels were used for histological analysis, and other 3 more modern kernels were added for iron quantification. The criteria to choose these samples were the preservation of the material and the number of kernels available for each site. In some cases, samples were limited. Then, we prioritized radiocarbon dating samples. The sample belonged to the following periods and archeological sites: Early Formative Period (ca. 3000- 1450 yr BP, Ramaditas), Late Formative Period (ca. 1450-750 yr BP, Pintados and Tarapacá 40), Late Intermediate Period (ca. 750-500 yr BP, Tarapacá 13), Late Period (ca. 500-400 yr BP, Tarapacá Viejo), Colonial Period (ca. 430-300 yr BP, Alero Cerro Colorado 7). The archaeological sites are four domestic contexts (Ramaditas, Pintados, Tarapacá 13, and Tarapacá Viejo), one funerary context (Tarapacá 40) and one ceremonial deposit (Alero Cerro Colorado 7). Archaeological kernels were cultivated locally near the sites mentioned and deposited as debris in the domestic contexts. For Tarapacá 40 and Alero Cerro Colorado 7 samples were deposited as offerings. The archaeological samples are corn types and floury types (Supplementary Figure 1). Modern samples were collected from Camiña basin (lat. 19°18`S, lon. 69°25`W) in traditional cultivars in 2014. The modern landraces were identified as Capio Chileno and Harinoso Tarapaqueño, and one European cultivar from INRAE-France.

Radiocarbon dating was made by DirectAMS radiocarbon dating service laboratory, Seattle, Washington, USA. The samples were calibrated using the OxCal v.4.3.2 online software service, using the SHCal13 atmospheric curve. All the technical parameters of the radiocarbon dating analysis are included in the Supplementary Table 1.

Sample preparations and Perls/DAB staining

Maize kernels were longitudinally sectioned using a blade before the fixation step. The fixation and Perls/DAB staining were performed following previous studies (13) (19) (20). Briefly, all samples were vacuum infiltrated with a fixation solution (2% w/v paraformaldehyde in 1 mM phosphate buffer pH 7.0) for 45 min and incubated for 16 h in the same solution. The fixated material was dehydrated with different concentration of ethanol (50%, 70%, 80%, 90%, 95% and 100%), then incubated 12 h with a solution of butanol/ethanol 1:1 (v/v), and finally incubated several hours with 100% butanol. The kernels were embedded in the Technovit 7100 resin (Kulzer) according to the manufacturer's instructions and thin sections (3 µm) were obtained. For Perls/DAB staining, slides were incubated with 2% (v/v) HCl and 2% (w/v) K-ferrocyanide (Sigma Aldrich) for 45 min. For the DAB intensification, each glass slide was washed with distilled water, and incubated in a methanol solution containing 0.01 M NaN_3 (Sigma Aldrich) and 0.3% (v/v) H_2O_2 (Merck) for 1 hour, and then washed with 0.1 M phosphate buffer (pH 7.4). For the intensification reaction, the glass slides were incubated in 0.1 M phosphate buffer (pH 7.4) solution containing 0.025% (w/v) DAB (Sigma), 0.005% (v/v) H_2O_2, and 0.005% (w/v) $CoCl_2$*6H2O. To stop the reaction the slides were rinsed with distilled water. The samples were observed and photographed with a Nikon Eclipse 80i microscope.

Iron quantification

Fifteen milligrams of ground material (dry weight) per sample (one kernel) was mixed with 750 mL of nitric oxide (65% [v/v]) and 250 mL of hydrogen peroxide (30% [v/v]) prior to homogenization. Following 10 min of incubation at room temperature, the samples were mineralized using the microwave digestion systems (Berghof). Once mineralized, the nitric oxide proportion present in the samples was adjusted to 5 to 10% of the final volume by adding ultrapure water. Iron content present in the samples was then measured by microwave plasma atomic emission spectroscopy (Agilent Technologies).

RESULTS

Dating of Archaeological Kernels

The kernels used in this analysis came from seven archaeological sites. All the locations are in the same area of the Atacama Desert, close to each other, in the Tarapacá region (see Material and Methods and supplementary Fig 1.).

To ensure the dates of Tarapacá maize we performed 19 radiocarbon dates directly on maize kernels and cobs from various sites of Tarapacá, including 10 dates for the samples reported in this study. The distribution of these age ranges is from 2260 to 280 yr BP (cal. 376 BC to 1799 AD, 95.4% of probability) (Figure 1 and Supplementary Table 1). This range covers a 2000 years' time span including five different periods of the Tarapacá culture (from the Early Formative Period; ca. 3000-1450 yr BP, to Colonial Period; ca. 430-300 yr BP; Figure 1).

The most ancient sample recovered for study is a popcorn kernel from Ramaditas site, dated on 2260 ± 28 yr BP (cal. 376-206. BC (95.4% of probability; Figure 1). These results show for certain that maize was already present at the southern part of Tarapacá at least from ca. 2400 yr BP and its production continue without interruption until ca. 1350 yr BP. Then, we noticed a disruption in maize around 1350 to 1050 yr BP, which could be due to a sample methodology bias, but almost for sure it is due to the temporary discontinuance of agriculture.

Thus, other organic material also lacks dates for these moments. Radiocarbon dates indicate a return to agricultural maize practices around 750 yr BP that continues intermittently to present times (Supplementary Figure 1).

Iron quantification and distribution in the archaeological kernels

Quantitative analysis of iron content in individual kernels from the six archaeological sites and the modern variety is shown in Figure 1. The kernels from Ramaditas site (2260 to 2041 yr BP , Figure 1) reached a high mean value of total iron concentration 36.6 ppm, while kernels from Alero Cerro Colorado 7 site (dated for 17th century, Figure 1), do not exceed a mean of 13.05 ppm, corresponding to less than 36% of total iron concentration compared with the kernels from Ramaditas (Figure 1). Our results indicate that there is subtle declination of iron total content in the old kernels versus new varieties indicated by the relation between iron concentration in kernels and radiocarbon dates of kernels (R^2=0.1297, p value < 0.043). Though this is small correlation it still allows us to postulate the diminished of iron through time.

Histological sections for kernels from the six archeological sites and the modern variety were obtained and the cellular structures and iron distribution were evaluated (Figure 2). The analysis of toluidine blue stained sections indicated that cellular structures are well preserved in all the samples, including cell wall, cytoplasmic contents and organelles like nuclei are easily identifiable. No nuclei were detected in the endosperm cells indicating that the analyzed kernels correspond to mature grains (Figure 2A, 2C, 2E, 2G, 2I, 2K, 2M). Regarding iron localization, Perls/DAB iron staining was performed on thin sections (Figure

2). Iron is detected in the cytoplasm and cytoplasmic structures embryo cells in all samples, while endosperm do not accumulate detectable iron by this histological method. No staining is observed in apoplast, indicating that iron pools are intracellular in embryo cells. Interestingly, nuclei are strongly stained in embryo cells from older kernels (ca. 1550 to 1350 yr BP ; Figure 2B, 2D, 2F). However, nuclear iron pools are no longer detected in embryo cells in samples from Tarapacá 13 ca. 450 yr BP to actual kernels (Figure 2H, 2J, 2L). It should be noted that the no detection of nuclear iron in the embryo cells in samples from ca. 450 yr BP to actual kernels is not due to differences in the samples preservation, as indicated by the toluidine blue staining where nuclei are perfectly observable (Figure 2G, 2I, 2K). Additionally, protodermis is less stained by Perls/DAB staining in the samples from ca. 450 yr BP to actual kernels compared to the older samples (Figure 1 and Figure 2).

