

Study of the Impact of Long-Term Ex Situ Conservation on Autochthonous Maize Populations and Factors Affecting Them

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Abstract

The conservation of genetic resources aims at preserving diversity within and between populations. In our case, the local maize populations are preserved through the periodic planting of seed, which does not ensure the preservation of diversity in the population. Genetic drift, as allele changing frequency in a population, occurs due to random sampling in each generation and has a stronger impact on small populations. It is a random evolutionary process that is caused by "wrong sampling". The phenomenon of genetic drift in our populations is natural one because of small populations, which do not contain all forms of plants of the initial population. The study presents plant height data and some traits of the ear of 16 local maize populations under in the study on 1975 and 2015 years, stored for nearly 40 years through their periodic sowing. The aim of the study was whether the populations retain the set of initial characteristics, after 40 years of reproduction through 30-35 plants. From the data of our study, we find that the significant changes in the values of the traits of the populations in the study are the result of the influence of genetic drift, but also of the genetic draft.

KEYWORDS: population, traits, inbreeding, genetic drift, genetic design, bottleneck

1.Introduction

The conservation of genetic resources aims aims preserving diversity within and between populations of the one taxon. Thus, the main task of genetic banks is to preserve the genetic diversity that exists in the plant species. But in our case, in the absence of a genetic bank, local maize populations have been preserved through periodic planting of the seed, with the aim of preserving its germinating power. This mode of conservation provides only the seed's power, but not the diversity of populations, because during population regeneration can lose many of their initial traits and characteristics.

It is well known that the high level of genetic diversity within the population is much needed to ensure that populations are genetically stable. The effectiveness of regeneration of plant collections is influenced by factors such as sample size, seed endurance, and also by random evolutionary factors, such as genetic drift, which has a much greater effect on the frequency of alleles in smaller populations (Crossa J. 1989, Piotibo B. 2010, Salillari A. 2001 etc.). Genetic drift, as a change in the frequency of alleles in a population from one generation to the next, occurs because of the random sampling of the initial populations, avoiding in this case sampling of all forms of plants that build the population. Therefore, it is acknowledged that the genetic drift is a random evolutionary process. The main source of this change is “wrong sampling”

in selecting alleles from the initial population to transmit them to the finite population. During this storage mode, it is more likely to lose the lower allele frequency in the population. This phenomenon is natural because small populations do not contain all forms of plants that represent the population that would bring in the population all the varieties of alleles of the initial population. According to Crossa J. (1989), the size of the sample depends largely on the allele frequency or the less common genotype. Genotypes that have allele frequencies of more than 10% can be stored with a sample size of 40 individuals. A sample size of 100 individuals will store genotypes with 5% allele frequency. If the allele frequency is below 5%, a larger sample size is required. Thus, samples of 300 to 400 individuals are required to store alleles present at a frequency of 1%.

According to Crossa J. (1992), finite populations contain, on the average, up to 11 neutral alleles at low frequency and from one to three neutral alleles at intermediate frequency. In general, there is no more than one neutral allele at high frequency. For a high mutation rate (5×10^{-3}), samples of 100 to 250 individuals will have a large proportion of the neutral alleles at low frequency and smaller samples will contain a lesser degree of neutral polymorphism. For a mutation rate of 5×10^{-4} collections of more than 250 individuals are required for a high degree of neutral polymorphism in a single locus. Overdominance is inefficient for maintaining a large number of alleles in a finite population. The usual sample for regeneration of 100-300 individuals would not maintain many overdominant alleles if the regenerations were affected by selection. If the original collection is small (less than 10 individuals), a bottleneck effect will result in the loss of some alleles present in the original population at low frequency. Therefore, the first priority is to insure appropriate sampling during collection. A sample of at least 100 individuals from the original population should be sufficient for this purpose. However, for the regeneration of cross-fertilized species collections (such as corn, in our case), which contain a high level of heterozygosity, it is recommended that 150 to 250 individuals and crossing procedures that maximize effective population size be utilized for the regeneration of collections of cross collections species containing a high level of heterozygosity. So the number of individuals (plants) used in our case for population regeneration is too small to maintain a copy of alleles of the original population. For further regeneration measures should be taken to use a balanced amount of individuals, thus avoiding changes in the frequency of alleles caused by the selection and effects of overdominant alleles in seed production. Mutation is not an important factor in regeneration relying on 150-250 plants (4).

