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Morphological and genetic characteristics allow the identification of a collection of garlic cultivars in the North-Central region of Mexico.

(With 1 Table & 2 Figures)

Características morfológicas y genéticas permiten la identificación de cultivares de ajo en las regiones Norte y Central de México

(Con 1 Tabla y 2 Figuras)

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Abstract. The objective of this study was to compare the yield and genetic relationships between two Perla garlic selections, obtained by individual selection of cloves, and commercial varieties and cultivars adapted to diverse regions of Mexico (which did not have a selection process). Varieties with fewer cloves showed higher yields. Coreano and California produced 20 ton/ha (fresh weight) and Perla selections between 17-19 ton/ha (fresh weight). Six primers of the series OPB were tested for genetic characterization and OPB-17 was selected. With the amplified DNA fragments, a bina-

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ry matrix was generated; afterwards, a dissimilarity matrix and dendrogram were developed. High genetic diversity was found among all varieties, which were separated into two groups through differential analysis. Varieties of smaller yield were included in one group. The other group was constituted by the best production varieties with fewer cloves per plant (Perla, California, Coreano and Chino). Dissimilarity was found among Perla varieties. The selection method used to obtain plants with better yield characteristics can be applied to the genetic improvement of garlic.

Key words: RAPDs, *Allium sativum*, selection, yield.

Resumen. El objetivo de este estudio fue comparar las relaciones genéticas y de rendimiento entre dos selecciones de ajo Perla, obtenidas por selección individual de dientes, y variedades y cultivares comerciales adaptadas a varias regiones de México (las cuales no tuvieron un proceso de selección). Las variedades con menor número de dientes mostraron mayores rendimientos. Coreano y California produjeron 20 ton/ha (peso fresco) y las selecciones de Perla entre 17-19 ton/ha (peso fresco). Seis iniciadores de la serie OPB fueron caracterizados genéticamente, y se seleccionó OPB-17. Con los fragmentos de DNA amplificados se generó una matriz binaria; posteriormente se desarrollaron una matriz de disimilitud y un dendograma. Se encontró una alta diversidad genética entre todas las variedades, que se separó en dos grupos. Un grupo incluyó las variedades de menor rendimiento. El otro grupo incluyó las variedades de mayor producción con menos dientes por planta (Perla, California, Coreano y Chino). Entre las variedades de Perla se encontró falta de similaridad. El método de selección utiizado para obtener plantas con mejores características de rendimiento puede ser aplicado a la mejora genética de plantas de ajo.

Palabras clave: RAPDs, *Allium sativum*, selección, rendimiento.

INTRODUCTION

Allium sativum L. ssp. *sativum* includes softneck garlic cultivars that are either non-bolting or produce only very weak stalks. In general, most softneck types have 12-20 cloves arranged in three to six layers within a bulb. Artichoke type softnecks tend to have early maturing cloves with coarse, thick clove skins, and yellow-green, horizontal leaves that emerge from all sides of the plant.

During stress conditions, asexual propagules called bulbils may be produced within the false stem, resulting in the classification of incomplete bolting types. In contrast, silverskin type softnecks have early vertical, blue-green leaves that display a bilateral symmetry. The tight clove skin and late maturation dates on silverskins enable these types to be stored for long time periods. Softneck cultivars often grow better under mild winter conditions (Engeland, 1995).

Garlic is a vegetatively propagated crop that does not set seed under standard growing conditions. Clonal lineages within this species show a remarkably high degree of phenotypic diversity. New genotypes have not been obtained through hybridizations, but through the selection of spontaneous mutations expressing traits of horticultural interest (Volk et al., 2004). The diversity of these clones is described by a set of phenotypic and morphological descriptors that have certain plasticity (Al-Zahim et al., 1997; Ipek et al., 2003).

Genetic markers have been used for better characterization. Garlic cultivated in Australia (Bradley & Collins, 1996), Korea (Eom & Lee, 1999), India (Shasany et al., 2000; Peiwen et al., 2001) and the United States (Ipek et al., 2003) has been characterized by the random amplification of polymorphic DNA fragment technique (RAPD). This characterization has been used to identify lines of garlic producing fertile pollen (Etoh & Hong, 2001), and the country of origin of garlic. Choi et al. (2003) analyzed 75 clones of garlic, and classified them in two large groups. The first group was formed by the Asian clones, and the second one by the European, American and Russian clones.

The Food and Agriculture Organization (FAO, 2000) reports garlic with a worldwide production of about 12 million tons, highlighting China with 74% of the total production. This country is followed by Korea, India and the United States. In Central and South America, 32,000 ha are cultivated with an approximate yield of 5.9 ton fresh wt/ha. This is quite below the 18.8 ton fresh wt/ha level recorded in the USA. Mexico is considered among the ten highest producers of garlic in the world, with a sown surface of 8,117 ha and a production of 65,200 tons (fresh weight) (SAGARPA, 2004). In Mexico, the most important producer states are Guanajuato, Aguascalientes, Zacatecas, Puebla, Sonora, Queretaro and San Luis Potosi. These states produce nearly 94% of the total Mexico production.