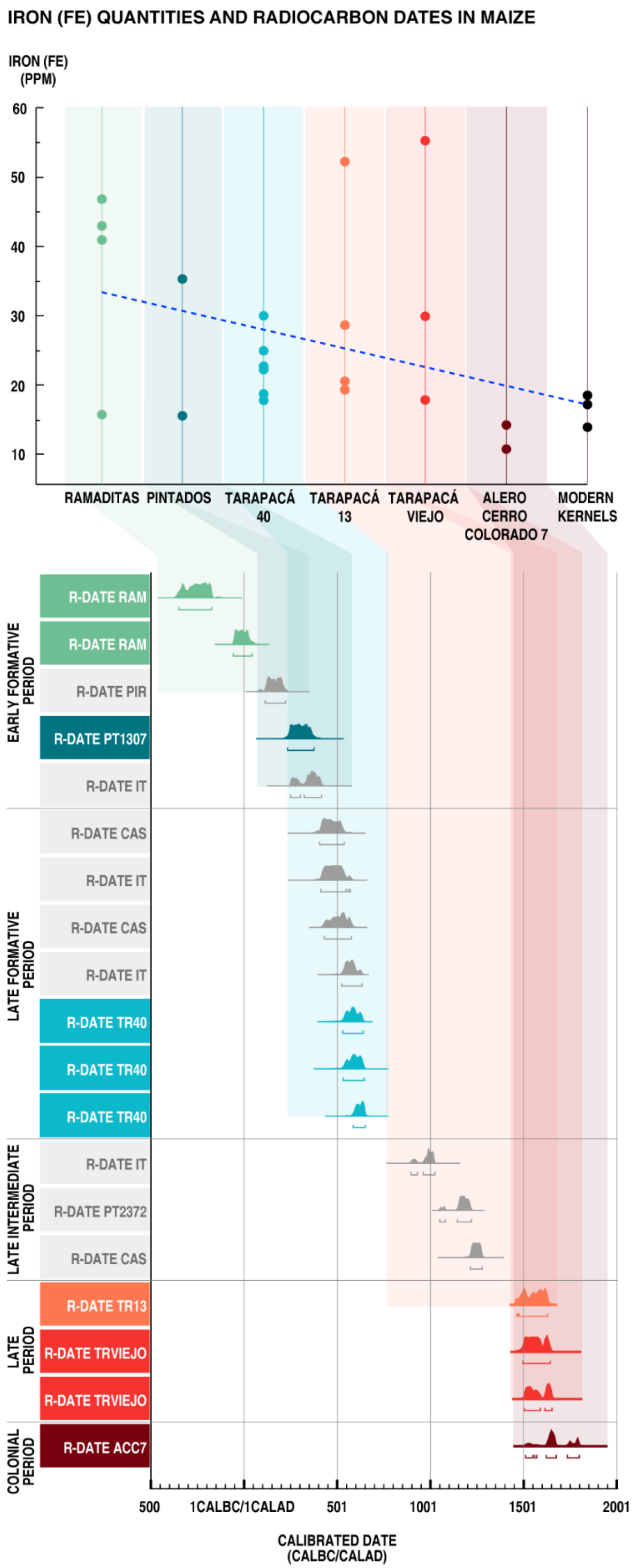

Figure 1. Iron (Fe) quantities and radiocarbon dates in maize. Quantifications of iron concentration in kernels are shown in color dots. Each site has been assigned to a different color and a time period. Lineal regression is expressed in the dotted line. Lineal regression results are: F-statistic=4.57(1.23), R^2 =.166, R^2adjusted=.129, p-value=0.0432. Radiocarbon dates are indicated in color bars which are the sum of probabilities for 19 dates. Dates in color are the maize kernels for the same archaeological site used in Fe analysis. Dates in grey are reported for maize kernels from other sites for Tarapacá region. Dates range from ca. 400 BC to ca. 1799 AD.

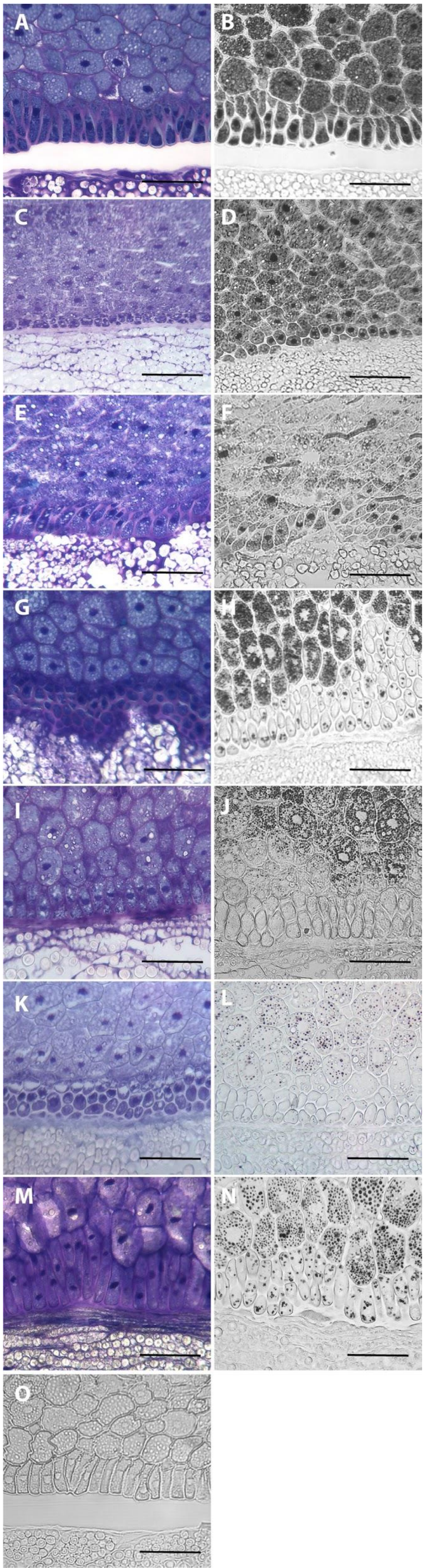

Figure 2. Iron distribution in kernels from archaeological sites and modern samples from Tarapacá revealed by Perls/DAB staining. A-B and O pictures from Ramaditas, C-D pictures from Pintados, E-F pictures from Tarapacá 40, G-H pictures from Tarapacá 13, I-J pictures from Tarapacá Viejo, K-L pictures from Alero Cerro Colorado 7, M-N pictures from modern sample. A, C, E, G, I, K, M correspond to toluidine blue stained sections, used to verify integrity of cells and subcellular structures in the section. B, D, F, H, J, L, N correspond to sections stained by Perls/DAB in order to detect iron, the black labeling correspond to iron. O is a negative control of the Perls/DAB staining (without K-ferrocyanide). For all pictures, embryo and endosperm correspond to Em End, respectively. Arrows indicate some nuclei (for simplicity only in Fig 2A). Bars: 50 µm.

DISCUSSION

In this study, we showed that iron accumulates in the cytoplasm of maize embryo cells (Figure 2). Interestingly, iron is not detected in nuclei of embryo cells in the kernels from ca. 750 yr BP (Tarapacá 13, Tarapacá Viejo, Alero Cerro Colorado 7) to modern kernels, while iron pool is clearly observed in embryo cells from kernels older than ca. 1350 yr BP (Ramaditas, Pintados, and Tarapacá 40; Figure 2). Iron accumulated in nuclei has been described in the literature, but its chemical speciation and function in this organelle remain unknown (13). It has been suggested that nuclei may be a reservoir of iron during seed development (14). Further studies should be performed to address these fundamental biological questions.