According to Heerwaarden J. et al. (2009), the loss of genetic diversity in a particular place over a given period of time, involves the loss of individual genes and specific gene combinations such as those that appear in cultivars or farmer varieties (landrace). In this point of view, genetic erosion can be seen as the disappearance of known varieties in the regions where they were present. Such was the loss of more than 200 local maize populations that were planted in Albania, resulting in a lack of conservation measures.

2. Materials and Methods

The object of the study was evaluation of the morphologic indicators and the production elements in the local maize populations, with the aim of finding any effect of ex situ conservation for a period of 40 years, through their periodic planting in Albania conditions. In the study, 16 maize populations were taken. Based on observations and data records on the morphological characteristics and growth and

development phases of the plants, as well as the production elements, the comparison of the values of the indicators of the two periods of study was made. Initial data were those of 1975 which are the data of the basic (original) populations obtained from the collection. Comparison of these data was done with those of the field test carried out in 2015. To understand the effect of ex situ conservation of the populations after a 40-year period, the indicators of the plant height and the elements of the ear, such as ear length of ear, number of rows in the ear, number of kernel per row, kernel length, kernel width and the number of kernel per ear. For plant, ear and kernel indicators, 5 plants were harvested randomly for each population. Kernel data are as average of 10 consecutive kernels from one row in the middle of the uppermost ear, measured by caliber (IBPGR, 1991). The data of field test were subjected to statistical variance analysis for the two years of study. Then a variance analysis was performed for the two years, using the average values of the traits, and comparing the data between the two study periods to see the impact of the ex situ conservation of local maize populations through periodic planting.

3. Results

Field test data with 16 local maize populations for the two years of study, 1975 and 2015, are quite interesting. Through variance analysis, the differences between the populations, at $P < 0.01$, are determined for the seven traits studied (Table No. 1 and No. 2). The purpose of this study was to finding any effect of ex situ conservation for a period of 40 years, through their periodic sowing, on the traits of the local maize populations. Thus, the average data of 1975 and 2015 years were analysed by variance analysis for the 7 traits in the study. According to the variance analysis (Table 3), in the seven traits under the study, data for genotype differences (between populations) were significant at $P < 0.01$. But in this case, it was of interest to confirm the differences between the two years of the test (1975 and 2015). According to the variance analysis for the study years (Table 3), the data of the 6 characteristics are significant by the variance analysis, of which 3 traits (plant height, ear length and kernel width) at $P < 0.01$ and 3 traits (number of rows per ear, kernel length and number of kernel per ear) at $P < 0.05$; only data of the number of grains in row were not significant.

Let's see the values of the traits in the study by the years, but also the differences between them. In an overview of the values of the traits recorded from both field tests, there are some phenomena. The general is that in 2015 yr., the values are lower than those of 1975; so the long period of conservation of the populations (40 years) through repeated planting has adversely affected, reducing the values of the traits. From the data in table no. 4 we can see that in 2015 the average plant height for the 16 local maize populations is 133.1 cm, from 189.6 cm in 1975, representing 70.2 percent of the initial value, or 56.5 cm less. 15 populations had lower plant height values, of which 10 populations had plant height 41.1 to 75 percent of their initial form, a significant decrease. Populations that have had the most significant decrease in plant height, compared to the initial form, are populations with no. 55 with 41.1%, Nr. 29 with 53.9% and no. 44 with 56.6% of initial value (1975); whereas the population with no. 10 has almost maintained the same level in the two years of study. Ear length values were lower than the original forms in 12 populations, out of 16 total, with the lowest values in populations no. 44 with 45.5%, Nr. 121 with 52.4% and no. 118 with 59.5% of the value of the initial forms. Two populations recorded very high values compared to the initial form, such as Nr. 29 with 149.0%, ie an additional 50% and no. 11 with 131.5% (Table 4).

