EVALUATING THE CORRELATION OF PLOIDY LEVEL, LEAF SIZE, STOMATA CHARACTERISTICS AND TUBER WEIGHT IN *Dioscorea* spp. POPULATIONS FROM JALISCO, MÉXICO †

[EVALUACIÓN DE LA CORRELACIÓN DEL NIVEL DE PLOIDÍA, TAMAÑO DE LA HOJA, CARACTERÍSTICAS DE LOS ESTOMAS Y PESO DE LOS TUBÉRCULOS EN POBLACIONES DE *Dioscorea* spp. DE JALISCO, MÉXICO]

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SUMMARY

Background. "Camote de cerro" (*Dioscorea* spp.) is a plant of great importance in West rural areas of Mexico as food source and alternative medicine. *Dioscorea* is an important crop around the world for its carbohydrate contribution to diets. Yams are polyploid plants, and species with modifications at ploidy level also present changes in leaf size and stomata characteristics. These variation can favor resistance to adverse conditions. **Objective.** In the present study, evaluation and correlation of ploidy levels, leaf size, stomata characteristics and tuber weight were carried out for *Dioscorea remotiflora* Kunth and *Dioscorea sparsiflora* Hemsley in accessions obtained from 11 localities in México. **Methodology.** Chromosome counting was carried out in root meristems. Measuring and counting stomata were carried out. Productivity rate (PR) was calculated dividing harvested tuber weight over seed tuber weight, for each experimental unit. Leaves width and length were measured. **Results.** Show that variation is higher among different localities than among specimens from the same locality. Significant differences were observed for, ploidy levels (P), stomata dimensions and number of chloroplasts in stomata. Differences in chloroplast numbers present in occlusive cells were also significant for different species. **Implications.** Ploidy level showed a relation with chloroplasts numbers in stomata and stomata width. Leaf size presented a relation with stomata dimensions, and leaf width showed a relation with tuber weight. **Conclusions.** These observations allowed us to determine that there are variation among populations; stomata width and chloroplast number in a population can help to determine ploidy levels, and leaf width is a response variable that allows to predict tuber weight.
Keywords: yam; Dioscoreaceae; polyploids; geophyte; population variation.

RESUMEN

Antecedentes. El camote de cerro (*Dioscorea* spp.) es una planta de gran importancia en las zonas rurales del occidente de México como fuente de alimento y medicina alternativa. *Dioscorea* es un cultivo importante en todo el mundo por su aporte de carbohidratos a las dietas. Los ñames son plantas poliploides, y las especies con modificaciones a nivel de ploidía también presentan cambios en el tamaño de las hojas y las características de los estomas. Estas variaciones pueden favorecer la resistencia a condiciones adversas. **Objetivo.** En el presente estudio se realizó la evaluación y correlación de los niveles de ploidía, tamaño de hoja, características de los estomas y peso del tubérculo para *Dioscorea remotiflora* Kunth y *Dioscorea sparsiflora* Hemsley en accesiones obtenidas de 11 localidades de México. **Metodología.** El conteo cromosómico se realizó en meristemos de raíz. Se realizaron medidas y conteo de estomas. La tasa de productividad (RP) se calculó dividiendo el peso del tubérculo cosechado entre el peso del tubérculo-semilla,

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para cada unidad experimental. Se midió el largo y ancho de las hojas. **Resultados.** Muestran que la variación es mayor entre diferentes localidades que entre especímenes de la misma localidad. Se observaron diferencias significativas para los niveles de ploidía (P), las dimensiones de los estomas y el número de cloroplastos en los estomas. Las diferencias en el número de cloroplastos presentes en las células oclusivas también fueron significativas para diferentes especies. **Implicaciones.** El nivel de ploidía mostró una relación con el número de cloroplastos en los estomas y el ancho de los estomas. El tamaño de la hoja presentó relación con las dimensiones de los estomas, y el ancho de la hoja mostró una relación con el peso del tubérculo. **Conclusiones.** Estas observaciones nos permitieron determinar que existen variaciones entre poblaciones; el ancho de los estomas y el número de cloroplastos en una población pueden ayudar a determinar los niveles de ploidía, y el ancho de la hoja es una variable de respuesta que permite predecir el peso del tubérculo.