During the Early Formative Period (3000- 1450 yr BP) the first agricultural display produced maize kernels with high contents of iron (Figure 1 and Figure 2). During this period, maize had a low degree of manipulation (7,15). Thus, there is a low amount of macrobotanical maize remains in archaeological sites that do not exceed 3% of the total macro botanicals recovered in the period's settlements (16). Also, isotope studies ($\delta^{13}C$ and $\delta^{15}N$) in human remains indicate a gradual transition to the consumption of C4 plants among the Tarapacá groups (17). At the end of the Late Formative Period (ca. 1450-750 yr BP.), cultivation fields are extended to the north and south of Tarapacá (18), kernels and cobs increased size (7), people are consuming maize (17), and we observed iron loss in maize embryos (this study).

Finally, our results suggest that a process of prehispanic agriculture of 2000 years in Tarapacá desert could modify maize kernels nutritional quality, what has also been observed as a consequence of the Green Revolution in the last 50 years (10). Changes in iron distribution at subcellular levels is an interesting focus of research to understand the causes of micronutrients loss in kernels maize by agriculture practices and selection. The use of archaeological seeds could help to understand the collateral effects on nutritional quality of crops under long term agricultural practices, which is a crucial trait and a global challenge for worldwide agriculture.

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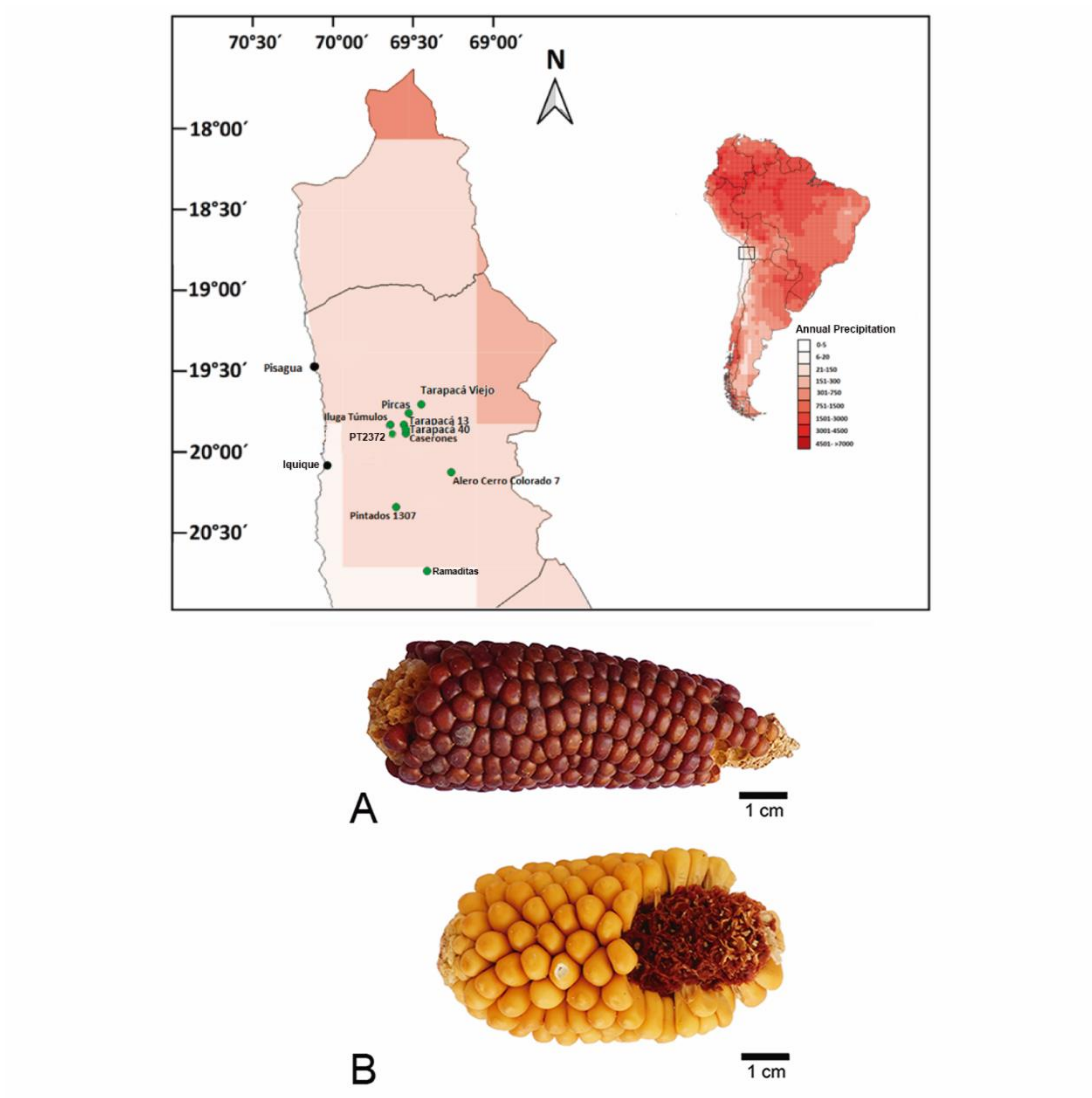
SUPPLEMENTARY INFORMATION



Sup. Figure 1. Map shows the general location of the Tarapacá Region (-19°0´21°0´Lat. South) in the Atacama Desert, northern Chile, and the approximate location of the archaeological sites and Camiña. Cob A belongs to Tarapacá 40 archeological site (example of the the first group of maize), and cob B belongs to Alero Cerro Colorado 7 (example of the second group of maize). Both samples were dated (marked with an asterisk in supplementary table 1 and used for histological and Fe analysis. Images of maps obtained from QGIS 3.16.

Samples ID and type			Radiocarbon age		Calibrated date (BC/AD)			Fraction of modern	
DirectAMS code	Archaeological Site	Sample type	BP	error	from	to	%	Pmc	error
D-AMS 033584[+]	**Ramaditas**	**corn kernel**	**2260**	**28**	**-376**	**-206**	**95,4**	**75.48**	**0.26**
D-AMS 033583[+]	**Ramaditas**	**corn kernel**	**2041**	**19**	**-56**	**45**	**95,4**	**77.56**	**0.18**
D-AMS 033582	Pircas	corn cob	1897	19	114	224	95,4	78.96	0.19
D-AMS 033569[+]	**Pintados**	**corn kernel**	**1778**	**30**	**234**	**376**	**95,4**	**80.14**	**0.3**
D-AMS 033573	Iluga Túmulo	corn cob	1730	28	250	416	95,4	80.63	0.28
D-AMS 033579	Caserones	corn kernel	1638	26	407	538	95,4	81.55	0.26
D-AMS 033571	Iluga Túmulo	corn kernel	1619	30	413	570	95,4	81.75	0.31
D-AMS 033578	Caserones	corn kernel	1592	24	432	577	95,4	82.02	0.25
D-AMS 033570	Iluga Túmulo	corn kernel	1545	23	525	634	95,4	82.5	0.24
D-AMS 033585*[+]	**Tarapacá 40**	**corn kernel**	**1522**	**32**	**530**	**640**	**95,4**	**82.74**	**0.33**
D-AMS 033924[+]	**Tarapacá 40**	**corn kernel**	**1533**	**27**	**533**	**645**	**95,4**	**82.63**	**0.28**
D-AMS 033586[+]	**Tarapacá 40**	**corn kernel**	**1478**	**24**	**586**	**652**	**95,4**	**83.19**	**0.24**
D-AMS 033572	Iluga Túmulo	corn kernel	1109	24	897	1025	95,4	87.1	0.26
D-AMS 033568	Pintados 2372	corn cob	920	23	1051	1221	95,4	89.18	0.25
D-AMS 033577	Caserones	corn cob	830	24	1214	1279	95,4	90.18	0.27
D-AMS 033567[+]	**Tarapacá 13**	**corn kernel**	**379**	**24**	**1464**	**1629**	**95,4**	**95.39**	**0.28**
D-AMS 033575[+]	**Tarapacá Viejo**	**corn kernel**	**352**	**25**	**1496**	**1643**	**95,4**	**95.71**	**0.3**
D-AMS 033580[+]	**Tarapacá Viejo**	**corn kernel**	**326**	**24**	**1504**	**1653**	**95,4**	**96.02**	**0.29**
D-AMS 033576*[+]	**Alero Cerro Colorado 7**	**corn kernel**	**280**	**29**	**1511**	**1799**	**95,4**	**96.58**	**0.35**

Sup. Table 1. Radiocarbon dates over maize used in this study. Nineteen Radiocarbon dates over maize are reported. They belong to 10 archaeological sites from the Tarapacá region. Dates highlighted in bold letters with crosses were previously indicated in Figure 1 of the main text and belongs to the sites used for this study. Dates with an asterisk correspond to sample A and B presented in supplementary Figure 1. Calibration date (BC/AD) was made using OxCal v.4.3.2 (online software service) and the SHCal13 atmospheric curve.