The number of rows in the ear also have low value in 2015, with an average of 9.4 rows, from 10.7 in 1975, or 87.6 percent. For this trait there were 13 people with lower values than the initial form, of which, population Nr. 118 had the lowest value, with 54.1 percent compared to 1975. For the number of rows per ear there were three populations with higher values than 1975, of which populations no. 83 and Nr. 53 had the highest values, respectively 135.0 and 130.0 percent, compared to 1975 (Table 4).

Kernel length values (Tables 5) have been significantly reduced compared to the initial forms (1975), with a reduction of approximately 20 percent. Only two populations, those with no. 10 and Nr. 11 have almost maintained the same level of values in both years, respectively with 102.1 and 101.4 percent. For this trait the lowest value was in the population Nr. 124 with 52.7 percent compared to 1975.

Kernel width is almost the only feature where all populations had the lowest values in 2015 than the initial populations (data of 1975). Of these, the four populations had the lowest values, populations no. 146 and Nr. 118, respectively with 52.3 and 52.4 percent, followed by populations no. 121 with 53.5 and no. 55 with 58.8 percent (Table 5).

The number of kernel per ear recorded the lowest values in 2015, compared to 1975 in the 11 populations, with the lowest values in populations no. 118 and Nr. 44, respectively by 30.2 and 38.3 percent. Although there are fewer populations with lower values than 1975, 11 populations in total, 5 populations have higher values, of which populations no. 29, Nr. 146 and Nr. 53 recorded the highest values, respectively 175.1, 172.2 and 151.0 percent (Table 5).

4. Discussion

The data obtained from the population traits in the study give an interesting picture. Values measured in 2015 are lower than those of 1975 for all traits under the study. The assessment of experimental data based on this point of view may lead to the judgment that this phenomenon is the result of environmental conditions represented by those of 2015 yr. This judgment would not be accurate and does not rely on scientific reasoning. It is known that different plant traits are controlled by two sets of factors, from genetic and environmental factors. But in this case we are not dealing with uncontrolled environmental conditions because, in the case of field trials, the best levels of plant cultivation are used in such a way that the populations in the study best express their traits. On the other hand, the fact that population behavior after 40 years of conservation through cultivation has not reacted equally to the experimental test conditions means that the significant decrease in the values of the features is not due to environmental conditions. Thus, eg, while a population, it with no. 55, in 2015, expressed the plant height about 60 percent over 1975 (respectively 203.2 and 83.6 cm), while population no. 10 has maintained almost the same level of plant height in the two test years (139.6 and 141.4 cm), excludes the possibility of influencing environmental factors in this population behavior. The same thing is noticed with other traits. For example, the ear length to the population Nr. 44 was 19.8 and 9.0 cm, respectively for the years 1975 and 2015, at the same time when population no. 29, for example, there has been a pronounced increase in ear length by almost 50 percent over 1975 yr., respectively 7.0 and 10.4 cm. The same thing can be said for the number of rows per ear, for the size of the kernel but also for the number of kernel per ear, an indicator that has the most pronounced differences, such as population Nr. 118 has the most significant decrease in the number of grains, respectively 492.4 and 148.8 kernel per ear, while population no. 29, for example, has increased over 75 percent the number of kernel (from 93.2 to 163.2 kernels) or population no. 146 with,

respectively 301.6 and 516.4 kernels, or an increase of over 72 percent compared to 1975. All these examples exclude environmental impact on the traits of populations under experimental conditions. In this case, the most reliable reasoning for these impacts is that they are random genetic factors, such as genetic drift.