Palabras clave: ñame; Dioscoreaceae; poliploides; geófitas; variación poblacional.

INTRODUCTION

Family Dioscoreaceae presents six genera and close to 850 species; *Dioscorea* is the most abundant genus with 650 species (Jiang *et al*. 2014). In Mexico, close to 73 species of this genus have been reported (Ramírez-Amezcua *et al*., 2012). Their distribution is found exclusively in tropical areas and it is limited to 20° N and 20° S, in the African, Asian, and American continents (Asiedu and Sartie, 2010). Genus *Dioscorea* plays an important role as a food source and it includes some of the most used tubers and roots in the planet (Bradshaw, 2010); 72´580,851 t of *Dioscorea* tubers are produced annually in the world, in a surface of 8´690,716 ha (FAOSTAT, 2018). In Mexico around 60 and 80 different *Dioscorea* wild species grow; among them are *D. dugesii*, *D. nelsonii*, *D. remotiflora* and *D. sparsiflora* (Mc Vaugh, 1989). These two last species are commonly known in Mexico as “camote de cerro”. They are collected in their natural habitats and the tubers are sold for human consumption (Jones *et al*., 2011). Their habitat is located mainly in hill slopes and they require a nurse plant for shade and support for the climbing stems (Mostul and Cházaro, 1996).

A characteristic in *Dioscorea* genus is that plants produce diosgenin (Janicka *et al*., 2016). This compound presents properties for several treatments as in the case reported by Li *et al*. (2010) who observed that total saponin extracts have the potential to reduce cardiovascular disease risk due to the antithrombotic effects they present in mice. Other works have studied diosgenin as an inhibitor of cancer cells development with good results (Lu *et al*., 2009; Raju and Mehta, 2009). Camote de cerro (*Dioscorea* spp.) presents characteristics healthy eating and additionally contains diosgenin (Contreras-Pacheco *et al*., 2013), Mexico was considered a rich source of diosgenin a few decades ago (Martin, 1969).

Dioscorea is a plant group which presents different ploidy levels among the same species, there are reports of 2n=80 chromosomes present in somatic cells (Martin and Ortiz, 1963; Ramachandran, 1968). Polyploidization, as well as interspecific hybridization play an important role in mechanisms of speciation (Hegarty and Hiscock, 2008). Polyploid organisms can adapt in less time because they can present greater variation in the genetic sequence (Selmecki *et al*., 2015). In different species, it has been observed that increment in the number of chromosomes pairs accounts for modifications in stomata size, length, and width of leaves as well as an increment in cells size for different tissues (Janaki and Singh, 1962; Abraham, 1998; Heping *et al.*, 2008; Yoshizumi *et al*., 2008). These changes can give advantages or disadvantages in terms of adaptation, compared to plants with lower ploidy levels. In the case of recently formed polyploids they present a cytotype with less probability for pairing (Hegarty and Hiscock, 2008). Some of the advantages present in polyploid organisms are greater resistance to abiotic factors, where polyploid organisms present mechanisms allowing more efficiency in the use of water (Xiong *et al*., 2006). Other effect presented in these variation is biomass production, as ploidy levels increases, also yields increases and hence dry biomass (Bragdo, 1962). Regarding polysaccharides production, a difference in lignin quantity has been observed, where diploids have a greater quantity compared to triploids and tetraploids (Serapiglia *et al*., 2014). In this work, wild plants of *Dioscorea* spp from eleven localities from different regions of the state of Jalisco were studied, variation in ploidy levels, leaf size, stomatic characteristics, species, and tuber yield variables were evaluated, and all these variables were correlated. The objective of this work is to determine the variation between populations in ploidy levels, leaf dimensions and stomata, in addition to correlating these variables with the tuber, to predict tuber weight using some variables.

MATERIALS AND METHODS

Vegetable material management

Ninety-nine accessions from eleven localities in Jalisco, México (Figure 1), for two different species of *Dioscorea* (*Dioscorea remotiflora* and *Dioscorea sparsiflora*) were used. They were planted in shaded greenhouse conditions at the greenhouses area from

Centro Universitario de Ciencias Biológicas y Agropecuarias, located in the geographical position 20°44'47.8" N 103°30'42.4" W. All plants originated from tuber fragments and were cultivated in plastic containers of 40 x 60 x 25 cm, leaf mulch was used as substratum and containers were placed under mono thread dark shade mesh allowing a luminous intensity of 26000 lux. Raffia strips attached to the greenhouse roof were used as tutors. They were fertilized every two weeks with hydrosoluble fertilizer 20-10-20 Peters Professional® brand at a dosage of 0.25 g, per container. The growing period was 35 weeks (May to December).