CHAPTER 3

AGRONOMIC AND WATER CONDITIONS FOR AGRICULTURE OF PREHISPANIC MAIZE OF THE ATACAMA DESERT INFERED BY $\delta^{18}O$ ISOTOPES.

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Key Word: pre-Columbian agriculture, maize, stable isotopes of oxygene ($\delta^{18}O$), irrigation, Atacama Desert.

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INTRODUCTION

In present times desert agriculture is a defiant topic and enormously accomplished for modern societies. As the Anthropocene has revealed as the new challenge for humanity (Bonneuil & Fressoz, 2017), we looked to the past to learn from Pre-Columbian communities, we fixed our gaze through their technical developments, cultural knowledge and the way they inhabited the land. Accordingly, in the Atacama Desert around 2000 yr BP, specifically in the intermediate plateau called *Pampa del Tamarugal* (Pdt) (19°-21° Lat. S 69°-70° Long W), prehispanic farmers developed a maize agriculture, displaying extended agricultural fields and a complex irrigations system (Bollaert, 1975[1860]; Rivera & Dodd, 2013; Santoro et al., 1998) (Figure 1). Though there has been good progress into the understanding of soils conditions for this early agriculture (Segura et al., 2021), irrigation technologies (Rivera & Dodd, 2013; Santoro et al., 1998; Vidal et al., 2012), the spectrum of crops and plants produced by early farmers (García et al., 2014; Núñez, 1966) and the transformation of crops due to human manipulation (Vidal Elgueta et al., 2019; Vidal-Elgueta et al., 2016) there is still few information of the water source and the water status used by maize agriculture in this desert environment

Since PdT plateau shows less than 1 mm of precipitation per year, the perennial run off Tarapacá river, groundwater, and occasional runoff caused by summer precipitation in high altitudes are the only three potential water sources that could have been used in Pdt agriculture. However, nowadays Tarapacá river feeds only small farms in the lower part and there is no agriculture in the Pdt of any kind. Additionally, groundwater does not surface at the northern or southern part of PdT, appearing only at Pica oasis surroundings. However, there is evidence of an old canal that diverted water from Tarapacá river to the rest of the Pdt to irrigate prehispanic agricultural plots. This canal dated around 400 yr BP (Barnard & Dooley, 2017).

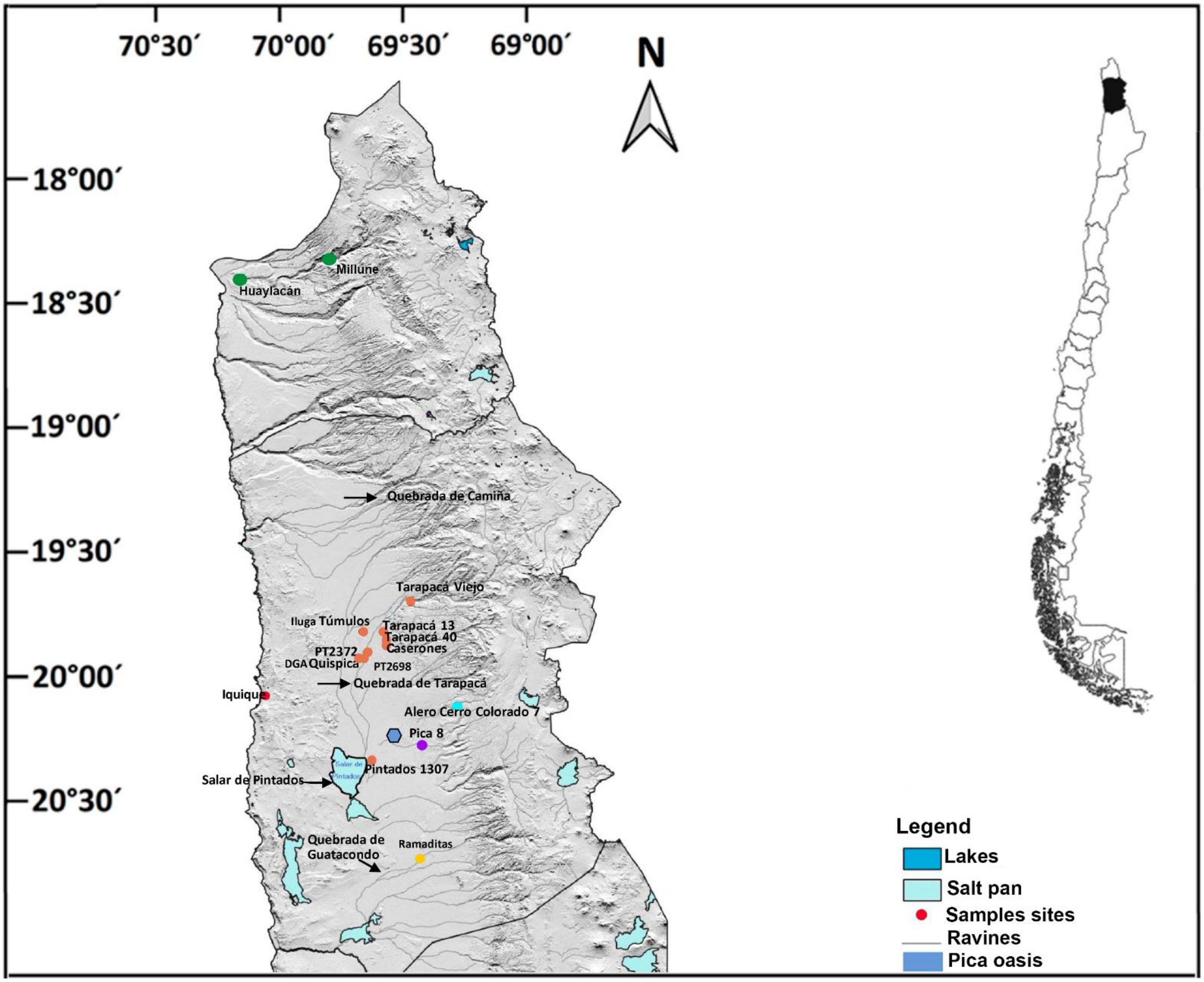

Figure 1. Map of Pampa del Tamarugal. Archeological sites, ravines and salt pan are indicated.

Moreover, in Northern Pdt the irrigation canals flow from east to west to round structures that are tentatively suggested as open water reservoirs *(cochas*). In fact, in the core of Iluga Tumulo site, there are structures with this characteristic reused as a public space (Uribe et al. 2020). In southern Pdt in Quebrada de Maní, one of this structure was dated to 2300 to 2000 years BP (Workman et al. 2020), and several others are reported for Quebrada de Guatacondo (Segura et al. 2021). These reservoirs would have played an essential role in supplying and maintaining water resources during the driest season. If the only primary source´s water was the Tarapacá river or Guatacondo basin, then water reservoirs allowed to conduct water up to 25 km to the west of Pdt (Figure 2 and 3).