It is known that genetic drift is the change in the frequency of an existing gene variant (alleles) in a population due to the random sampling of organisms, which means erroneous sampling that does not fully represent the population from which they were taken. The alleles in the offspring are a sample of those in the parents and chance has a role in determining whether a given individual survives and reproduces. A population's allele frequency is the fraction of copies of one gene that share a particular form. Genetic drift may cause gene variants to disappear completely and thereby reduce genetic variation. It can also cause initially rare alleles to become much more frequent and even fixed. This change in the frequency of the allele or gene variation must occur randomly in order for genetic drift to occur. There are no environmental influences that cause genetic drift to occur (5, 6). In other words, genetic drift is a random evolutionary process, so it acts randomly and in a way that is not correlated with selection at the gene in question and depends largely on the size of the population, which is defined as the actual number of individuals in an idealised population (7). This definition of genetic drift fully supports our reasoning because local maize populations of our study have been regenerated for 40 years based on a sample of 30-35 plants through SIB pollinated. This is such a number of individuals that leads to the loss of some low frequency alleles present in the original population (4). The fact that not all populations have reduced the values of the traits in our study at the same level is explained to the random individuals of the population. Finite populations, such as those in our study after 40 years of reproduction, contain, on the average, up to 11 neutral alleles at low frequency and from one to three neutral alleles in intermediate frequency alleles. In general, there is no more than one neutral allele at high frequency. According to Crossa, J. et al (1992), a sample of at least 100 individuals from the original population should be sufficient for this purpose. However, for the regeneration of cross-fertilized species (such as maize), which contain a high level of heterozygosity, it is recommended to use 150 to 250 individuals as well as appropriate crossing procedures that maximize effective population size. In this case, the mutation is not an important factor (4). So the number of individuals (plants) used in our case for population regeneration is too small to maintain a copy of alleles of the original population.

Cases where, in our study, some traits in certain populations have shown very high indicators in 2015, i.e. after a 40-year reproduction period with an unsuitable number of plants, for example, ear length to the population Nr. 29 with approximately 50% more compared to 1975, or populations with no. 29 and Nr. 146, which have given over 70 percent more kernel per ear, we believe it is the result of the action of another random factor of the **genetic draft**. The genetic draft is when an allele changes frequency not because it itself is under natural selection, but because it is near another gene that is undergoing a selective sweep during selection and that is in the same DNA chain. So the genetic draft is the change in the frequency of an allele due to the randomness that occurs in any other non-neutral allele associated with it. When one gene goes through a selective sweep, any other nearby polymorphism that are in linkage disequilibrium will tend to change their allele frequencies. Selective sweep happens when newly appeared (and hence still rare) mutations are advantageous and increase in frequency. The frequency of alleles due to the genetic draft is more likely to go up than down in the next generation. Thus, the genetic draft

generates a different allele frequency spectrum in contrast to genetic drift, it does not depend on the size of the population (7).

Tabela nr. 1: Analysis of variance for genotype distinction of maize populations, Tirana 1975

Sources variation	df	ms						
		Plant height	Ear length	Rows /ear	Grain /rows	Kernel length	Kernel width	Number of kernel per ear
Popualation	15	6437.5**	98.7**	35.4**	429.0**	0.05**	0.004**	53350.7**
Replication	4	635.3	26.9**	1.7	54.5**	0.01	0.002	19991.5*
Error	60	598.8	2.7	4.13	11.01	0.01	0.001	7443.3
D01		41.17	2.7	3.42	5.58	0.14	0.07	145.2
D05		30.96	2.1	2.57	4.20	0.10	0.05	109.1

** P≤0.01; *P≤0.05

Tabela nr. 2: Analysis of variance for genotype distinction of maize populations, Tirana 2015

Sources variation	df	ms						
		Plant height	Ear length	Rows /ear	Grain /rows	Kerne l length	Kerne l width	Number of kernel per ear
Popualatio n	15	5893.6*	21.2*	10.99*	281.6*	0.07*	0.08*	57499.9*
Replicatio n	4	22.9	1.7	2.71	64.5	0.01	0.007	11666.3*
Error	60	234.2	1.8	1.23	22.5	0.01	0.006	4386.7
D01		25.75	2.25	1.86	7.97	0.14	0.14	111.4
D05		19.36	1.70	1.40	5.99	0.11	0.10	83.8