Cytogenetic analysis

Chromosome observations were carried out in radicular meristems. Roots of ±1 cm were collected between 7:00 and 9:00 AM, and placed in plastic tubes of 2 mL, containing 8-hydroxyquinoleine 2 mM. They were placed in an ice recipient to provide an approximate temperature of 4 °C, during 6 h. To fix the samples, a mixture of ethanol and acetic acid at 3:1 was used. Pectinase from *Aspergillus niger* (N. C. P-2736 SIGMA), combined with citrate buffer 1:1 was used for cell wall digestion for 90 min at 37 °C. Roots were then transferred to hydrochloric acid (HCl) 1N for 10 min at 60 °C. The root apex was placed on a microscope slide with a drop of acid dye acetoorcein and squeezed with cover slide. Preparations were heated with an alcohol burner for 4 s, avoiding dye to reach boiling point. Ten metaphase photographs were taken per accession with 100X in microscope ZEISS Axiostar Plus equipped with AxioCam camera model ICc1. Photographs were processed with program AxioVision 4.8.1 for chromosome counting latter on.

Stomata density and chloroplast number

For counting stomata and chloroplasts per stoma, four leaves per accession were used, they were collected during August and September between 8 and 10 AM, from the most vigorous stems at ± 1.1 m height avoiding old or very young leaves; after collecting, they were placed in plastic containers containing moist paper to avoid dehydration. At the laboratory, with dissection players, an area of 0.5 cm^2 epidermis was removed from the back central part of the leaf lamina and placed in a microscope slide over a drop of potassium iodine (KI). Stomata counting in five fields of the leaf was then carried out, with a total of 20 observations per accession. The central stoma from each field was chosen for chloroplast counting. Twenty stomata were counted per accession. These observations were accomplished in a compound LEICA microscope model DME with objective 40X for stomata density, and 100X for chloroplast counting.