Though, prehispanic agriculture suggests that paleoenvironmental conditions were wetter in the past. So far, humid periods are recognized between 2.500-2040, 1615-1350, and 1050 - 680 yr BP. (Gayó et al. 2012a, Maldonado y Uribe 2012, Maldonado et al. 2016). In southern Pdt, riparian vegetation observed in macro-fossils plant remains indicate similar wetter conditions for the Formative Period (Gayo et al., 2012; Latorre et al., 2013; Nester et al., 2007). Corn stalks observed in situ in the prehispanic fields of Quebrada de Mani dated between 1050 -730 cal. yr. BP support corn agriculture during the Formative and Late Intermediate period (Gayó et al. 2012b). Concordantly, vegetal remains of packrats middens, indicate higher humidity in the altiplano (> 3000 masl) associated with the Medieval Climatic Anomaly (Maldonado and Uribe 2012, Latorre et al 2013). For the Late Period, between 500 yr BP to present times, aridity increased (Maldonado et al. 2016). Additionally, an ongoing study of dH over ancient rodden middens materials, indicates fluctuations of -59.7‰ to -81.7‰ (dH) for the highlands of northern Chile. These results show more drier period or wetter period depending on the positive or negative values for dH. Thus, the great variability of more than 20‰ suggest during the last 1.700 years it is suggestive for shifting on moisture sources (Latorre et al. 2018). In summary, fluctuations between wetter and dryer conditions were a constant challenge for prehispanic agriculture in the Pdt depression, also the different sources for rain could vary the isotopic values for $\delta^{18}O$ and $\delta^{2}H$ for the past.

Understanding the water availability for past agriculture can give us insight into the type of crops adapted to desert environments, explain changes in crops, and the dynamics of social and political aspects of water management resources. Thus, in the absence of precipitations, the control to water access and, therefore to agriculture production could have led to significant social changes and challenges to the social organization of Tarapacá communities. Moreover, changes in the water regime could have had an essential effect over maize itself. After all, agriculture is a history of failures and successes that is not necessarily linear or progressive. These complex desert agriculture scenarios led us to several questions: What were the sources of water used by early farmers in Pdt? The water status for maize agriculture changed during these prolonged time of desert agricultures?. If so, maize agriculture production was altered or adapted to different water regimes? To answer these questions, we resort to the use of stable isotopes of water ($\delta^{18}O$) analyzing cobs and kernels from the Pdt plateau's archaeological sites. Since $\delta\ ^{18}O$ are especially useful in arid environments where the relative humidity is scarce and the water of plants reflects the water soil (Brunel et al., 1995), the aim of this study was to evaluate the water availability and water source used in maize agriculture for the different moments of the agrarian history of *Pampa del Tamarugal*.

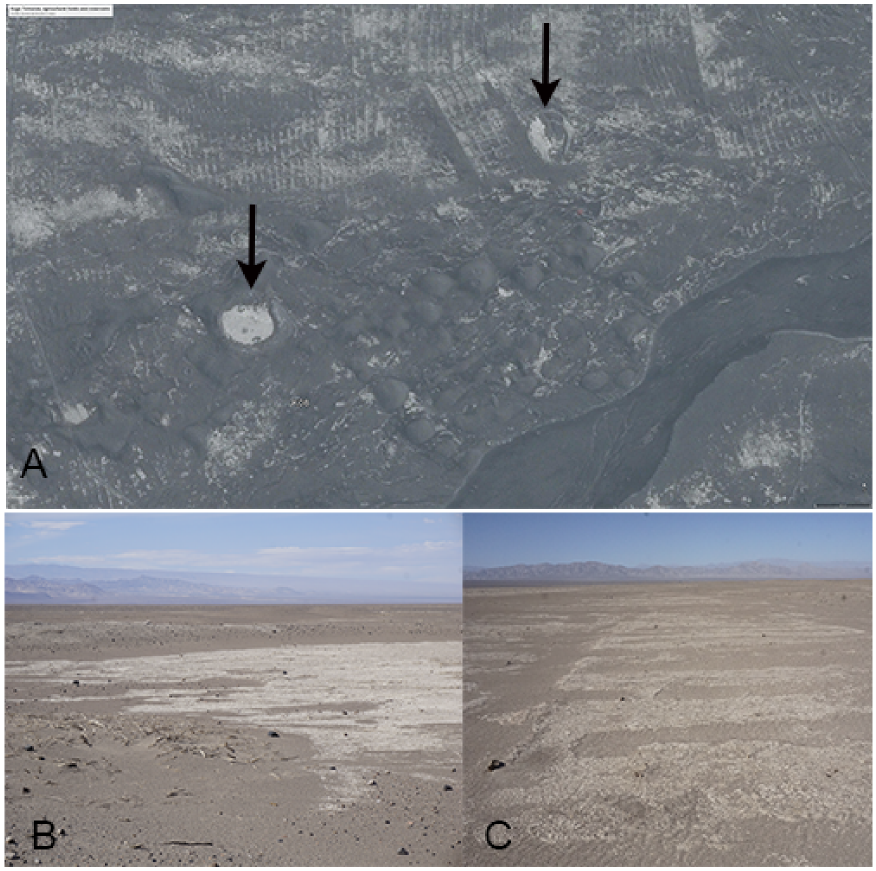

Figure 2. Archaeological fields and water reservoir in northern part of Pampa del Tamarugal. A: Satellite image of Iluga Túmulo, showing water reservoirs with black arrows and agricultural fields surrounded (source Google Earth 2021). B: Water reservoir indicated above in figure A. C: Agricultural fields shown in figure B.

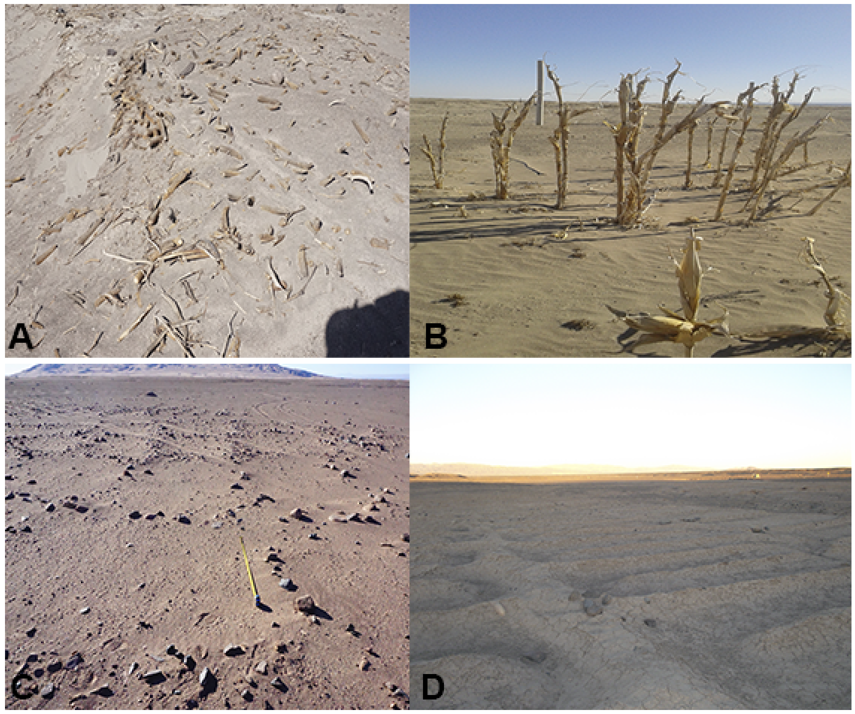

Figure 3. Maize cultivation in northern PdT and archaeological fields southern part of Pdt. A: archaeological cobs from a Late Period site called "Casita de maíz" (Rivera, 2018). B: DGA Quipisca modern cultivation maize. C: Prehispanic field of Guatacondo ravine D: Colonial fields of Guatacondo ravine (campos G-1 site) (Mostny, 1971; Segura et al., 2020).