** P≤0.01; *P≤0.05

Tabela nr. 3: Analysis of variance for genotypes distinction of maize populations, for two study ears Tirana, 1975-2015

Sources variation	df	ms						
		Plant height	Ear length	Rows /ear	Grain /rows	Kern el length	Kern el width	Number of kernel per ear
Popualati on	15	10363.34**	75.67*	27.29*	425.26*	0.07*	0.04*	65986.1*
Replicatio n	9	14337.10**	59.12*	2.16*	79.0.3	0.16*	0.27*	22326.2*
Error	135	446.98	6.90	4.51	46.59	0.01	0.01	10242.8
D01		24.70	3.07	2.48	7.97	0.12	0.11	118.3
D05		18.70	2.32	1.88	6.04	0.09	0.08	89.5

Tabela nr. 4: Plant height, ear length, number of rows per ear, during study years, Tirana 1975, 2015

Nr.	Population	Characters of maize population											
		Plant height (cm)				Ear length (cm)				Number of rows per ear			
		Value		Distinctions		Value		Distinctions		Value		Distinctions	
		1975	2015	Vlera	%	1975	2015	Vlera	%	1975	2015	Vlera	%
1	Nr. 10	139.6	141.4	-1.8	100.7	12.2	11.8	0.4	96.7	17.2	11.6	5.6	67.4
2	Nr. 11	173.0	152.0	21.0	87.9	9.6	12.6	-3.0	131.5	14.0	10.8	3.2	77.1
3	Nr. 19	165.8	145.2	20.6	87.6	12.7	9.6	3.1	75.4	10.8	8.0	2.8	74.1
4	Nr. 21	168.0	128.2	39.8	76.3	12.1	10.0	2.1	82.6	8.4	8.0	0.4	95.2
5	Nr. 29	150.2	81.0	69.2	53.9	7.0	10.4	-3.4	149.0	8.4	8.0	0.4	95.2
6	Nr. 31	142.0	88.4	53.6	62.3	9.5	10.4	-0.9	109.7	12.0	10.8	1.2	90.0
7	Nr. 44	231.2	130.8	100.4	56.6	19.8	9.0	10.8	45.5	8.8	8.0	0.8	90.9
8	Nr. 50	140.6	84.8	55.8	60.3	12.4	9.0	3.4	72.6	10.4	8.0	2.4	76.9
9	Nr. 53	187.0	121.4	65.6	64.9	12.0	11.4	0.6	95.0	8.0	10.4	-2.4	130.0
10	Nr. 55	203.2	83.6	119.6	41.1	14.4	14.6	-0.2	101.4	10.8	8.0	2.8	74.1
11	Nr. 83	255.6	167.4	88.2	65.5	21.0	14.4	6.6	68.6	8.0	10.8	-2.8	135.0
12	Nr. 86	201.2	127.2	74.0	63.2	20.6	13.8	6.8	67.0	10.0	8.8	1.2	88.0
13	Nr. 118	228.0	175.6	52.4	77.0	14.8	8.8	6.0	59.5	14.8	8.0	6.8	54.1
14	Nr. 121	219.8	169.0	50.8	76.9	20.6	10.8	9.8	52.4	9.2	10.8	-1.6	117.4
15	Nr. 124	214.2	133.0	81.2	62.1	13.8	9.2	4.6	66.7	11.6	8.8	2.8	75.9
16	Nr. 146	214.8	200.6	14.2	63.4	19.0	14.0	5.0	73.7	9.2	11.6	-2.4	75.9
Means		189.6	133.1	56.5	70.2	14.5	11.2	3.2	84.2	10.7	9.4	1.3	87.6

Tabela nr. 5: Kernel length, kernel width, number of kernel per ear, during study ears, Tirana 1975, 2015