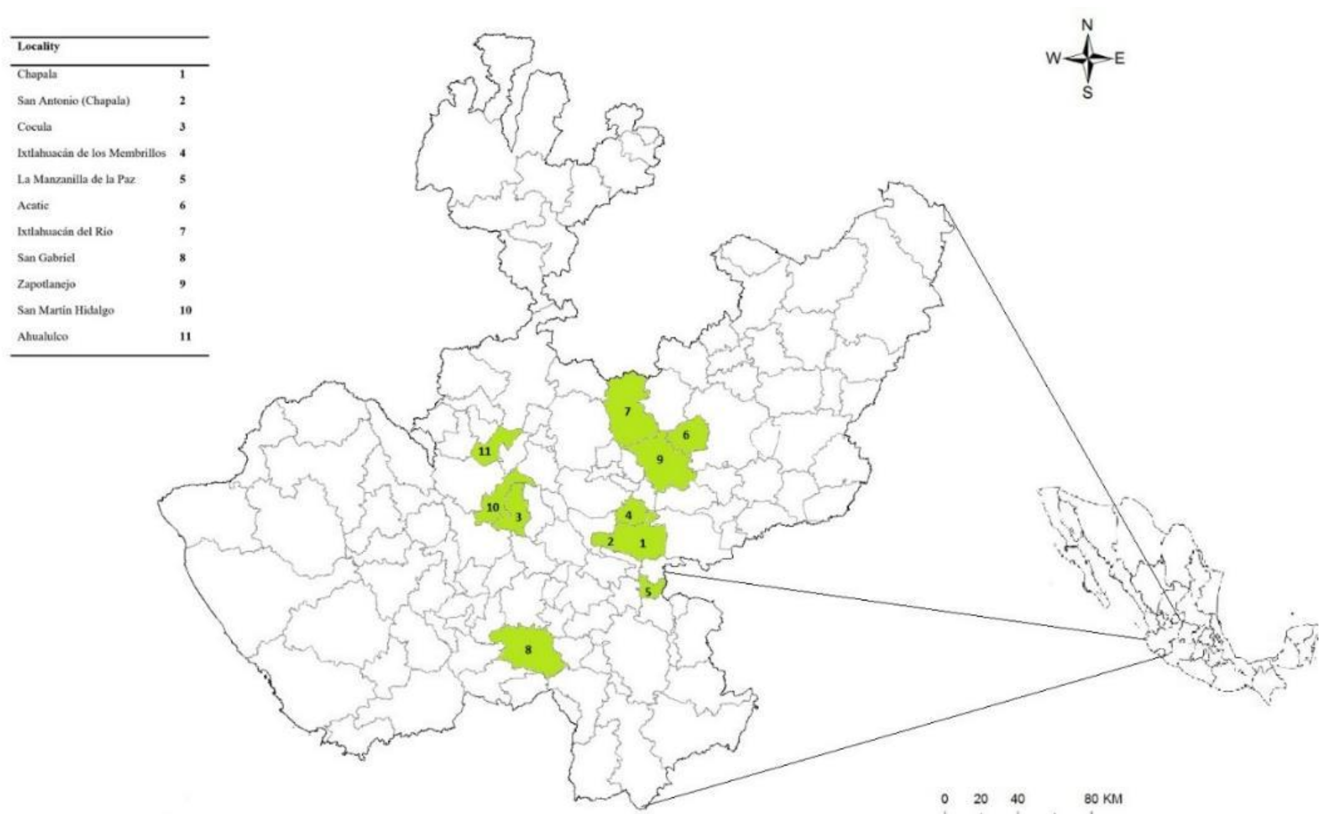

Figure 1. Distribution of the eleven localities of origin for the accessions of *Dioscorea* sp. in Jalisco, Mexico.

Stomata size

Ten leaves per accession were used, chosen from the most vigorous stems. The leaves chosen were healthy and mature to avoid measuring stomata that were not completely developed. They were kept in a refrigerator at 5 °C for less than 4 h. Potassium iodide (KI) was used to dye stomata. Two stomata were used from each leaf, they were photographed with AxioCam ICc1 camera with 100X objective in a ZEISS Axiostar Plus microscope. Measurements of stomata length and width were carried out with measuring tools from program ZEISS AxioVision 4.8.1.

Leaves measurement

To determine leaf width, length and area ten leaves were used per accession, collected in the months of August and September between 8 and 10 AM. At the laboratory they were placed in refrigerator at 5 °C to preserve them and avoid dehydration. Each leaf was digitalized in scanner HP Model Scanjet 3970, with a standard rule placed in the area of digitalization as a reference for scale determination. Digitalized files were kept in PDF format. Files were analyzed and leaf width, length and area were determined with the measuring tools of Adobe Acrobat 8 Professional program. Data was captured in Excel 2007 in order to manage it and obtain measurement equivalences.

Tuber evaluation

Tuber fragments (seed tubers) were weighed before planting. When plants ended their crop cycle (180 d) tubers were harvested and weighted in a digital balance Torrey, model EQ-10/20. Productivity rate was determined using the following formula:

$$PR = \frac{WTH}{WST}$$

where:
PR: *Productivity rate*
WTH: *Harvest tuber weight*
WST: *Seed tuber weight*

Study variables and statistical analysis

Variables considered for this work were: harvest tuber weight, productivity rate, leaf width, length and area, stomata density, chloroplasts number, and ploidy level. Considering that all 99 accessions were planted in the same conditions, differences among the localities, species and ploidy levels were analyzed by one-way Analysis of Variance using the GLM procedure of the Statistical Analysis System (SAS) version 9.0.To determine the degree of association between variables, simple correlation coefficients were calculated among all pairs of variables using and the CORR procedure from SAS.

RESULTS

Ploidy level

When analyzing the cytologic preparations, four levels of ploidy (Figure 2) were found among the 11 populations distributed in the state of Jalisco; 16 were diploid, 34 triploid, 47 tetraploid and two hexaploid (Table 1), basic chromosome number is n=10. Ploidy of populations from San Martín Hidalgo, San Gabriel, Ixtlahuacán del Río, Ixtlahuacán de los Membrillos and Cocula was mainly triploid and tetraploid.