Theory and use of $\delta\ ^{18}o$ in archaeological studies

The proportion of oxygen isotopes according to the standard (SMOV) varies in plants from 10 ‰ to 47 ‰ (Squeo & Ehleringer, 2004). The fundamentals of $\delta\ ^{18}O$ isotopes in plant tissues indicates that oxygen isotope ratio up taken by a plant depends on the temperature of droplet formation for rain, where precipitations are more depleted in oxygen isotope as temperature decreases (Barbour, 2007). A second basis is that there is no fractionation effects for oxygen during plant absorption of water soil, then oxygen of plant tissues reflect the isotopic composition of water soil (Brunel, 2009). The exception to this last fundamental is leaf vicinity composition, where transpiration can cause an enrichment of leaf tissue. Also, the enrichment of water soil surface can occur because of evaporation which is factor to consider in desert environment (García & Andrade, 2007). In our case, we assume all these fundamentals are present in the Atacama Desert, but aditionally since the relative humidity of Atacama in its norther portion is quite reduced (10% to 30%) an there is no precipations, the isotopic water composition is not related to rainfalls in the site. Another assumptions of our studies is that isotope composition of water for the Pdt has kept stable for the last 2000 years, and that humidity and precitpitations values have also not changed dramatically.

Stable isotopes of water ($\delta^{18}O/^{2}H$) have been used in archaeology and archaeobotany studies to established three main aims: the water availability of crops (Cabrera-Bosquet et al., 2009; Ferrio et al., 2006), the sources of water used in agriculture (Williams et al., 2005), and the water source for human consumption (Buzon et al., 2011). The increasing interested $\delta^{18}O$ among archaeobotanical community, it is due to the limitations of analyzing C4 plants through $\delta^{13}C$, because of the photosynthesis pathway they present (Squeo & Ehleringer, 2004). Instead, $\delta^{18}O$, such as maize an others cereals, can be analyzed through oxygen isotope in a analogous way as ^{13}C does for water availability. (Fiorentino et al., 2015). According to this, Cabrera-Bosquet (2009) and co-workers, showed that in plants grown under water limited conditions the leaf and kernels $\Delta\ ^{18}O$ is higher than in well water conditions, and thus indicated the water status of the plant during filling grains.

For water sources determination the use of Hydrogen ($\delta^{2}H$) and Oxygen analyses is common. The principles, already explained, is that water isotopes composition taken up by plants reflects the isotope composition of water sources of soils, underground, runoff or rainfall (Dawson et al., 2002; Williams et al, 2005; Buzon et al., 2011).

METHODS AND SAMPLES

Archaeological samples, locations and presumably water sources.

The total sample reached 118 between cobs and kernel. These belongs to 10 archaeological sites from Tarapacá, one modern location (DGA Quipisca) and two archaeological sites from Valle de Lluta (Huaylacán and Millune), which served as control sample.

Sample are ages range from ca. 400 BC to the 17th century. The archaeological sample come from different environments: Ramaditas is located at the southern part of Tarapacá in the Guatacondo ravine, thus the water source should be completely independent from Tarapacá northern samples. At the northern part of Tarapacá, in the core of PdT we found Iluga Túmulos, PT2373, PT2698. In Tarapacá ravine we found Caserones, Tarapacá 13, Tarapacá Viejo and Tarapacá 40 sites. PT1307 is situated at the west of PdT near Pintados salt pan. We presume corn samples from these sites were planted in PdT plains according to fields of the different domestic sites of PdT. Therefore, the water is probably from the same sources which could be the perennial Tarapacá river or groundwaters naps. Pica 8 is situated at the oasis of the same name to the southeast of PdT and the potential source water is the underground water that at this area emerges forming water wells. Today modern farmers still use the underground water in Pica and the near sector of La Huaica. DGA Quipisca was a contemporaneous experiment consisting in planting corn in a small farm in the middle of the

PdT (near PT2373 an PT2698) using water of the Tarapacá ravine. The reservoir for DGA experiment was kept in sealed water containers. Finally, Millune and Huaylacán were watered with Lluta river perennial flow (Mendes-Quirós personal communication 2019).

We clustered the $\delta^{18}O$ mean values of organic matter in different groups according to the potential irrigation water sources. These groups are Quebrada de Guatacondo (group 1), Pampa del Tamarugal y Quebra de Tarapacá (group 2), oasis de Pica (group 3), Cerro Colorado (group 4), and Valle de Lluta Arica (control group 5).

There are significant difference of $\delta^{18}O$ values in cobs and kernels, so samples were analyzed separated (Table 1 and 2 sup. Figure 1). We run Nested ANOVA analysis and Tukeys post hoc test to establish the main factor of variance $\delta^{18}O$ values for kernels and cobs.

$\delta^{18}O$ of Stable isotope analysis of organic matter (OM) and cellulose.

Samples of cobs and kernels were processed to obtain the $\delta^{18}O$ from organic matter (OM) and cell cellulose at the Institute of Bio- and Geosciences, Germany

For stable isotope analyses of organic matter of kernels and cobs a subsample of the milled material was treated with hydrofluoric acid (HF, 10 %) to reduce the amount of inorganic contamination of samples. However, HF treatment is insufficient to remove inorganic matter completely, and this does not entirely preclude a negative bias of measured $\delta^{18}O$ values from traces of inorganic bound oxygen released during pyrolysis. Therefore, cellulose was chemically extracted from the subsamples using the cuprammonium solution (CUAM) protocol as described in Wissel et al., 2008. In short, the CUAM protocol comprises a wet oxidation step with sodium chlorite (NaClO2, pH 3, for 10 h at 60 °C) followed by a purification step via dissolution in cuprammonium solution and subsequent precipitation of cellulose. This protocol ensures that the extracted cellulose is free of any organic and inorganic contamination, which otherwise would distort the genuine cellulose oxygen isotope value. Especially for small sample amounts, e.g. from kernels cellulose yields are often low or insufficient for oxygen isotope analyses and are best complemented by the respective OM measurements.

Approximately 275µg of freeze-dried organic matter or cellulose was weighed in silver capsules for stable oxygen isotope analyses. Samples were crimped and stored for >24h in a vacuum drier at 100°C before measurement to ensure complete drying. Samples were pyrolysed at 1450°C in a high temperature pyrolysis oven (HT-O, HEKAtech) and measured with a coupled isotope ratio mass spectrometer (IRMS) (Isoprime, GV Instruments).

Isotope data are reported in δ-notation [‰]

$\delta = (R_S/R_{St} - 1) \bullet 1000$

with R_S as the isotope ratio ($^{18}O/^{16}O$) of the sample and R_{St} as the isotope ratio of the respective standard. Laboratory standards IAEA-CH6 cellulose powder ($\delta^{18}O$=37.09±0.09‰), technical cellulose powders from Merk ($\delta^{18}O$=29.97±0.08‰) and Fluka ($\delta^{18}O$=28.84±0.12‰), and the in-house standards rice cellulose ($\delta^{18}O$=23.64±0.15‰) and peanut cellulose ($\delta^{18}O$=23.93‰±0.11‰) were included into autoruns. The precision of replicate analyses is <0.25‰. International Atomic Energy Agency (IAEA) reference standards IAEA-601 ($\delta^{18}O$=23.14‰) and IAEA-602 ($\delta^{18}O$=71.28‰) were used to calibrate laboratory standards and to scale normalize the raw values to the Vienna Standard Mean Ocean Water (VSMOW) scale.