Nr.	Population	Characters of maize population											
		Kernel length (mm)				kernel width (mm)				number of kernel per ear			
		Value		Distinctions		Value		Distinctions		Value		Distinctions	
		1975	2015	Value	%	1975	2015	Value	%	1975	2015	Value	%
1	Nr. 10	8.4	8.6	-0.2	102.1	7.4	5.0	2.4	67.3	482.0	380.4	101.6	78.9
2	Nr. 11	8.9	9.0	-0.1	101.4	8.0	4.1	3.9	51.8	307.2	385.6	-78.4	125.5
3	Nr. 19	9.0	6.8	2.2	75.4	6.2	4.6	1.6	74.8	289.2	188.8	100.4	65.3
4	Nr. 21	10.0	7.4	2.6	74.1	6.2	4.3	1.9	70.0	218.4	211.2	7.2	96.7
5	Nr. 29	7.3	6.2	1.1	85.4	5.2	4.7	0.5	90.8	93.2	163.2	-70.0	175.1
6	Nr. 31	8.5	6.8	1.7	79.6	6.0	5.2	0.8	86.7	222.8	284.0	-61.2	127.5
7	Nr. 44	10.4	7.0	3.4	67.3	7.8	4.8	3.0	61.0	355.2	136.0	219.2	38.3
8	Nr. 50	9.2	5.8	3.4	63.0	5.0	4.8	0.2	96.0	253.6	145.6	108.0	57.4
9	Nr. 53	8.4	7.4	1.0	88.1	6.6	4.6	2.0	69.7	193.6	292.4	-98.8	151.0
10	Nr. 55	9.8	9.0	0.8	91.8	8.6	5.1	3.5	58.8	294.0	243.2	50.8	82.7
11	Nr. 83	10.2	8.4	1.8	82.4	7.8	4.8	3.0	61.8	356.8	318.8	38.0	89.3
12	Nr. 86	10.4	8.6	1.8	82.7	8.0	4.8	3.2	60.0	408.0	308.4	99.6	75.6
13	Nr. 118	10.2	8.6	1.6	84.3	8.4	4.4	4.0	52.4	492.4	148.8	343.6	30.2
14	Nr. 121	10.8	8.6	2.2	79.6	8.6	4.6	4.0	53.5	363.2	344.0	19.2	94.7
15	Nr. 124	11.0	5.8	5.2	52.7	5.6	4.4	1.2	78.6	316.8	191.6	125.2	60.5
16	Nr. 146	9.8	9.0	0.8	91.8	8.8	4.6	4.2	52.3	301.6	516.4	-214.8	172.2
Mesatare		9.52	7.69	1.83	81.4	7.14	4.68	2.46	67.8	309.3	266.2	43.1	95.0