Analysis of Variance

The Analysis of Variance (ANOVA) using Generalized Linear Model (GLM) showed variability for accessions obtained from the different classification criteria: localities, species, and ploidy levels (Table 2). Localities showed greater variability than species and ploidy levels. Mean squares for localities were significant for productivity rate, ploidy level, stomata density, chloroplast number, and stomata width and length. The model considering ploidy levels was significant for chloroplast number and stomata width, while for the model considering species, presented significance for chloroplast number only. A nested model including localities and species among localities, was significant only for localities in the variables ploidy, stomata density, chloroplasts in stomata, stomata width and stomata length.

Table 1. Ploidy levels present in different accessions of *Dioscorea* from the 11 localities in the state of Jalisco, México.

	Ploidy level			
Locality	2*x*	3*x*	4*x*	6*x*
Chapala	2	3	1	
San Antonio (Chapala)	6	6		
Cocula	1	3	1	
Ixtlahuacán de los Membrillos		4	1	
La Manzanilla de la Paz	2	1	8	
Acatic	1		4	
Ixtlahuacán del Río	1	8	3	
San Gabriel		6	4	
Zapotlanejo	1	1	10	
San Martín Hidalgo	1	2	7	
Ahualulco	1		8	2
Total	**16**	**34**	**47**	**2**

2*x*- diploid; 3*x* - triploid; 4*x* - tetraploid; 6*x* – hexaploidy.

Table 2. Variance analysis and mean squares for different localities, species, and ploidy levels.

Variable	M. S. Species	M. S. Localities	M. S. Ploidy	Factor model	
				Species (Loc)	Locality
PR	4.41	87.92*	51.77	1.35	77.24
P	29.44	222.58**	-----	13.03	214.30**
LW (cm)	0.27	2.61	1.08	5.50	2.48
LL (cm)	0.70	3.44	0.73	5.00	3.29
LA (cm^2)	130.39	31.18	186.97	997.27	496.53
SD	11.31	288.13**	89.71	218.24	269.93**
CS	18.51*	18.64**	11.11*	4.01	15.66**
SW (μm)	0.02	6.59**	7.42**	2.20	6.67**
SL (μm)	1.11	18.57**	10.22	0.32	18.54**

MS - mean squares; PR- productivity rate; P- ploidy; LW - leaf width; LL - leaf length; LA - leaf area; SD - stomata density; CS - chloroplasts in stomata; SW - stomata width; SL - stomata length; *, $p \leq 0.05$ significant; **, $p \leq 0.01$ highly significant

Correlation

Ploidy levels presented associations with number of chloroplasts in stomata and with leaf width. Harvest tuber weight showed correlation to productivity rate, leaf width and number of chloroplasts in stomata. Productivity rate showed correlation with leaf length, width, and area. Leaf width, length and area showed correlation with stomata width and length; leaf area also showed correlation with stomata density. Stomata density presented correlation with chloroplast number in stomata (Table 3).