Cultivated varieties in Mexico have been preferably introduced from the United States; other genotypes have been selected through cultivation. This is the reason why there are many garlic phenotypes that differ in number, weight, color and size of cloves and bulbs. In the present study, twenty one varieties of garlic cultivated in the Central-North region of Mexico were analyzed to assess their productive performance and genetic relationships using RAPD markers.

MATERIALS AND METHODS

The following varieties were used in this study: Coreano, California, Chino, Español, Cortazar, Positas, Pepita, Massone, Durango, Chilean, Hermosillo, Sonora, Napuri, Nacajuca, Nicaragua, Ixmiquilpan, Pata de Perro, Guatemala, Criollo Aguascalientes and the two cultivars Perla C-3-1/25 and Perla C-37-1/8. Perla's cultivars were obtained after a period of six cultivation cycles, using a method of individual selection which included bulbs with fewer and bigger cloves.

Field evaluation. Garlic varieties were cultivated in the "Campo Experimental Pabellón" (Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias, INIFAP) located in Pabellón de Arteaga, Aguascalientes, Mexico. Garlic was sown on 30 October 2004 and harvested in May 2005. Seeding was conducted using 80 cm double line furrows with a distance of 10 cm between plants and 20 cm between lines. A 180-100-100 (N,P,K) fertilization was applied. The experiment was completely randomized with samples in a subdivided block design. Each subsample was represented by a variable number of plants, according to clove/bulb number (7-21) produced by each variety. The total number of subsamples for each variety was 100. The number of days to harvest was determined and then, 100 plants obtained at random were analyzed. Length, fresh weight and number of cloves in the bulbs, and yield expressed in ton fresh wt/ha were determined. These variables were measured at various growth stages using the standard descriptors for garlic developed by the International Plant Genetic Resources Institute-IPGRI (IPGRI, 2001).

DNA extraction and RAPD analysis. Genomic DNA was extracted from frozen leaves according to Doyle & Doyle (1990). DNA samples were run on 0.8% agarose gel, and DNA concentration was measured with a spectrometer (model GBC Cintra 10e UV-visible). RAPD reactions were performed in a 25 ml volume, consisting of 10X buffer solution [10 mM Tris-HCl buffer (pH 8.0), 50 mM KCl_2], 2.5 mM $MgCl_2$, 2.5 units of Taq DNA polymerase (Promega), 100 µM dNTP, 50 ng genomic DNA and 0.4 µM OPB series (OPB-8, OPB-9, OPB-10, OPB-11, OPB-15 and OPB 17) primer (Operon Technologies, Alameda, CA, USA). A total of 20 µl of mineral oil was placed over the reaction mixture. Amplifications were carried out in a DNA thermocycler (Model FPR0G02Y Techne Progene, England),

under the following conditions: an initial denaturalization step of 2 min at 94 °C, followed by 35 cycles of 1 min at 94 °C, 1 min at 35 °C, and 2 min at 72 °C, with a final extension step of 7 min at 72 °C.

Scoring and analysis of RAPD data. Amplification products were analyzed by electrophoresis in a 1.2% agarose gel. It was run at 100 V for 4h, and detected by staining the gel with ethidium bromide (10 ng/100 ml of agarose solution in TBE). All visible and unambiguous fragments amplified by the chosen primers were entered under the heading of total visible fragments. Fragment data were entered on a spreadsheet to form a binary matrix, where (1) represented fragment presence and (0) fragment absence for each fragment-accession combination. Cluster analysis was conducted by converting the data matrix into a similarity matrix using a simple matching coefficient. This coefficient was calculated by dividing the number of matches (0-0 and 1-1) by the total number of comparisons (Nei and Li, 1979). A cluster analysis was then conducted using the unweighted pair group method, with arithmetical averages (UPGMA) process using the S–Professional Plus 2000 program.

RESULTS AND DISCUSSION

Field performance. Greatest values for bulb perimeter were found in the California (23.1 ± 1.8 cm) and Coreano (20.4 ± 0.7 cm) varieties, as well as in the cultivars Perla C-37-1/8 (21.1 ± 0.6 cm) and Perla C-3-1/25 (20.5 ± 0.7 cm) (Table 1). Three varieties of white garlic, California (112.3 ± 22.8 g), Perla C-37-1/8 (84.3 ± 8.1 g) and Perla C-3-1/25 (79.2 ± 7.8 g), as well as a marbled one, Coreano (82.3±8.0 g), showed the greatest bulb weight. Regarding the number of cloves per bulb, variety Español produced 7.5 (±0.9), while cultivars Perla C-3-1/25 and Perla C-37-1/8 had 10.9 (±1.3) and 11.9 (±1.9), respectively.