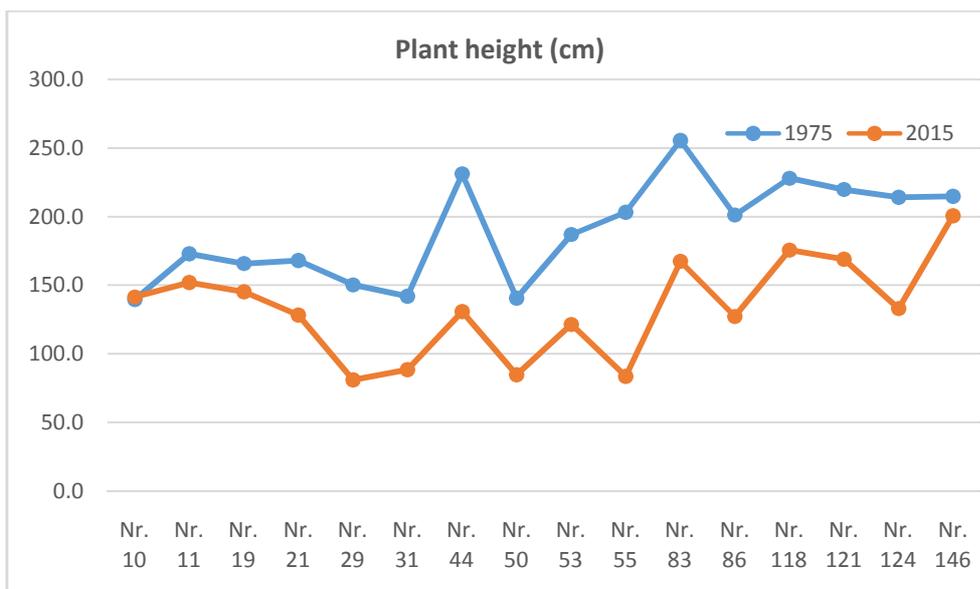


Figure a: Plant heights for 16 maize populations

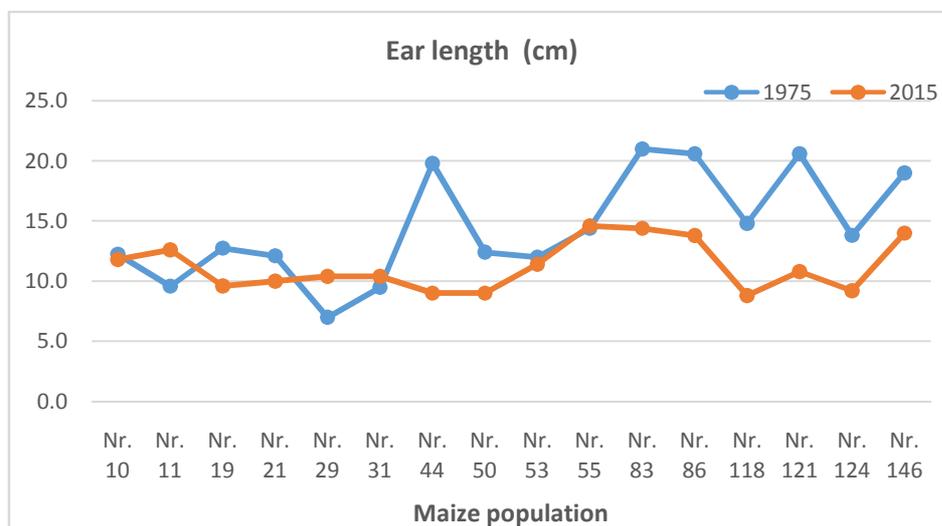


Figure b: Ear length for 16 maize populations

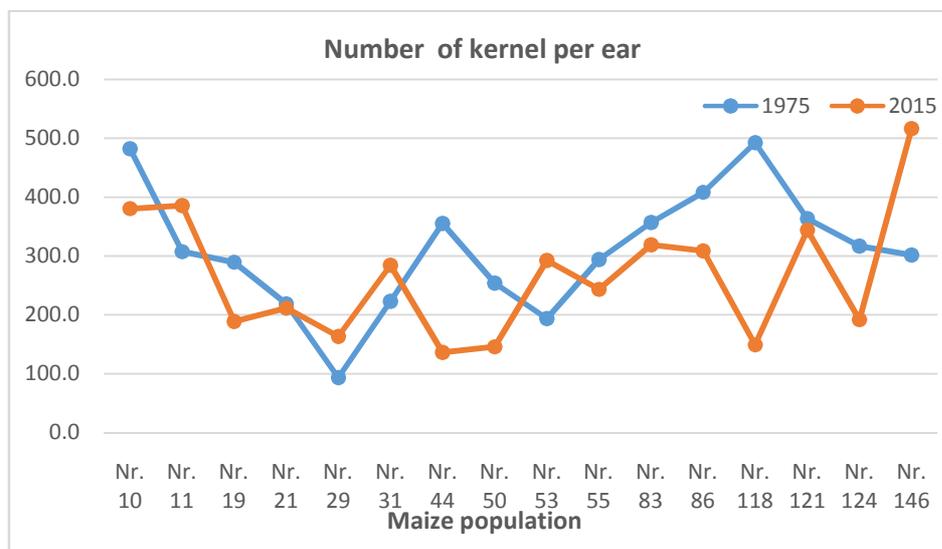


Figure c: Number of kernel per ear for 16 maize populations

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