Cellulose extraction was not always possible for all the samples due to the lack of enough material for analyses. This is a consequence of the preservation conditions of archaeological samples. Thus, in order to be able to use $\delta^{18}O_{OM}$ instead of $\delta^{18}O_{cellulose}$, we run a correlation between OM values and cellulose values from the samples we had both values. The OM and cellulose values correlates at R>0.97. Then, we decided to use the OM values because we obtained more samples for this source. $\delta^{18}O$ cellulose are reported in supplementary table 1, but not used in the statistical analysis. Finally, kernels and cobs organic matter show significant differences for which they are reported but not used in the statistical analysis.

RESULTS

δ ^{18}O of organic matter and ANOVA results.

The mean values of δ ^{18}O of organic matter for kernels and cobs are shown in table 1 (sup. Figure 1). The mean values of δ ^{18}O cellulose are shown in supplementary table 1. Kernels samples from Group 1 is in average 31.65 (‰), Group 2 range from 26.55 (‰) 29.21(‰), group 3 samples are in average 24.73 (‰), group 4 samples are in average 31.39 (‰). While gruop 5 (control samples) range significantly lower with an average of 25.5 (‰) and 25.6(‰).

An analysis of nested variance (n ANOVA) on kernels organic matter shows a significant effect of the group factor on δ ^{18}O values at the $p < 0.01$ level (F=32.03) (Table 2 and Figure 4). A post-hoc Tukey's test showed that there are significant differences between Group 1 (Ramaditas), group 3 (Pica 8) and partially with Group 2 (sites PT1307, Iluga Túmulo). Group 4 (ACC7) differs significantly from Group 2 and Group 3. As expected, Group 5 (control) differs significantly from all other groups, except with group 3 (Figure 4). In summary, tukey´s test corroborates that the aggregation based on potential water source explains the variance among δ ^{18}O values samples.

Groups	Location	Period	Site	Height masl	n	Mean (1SE) d^{18}O (‰) kernels OM	n	Mean (1SE) d^{18}O (‰) cobs OM
1	Q. Guatacondo	EFP	Ramaditas	1112	7	31.65(0.6)	-------	-------
2	Salar Pintados	EFP	PT1307	981	7	28.43(0.6)	-------	-------
	PdT	EFP	Iluga Tumulos	1145	8	28.66(0.5)	-------	-------
			PT2698	1111	-------	-------	6	30.47 (0.2)
	Q. Tarapacá	LFP	Caserones	1289	7	28.91(0.2)	2	32.77(1.3)
			Tarapacá 40	1291	9	29.21(0.2)	-------	-------
	PdT	LIP	PT2373	1117	-------	-------	7	32.60(1.1)
			Tarapacá 13	1395	-------	-------	6	30.08(1.2)
	Q. Tarapacá	LP	Tarapacá Viejo	1426	10	29.02(0.6)	9	28.32(0.6)
	PdT	MN.	DGA quipisca	1121	1	26.55	7	28.41(1.2)
3	Pica	LIP	Pica 8	1225	2	24.73 (1.52)	2	31 (0.6)
4	Cerro Colorado	Colonial	Cerro Colorado 7	2600	11	31.39(0.1)	-------	-------
5	V.Lluta, Arica	LIP	Huaylacán	143	7	25.55(0.2)	-------	-------
		LP	Millune	1500	7	25.60(0.4)	-------	-------

Table 1 Mean δ^{18}O values of organic matter for kernels and cobs. Groups assignation, Location, Period, Site, Height of sites, number of samples(n), δ^{18}O values of kernels and cobs.EFP: Early Formative, LFP: Late Formative, LIP: Late Intermediate, LP: Late Period, MN: Modern, Dotted line= no data obtained.

	DF	Sum sq	Mean	F value	Pr(>F)
Groups	4	309.22	77.31	32.303	<0.001***
Period	3	7.75	2.58	1,080	0.364
Weight	1	9.05	9.05	3,782	0.0562
Site	3	0.93	0.31	0.13	0..9421
Residuals	64	153.16	2.39		

Table 2 Nested Anova for δ^{18}O values of organic matter for kernels. F statistic= 32.303, p <0.001.***

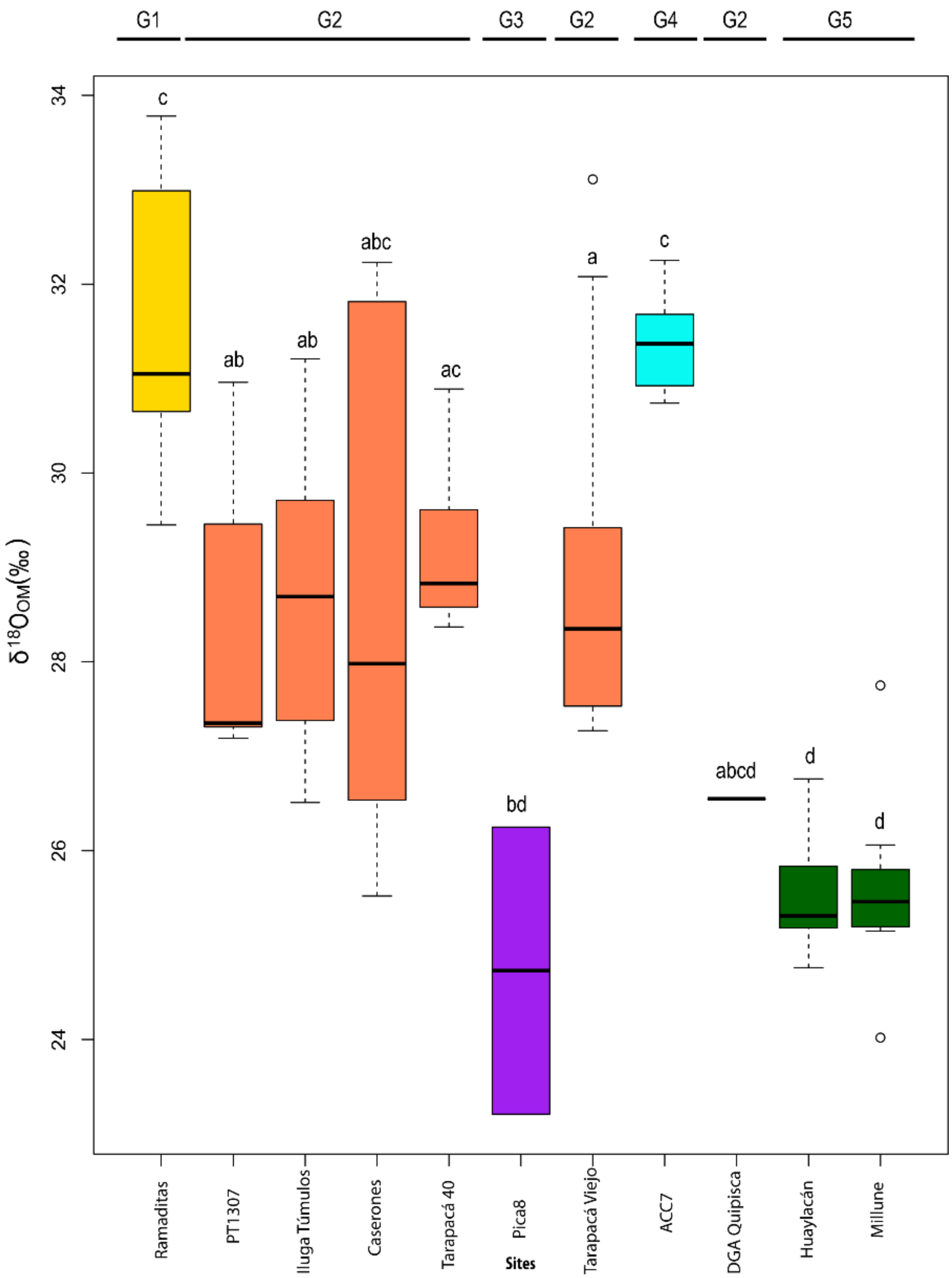

Figure 4. Box plot of δ ^{18}O (‰) organic matter over kernels and Tukey´s test comparison. All media values that do not share a letter are significantly different. Groups (G1 to G5) are colored similarly, and they are indicated in bars above the graphic.