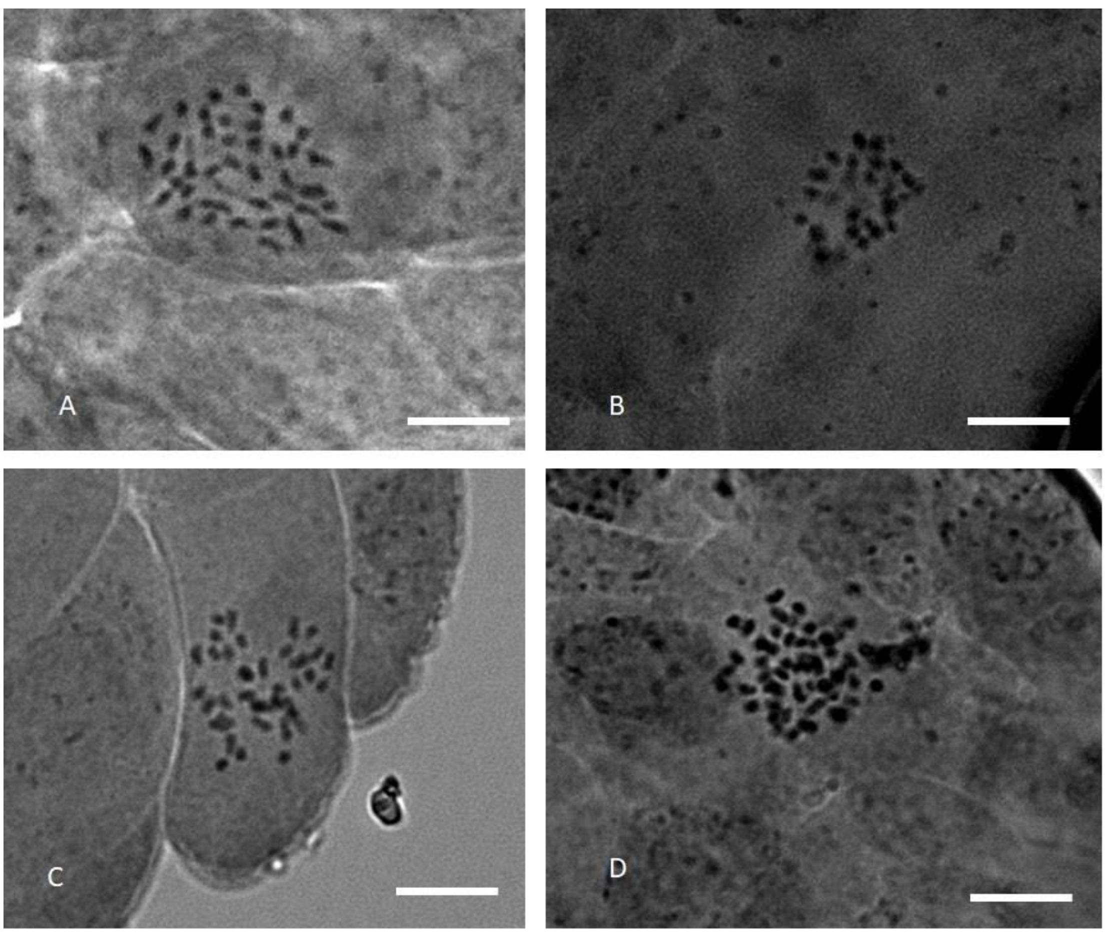

Figure 2. Ploidy level in populations of *Dioscorea* sp.: tetraploid (A); diploid (B); triploid (C); hexaploid (D); bar = 5 μm.

Table 3. Correlation analysis of weight tuber, productivity rate, ploidy, leaf width, leaf length, leaf area, stomata density, chloroplasts in stomata, stomata width and stomata length.

	WTH	PR	P	LW	LL	LA	SD	CS	SW	SL
WST	**0.624**	**-0.583**	-0.054	**0.276**	0.190	**0.217**	**-0.272**	**0.328**	0.156	0.097
p	0.000	0.000	0.593	0.006	0.063	0.033	0.006	0.001	0.126	0.345
WTH		**-0.301**	0.027	**0.245**	0.179	0.181	-0.155	**0.326**	0.191	0.119
p		0.002	0.790	0.016	0.080	0.076	0.126	0.001	0.061	0.244
PR			0.009	**-0.328**	**-0.281**	**-0.291**	0.069	-0.148	-0.161	-0.083
p			0.930	0.001	0.005	0.004	0.499	0.143	0.116	0.419
P				0.005	0.045	-0.040	0.128	**-0.279**	**0.208**	0.185
p				0.963	0.664	0.695	0.207	0.005	0.041	0.070
LW					**0.880**	**0.938**	-0.191	-0.019	**0.213**	**0.275**
p					0.000	0.000	0.061	0.853	0.036	0.006
LL						**0.939**	-0.146	-0.100	**0.282**	**0.335**
p						0.000	0.153	0.328	0.005	0.001
LA							-0.199	-0.038	**0.241**	**0.299**
p							0.050	0.715	0.018	0.003
SD								**-0.359**	-0.145	-0.044
p								0.000	0.156	0.672
CS									0.171	0.127
p									0.094	0.215
SW										**0.779**
p										0.000

WST - seed tuber weight; WTH - harvest tuber weight; PR - Productivity rate; P- ploidy; LW - leaf width; LL - leaf length; LA- leaf area; SD - stomata density; CS - chloroplasts in stomata; SW - stomata width; SL - stomata length; *p* - probability. Bold font indicates statistical significance.

DISCUSSION

Ploidy levels

The highly significant differences among localities for ploidy levels, seem to indicate that modifications at ploidy level occur in localities allowing each organism to a greater capacity for adaptation in its habitat (Wendel, 2000). Modifications at ploidy level allows for greater flexibility for processes and chemical, physiological, and morphological changes in order to have greater success in adaptation and in being more competitive with other plants in its habitat (Wendel, 2000). Another theory holds that polyploidy is a series of events involving morphological diversity and significant effect on population dynamics (De Groot *et al*., 2012).

Ploidy level refers to the number of chromosomes in somatic cells. In plants some changes in chromosome number or ploidy level, depending on the quantity of genetic material they carry are usually expected. In the case of the *Dioscorea* species used in this work the number of chromosomes pairs had a highly significant correlation with the number of chloroplasts present in stomata (Table 3), and a significant correlation with stomata width. Correlation coefficient was negative for chloroplasts, Thus, plants with the smallest number of chloroplasts have an increase in ploidy. Krishnaswami and Andal (1977) carried out a research with the number of chloroplasts present in stomata of *Gossypium spp.* and concluded that plants showed a positive correlation of ploidy level and chloroplast number in occlusive cells, different from what was found in *Dioscorea*. These observations, although relating to another species agree with the findings in this work, when ploidy levels increase, chloroplast numbers change. Correlation coefficient with stomata width was positive, which means that to an increase in the number of chromosomes pairs, corresponds to an increase in stomata width. This observation has also been reported in other species, when an increment in ploidy level is induced, stomata size also increases (Simmonds, 1948; Van Laere *et al*., 2011).

Table 2 shows ploidy correlation with other variables, where ploidy shows a relation with chloroplast number in stomata and with stomata width. In other works where an increment in ploidy levels has been induced there are changes not only in stomata size but also in leaf size (Huang *et al*., 2008; Xu *et al*., 2010). This relation was not found in this work. It is important to consider that polyploidy is reflected in effects on the growth and development of plants, plants with higher ploidy have been used as an efficient strategy to increase the level of compounds that act as secondary metabolites, which simultaneously improve the morphological characteristics (Moetamedipoor *et al*., 2022).

Tubers

Tubers are the structure that stores the greatest part of compounds elaborated by yams, potatoes, and other species with tubers. In *Dioscorea* species it is important to know which factors have an influence in the growth and development of tubers due to their importance as a source of food. In this study the correlation among seed tuber weight and harvest tuber weight was highly significant (Table 3). The correlation was positive so to a higher weight of seed tubers a higher weight in harvest tubers is obtained (Iseki and Matsumoto, 2019). Also, it has been observed in field that sprouts from tubers with higher weight have more robust and vigorous stems (Arsenault and Christie, 2004; Aighewi *et al*., 2020).
The aerial part of *Dioscorea* plants (leaves and stems) is lost at times of the dry season, only the tuber survives. Species of *Dioscorea* genus in the state of Jalisco start to produce new shoots in May using carbohydrates and other elements stored in the tuber (Contreras-Pacheco *et al*., 2013), allowing plants growing from big tubers to have greater capacity to compete for spaces getting light (Park *et al*., 2009). Productivity rate presents a highly significant negative correlation with tuber seed weight which means that tubers with less weight increased more times their weight. This characteristic of *Dioscorea* tuber has been exploited as a new propagation method for production named "minisett" (Morse and McNamara, 2020). When assigning a specific quantity of kilograms for seed, yield will be higher if the number of fragments is higher than if using tuber sections of higher weight and less quantity of seed tubers. Correlation between harvest tuber weight with seed tuber weight and productivity rate seem to be in contradiction; nevertheless, taking both observations into consideration a balance can be searched to reach the highest yield.