DISCUSSION AND CONCLUSION

The results of the study show that the variance between $\delta^{18}O_{OM}$ values is mainly explained by the geographical and potential water sources grouped. Thus, the ANOVA results indicates no significance interference from another variable such as the age sample. Despite the environmental changes reported for the PdT (Gayo et al., 2012; Maldonado & Uribe, 2011), and the values of $\delta^{18}O$ of tree ring of *P.tamarugo*, which indicates a sustained increase in aridity in the region for the past 10.000 years (Rivera and Dodd 2013), we observe no modifications on the $\delta^{18}O$ values for the maize produced in Pdt and nearby ravines. We can infer that irrigation sources were conservative, and independent of the occasional drought or scarce water supplies, they did not affect Tarapacá agricultural prehispanic system.

We propose that Tarapacá farmers found technological solutions to deal with water scarcity moments, such as maize adapted to dry environments, creation of channels to divert water from the Tarapacá river, and one particular solution: keep water in opened reservoir. Thus, the enriched values of Pdt samples and Ramaditas could due to the irrigation system adopted. When we observe the value of DGA Quipisca maize, which was irrigated by watered kept in a tank, these values are lower compared to the rest of the archaeological sample (26.55 ‰ DGA, 28.85 ‰ Pdt and 31.65‰ Q. Guatacondo). In Pdt and Quebrada de Guatacondo plain, water transportation through open canals could have led to oxygen evaporation resulting in

$\delta^{18}O$ enriched isotopic values. Evaporation effects for Tarapacá lower part have been documented by Fritz et al.1981. Moreover, this is concordant with the large round structures that have been described as water reservoirs *(cochas*) (Workman, 2021). Then, water could be maintained in reservoirs for an undetermined time before irrigating the fields. This technological solution fixed the problem of scarcity of water during wintertime or short drought. However, this hypothesis has to be corroborated by further analysis of $\delta^{2}H$.

Though it is not possible for now to establish the exact source of water for maize irrigation, we suggest that the difference between groups is mainly caused by the origin of water. The results indicate a partial distant between Quebrada de Guatacondo (G1) and the northern part of PdT and Quebrada de Tarapacá (G2) $\delta^{18}O_{OM}$ values for maize. This contraposition is expected since both ravines feed on different sources suggesting maize was irrigated locally (Aravena et al. 1999, Aravena and Suzuki 1990).

Conversely, the values of $\delta^{18}O_{OM}$ of maize differ significantly between Cerro Colorado and PdT/Quebrada de Tarapacá. We have no insight yet of the origin of Alero Cerro Colorado maize. (G4). It is a type not found previously in the archaeological record, and because of its colonial date, we presume it could be a foreign introduction. However, the $\delta^{18}O_{OM}$ supports the idea it was not irrigated by the same source as the rest of Pdt and Tarapacá maize. Alero Cerro Colorado's values are closer to Ramaditas $\delta^{18}O_{OM}$ maize values, suggesting it was could have been cultivated in higher altitudes.

Pica 8 samples (G3) also shows a significant variance from Quebrada de Guatacondo, Cerro Colorado and Tarapacá 40 and Tararapacá Viejo maize. This is coherent with the fact that Pica area has the most depleted values for $\delta^{18}O$ of underground water, which varies between

-13.4 ‰ and -9.0 ‰ (Aravena 2005). On the contrary, Pdt underground water is most enriched, with values near -6.0‰ to -3.8‰ (Aravena 1995). The depleted values of Pica 8 maize suggest that it was irrigated by underground water rather than superficial waters. However, Pica 8 results must be carefully considered since we have a small sample from this area.

We can not establish if Pdt northern agricultural fields were irrigated by underground water, as was suggested in the past or by Tarapacá river. The large difference between $\delta^{18}O$ isotopic values of Tarapacá river (-11.5‰ to -10.2 ‰, Aravena & Suzuki, 1990) versus the $\delta^{18}O$ isotopic values of underground water for this area (-6.0‰ to -3.8‰ , Aravena 1995), makes possible, in the future, to try to correlate these water values to that of $\delta\,^{18}O$ isotope for maize. However, other local plants like *P. tamarugo,* which obtains all the water from the phreatic aquifer, have more depleted $\delta\,^{18}O$ values at leaf level ($\delta^{18}O$ 11.8‰ to 22.1‰) compared to our samples' maize values (Aravena y Acevedo 1985). Indirectly, this permits us to hypothesize that maize isotopic values could be due to the Tarapacá river´s irrigation water rather than underground water. However, as mentioned before if isotopic values of runoff water had different values in the past, due to different sources of rainfall or/and fluctuations in moisture conditions, these associations must be taken carefully.

We suggest that maize agriculture's water status did not change during Pdt prehispanic agriculture until Colonial and early Republican periods when maize plantation possibly moved to other environments. Technological developments controlled the water regime that irrigated maize, as suggested above.

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SUPPLEMENTARY INFORMATION

Site	n	Cobs mean value $\delta^{18}O$ (‰) (1SE)	n	Kernels mean value $\delta^{18}O$ (‰) (1SE)
Iluga Túmulos	0	------	1	31.8
PT2698	7	32 (0.4)	0	------
Tarapacá 40	0	------	1	30.7
Caserones	2	34.7(1.0)	3	27.9 (0.2)
Pica 8	2	33.1 (0.6)	2	26.2(1.5)
PT2373 (ILP015)	7	34.3 (1.0)	0	------
Tarapacá 13	7	32.5 (1.0)	0	------
Tarapacá Viejo	9	30.3 (0.4)	7	30.4(0.6)
Alero Cerro Colorado 7	0	------	11	31.9 (0.2)
DGA Quipisca	8	29.17(0.7)	1	27.6
Huaylacán	0	------	6	27.3(0.2)
Millune	0	------	7	26.5(0.5)

Sup. Table 1 Mean values $\delta^{18}O_{cellulose}$ (cobs and kernels). Site and number of samples per site (n). Values are reported against VSMOW per mil (‰).

FINAL WORDS

Our research contributes to the understanding of the archaeological scenario of ancient maize agriculture in Tarapacá. This was, and still is, a challenge because of the complexity of the relationship we are interested in exploring. The aims are partially accomplished: we have given some insights into the changes suffered by maize agriculture, the introduction of new varieties, changes in the chemistry of maize, and explore some of the conditions of irrigation. All these results are correlated to a complete ^{14}C data set that enriched Chilean maize's archaeological data sequence.

The most surprising result was to observe how conservative agriculture in Desert Atacama was, probably due to the hyperarid conditions that limited more radical changes. On the opposite, Tarapacá farmers created impressive technological displays of canals, water reservoirs, adopted specific maize varieties and made corn grown in time to endure the demanding climate and social conditions. These strategies confirm that prehispanic farmers were an active agency over their territories and natural resources.

Although, we think the cultural taste and the social dimension of tarapacá societies were present at all times, some changes occurred in maize that were not predicted. This is the case for the loss of iron, which challenges us to consider if intensive agriculture ends in a convergent path regardless of time.

In conclusion, we propose that desert agriculture, present or past, is an appropriate way to research the Domestication Syndrome from an original perspective and engage with prehispanic knowledge.