Correlation between tuber weight and chloroplast number, and leaf width (Table 3) shows the role they play in photosynthesis since it reflects in tuber weight, where captured energy is stored (Schwartz and Zeiger, 1984). For that reason, if what is preferred is a higher tuber weight in harvest, it is necessary to select plants with a bigger leaf size, which will allow to increase yield due to the positive correlation among those variables. Nevertheless, if the facts that productivity rate and harvest tuber weight are correlated negatively, and harvest tuber weight and leaf width are correlated positively are taken into consideration, the importance of the seed tuber fragment size and its impact in the development of the leaves and in the yield of each plant is highlighted. As *Dioscorea* species are important due to tuber consumption and obtaining pharmaceutic and cosmetic products from it, it is important to study the tuber development considering production (Cronin and Draelos, 2010). In this work no correlation between tuber and level of ploidy was observed; in other species of *Dioscorea* it has been observed that when increasing ploidy level, the number of aerial tubers also increases (Girma *et al*., 2017).

Stomata

Stomata are structures of great importance in the photosynthesis process. This work evaluated their length, width, and density. Results show that there was a highly significant correlation among stomata density and chloroplast number (Table 3). The correlation coefficient was negative which seems to imply that to a greater number of stomata present in a specific area, a lesser number of chloroplasts. This has also been reported by Abdoli *et al*. (2013) who when inducing an increment in ploidy level found variation in the number of stomata per cm^2 and in the chloroplast number in occlusive cells. Generally, in herbaceous plants, higher levels of ploidy are associated with lower stomatal densities, which may favor certain species to adapt to specific environments or as a biological strategy (Rhounghani *et al*., 2021). Variation in stomata and chloroplasts have been observed in other species; when comparing hexaploids with triploids it was found that triploids present higher stomatic density, but chloroplast numbers decrease (Liu *et al*., 2018). These observations allow us to determine that the relation between stomata and chloroplasts is adaptative according to the environmental conditions of the place each accession comes from (Lichtenthaler *et al*., 1981; Lawson, 2009). In some grape varieties it has been found that at higher levels of ploidy the length and width of the stomata increase. These changes in morphology are used to adapt to different environments (Catalano *et al*., 2021).

Leaf

Leaves are of great importance in plants, being the organ in charge of photosynthesis. Their shape and size are variable depending on species. Table 3 shows that leaf width showed a significant correlation with stomata density and width, and a highly significant correlation with stomata length. Stomata density correlated negatively; that is, plants having wider leaves presented a lower stomata density than those with smaller leaves. Correlations between leaf width and stomata width and length were positive. This behavior regarding correlation between leaves and stomata has been reported in evolutionary development in other species where the correlation between size and density was negative (Franks and Beerling, 2009).

In the case of the species on this study, correlation between stomata width and length, and leaf length was highly significant and the coefficient was positive. The correlations between leaf area with stomata width and length were also evaluated in this work and resulted positive and significant and highly significant, respectively. Taking Table 3 into consideration it could be said that stomata and leaf characteristics (length, width and area) were closely related (Heping *et al*., 2008; Yoshizumi *et al*., 2008).

CONCLUSIONS

In the present study, *Dioscorea* ssp. variation were higher between populations than between species, there were differences in ploidy levels which were associated with populations of origin. In the same way, ploidy level was found to be associated with other variables and these were associated among themselves, as could be seen with harvest tuber weight which was associated with seed tuber weight as well as with leaf width. Another finding is that the chloroplasts in the stomata can be used to discriminate between the ploidy levels as well as to determine which plants present the highest yield of the harvested tuber. Likewise, accessions with high or low stomatal densities, different ploidy levels, leaf shapes and sizes may be considered as potential genetic resources for *Dioscorea* breeding with the aim of drought resistance and increasing tuber weight.

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Conflict of interest. The authors declare that they have no conflict of interest.

Compliance with ethical standards. Does not apply.

Data availability. All data was presented in this manuscript. Experimental data are available upon request to the corresponding author.

Author contribution statement (CRediT). J. J. Castañeda-Nava - Conceptualization, Investigation, Methodology, Resources, Software, Writing original draft, Writing – review & editing. **F. Santacruz-Ruvalcaba** – Conceptualization, Funding acquisition, Methodology, Project administration, Supervision, Resources, Writing original draft, Writing – review & editing. **R. Barba-Gonzalez -** Investigation, Supervision, Validation. **J. J. Sánchez González** - Formal analysis, Software, Validation, Writing original draft. **L. De la Cruz Larios -** Formal analysis, Methodology, Resources.

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