Plants showing a smaller number of bulb cloves appeared to have greater clove weights (Table 1); Perla cultivars were obtained by individual selection during six cycles of cultivation. Chino and Coreano varieties also showed a good clove weight performance. However, they are more susceptible to diseases and

they require more time for bulb formation. Varieties with greater bulb weights appeared to be taller than those with smaller bulb weights (Table 1).

Days to harvest and yield. Varieties of garlic can be harvested at either 150 (early cycle), 180 (intermediate cycle) or 210 (late cycle) days after being sown. Late cycle varieties showed greater bulb and clove weights [i.e., California and Coreano varieties, and Perla cultivars (Table 1)]. Greatest bulb weight variaties (i.e., California) showed more than 75% greater bulb weight than lowest bulb weight varieties (i.e., Pata de Perro). Perla cultivars (C-3-1/25

Table 1. Size and weight analysis of garlic varieties cultivated in the Central-North Region of Mexico. Data are presented according to standard descriptors for garlic (IPGRI, 2001). Each value is the mean ± 1 s.e.

Tabla 1. *Peso y tamaño de variedades de ajo cultivadas en la Región Norte-Central de México. Los datos se presentan de acuerdo a descriptores estándar para ajo (IPGRI, 2001). Cada valor es el promedio ± 1 e.s.*

Varieties	Bulb length (cm)	Bulb weight (g)	Clove number/bulb	Plant length (cm)	Clove weight (g)
California 20/1	23.1 ± 1.8	112.3 ± 22.8	17.4 ± 4.6	71.1 ± 2.9	8.1 ± 2.7
Coreano	20.4 ± 0.7	82.3 ± 8.0	12.9 ± 3.5	67.6 ± 4.9	9.9 ± 2.0
Perl C-3 - 1/25	20.5 ± 0.7	79.2 ± 7.8	10.9 ± 1.3	74.3 ± 3.4	12.4 ± 1.1
Perla C-37 - 1/8	21.1 ± 0.6	84.3 ± 8.1	11.9 ± 1.9	73.9 ± 3.4	12.8 ± 3.7
Chino	18.7 ± 0.9	62.5 ± 6.7	12.2 ± 1.4	49.2 ± 3.8	7.2 ± 1.1
Ixmiquilpan	18.7 ± 1.6	60.8 ± 10.4	21.8 ± 1.3	72.9 ± 2.4	3.7 ± 1.1
Durango	19.9 ± 1.0	71.9 ± 7.7	19.9 ± 3.3	75.7 ± 3.6	4.8 ± 1.0
Criollo Ags.	17.4 ± 1.1	49.7 ± 8.3	14.5 ± 1.4	63.9 ± 2.9	5.3 ± 2.6
Cortazar	18.4 ± 0.7	57.4 ± 5.8	20.7 ± 2.3	64.7 ± 2.9	3.3 ± 0.5
Sonora	14.9 ± 0.7	36.4 ± 5.2	16.9 ± 3.5	47.7 ± 5.4	2.5 ± 0.8
Guatemala	14.8 ± 1.3	33.8 ± 8.8	14.8 ± 2.5	62.0 ± 5.4	3.0 ± 0.7
Positas	14.3 ± 0.7	34.6 ± 4.9	14.7 ± 3.5	51.5 ± 3.2	3.7 ± 0.9
Hermosillo	13.9 ± 1.3	31.9 ± 6.7	12.6 ± 3.7	60.1 ± 6.6	2.8 ± 0.8
Español	13.6 ± 1.4	24.6 ± 5.7	7.5 ± 0.9	59.9 ± 2.4	4.2 ± 1.0
Pepita	14.0 ± 0.6	32.9 ± 4.2	20.0 ± 4.0	48.3 ± 5.6	2.5 ± 0.6
Massone	13.2 ± 0.6	30.2 ± 3.6	15.0 ± 2.1	48.1 ± 4.6	2.9 ± 0.8
Nicaragua	14.1 ± 0.9	29.6 ± 6.6	13.0 ± 2.7	51.5 ± 3.2	2.7 ± 0.8
Nacajuca	14.4 ± 1.1	31.9 ± 6.7	18.4 ± 3.7	54.7 ± 7.0	2.9 ± 0.8
Chileno	14.2 ± 1.2	32.4 ± 5.8	17.6 ± 5.7	49.2 ± 3.8	2.8 ± 0.5
Napuri	13.5 ± 1.3	31.3 ± 7.1	14.8 ± 4.9	47.0 ± 2.9	3.0 ± 0.7
Pata de Perro	13.3 ± 0.7	27.7 ± 4.4	8.4 ± 1.2	55.0 ± 3.6	3.4 ± 1.3

and C-37-1/8) had a better tolerance to environmental conditions (Fig. 1), their bulbs had fewer cloves (10-12) (Table 1), and their bulb and clove weights were favorably compared with those of commercial varieties (i.e., Chino and Coreano: Table 1). Sonora, Positas, Hermosillo, Español, Pepita, Massone, Nicaragua, Nacajuca and Chileno cultivars showed very similar patterns in morphological characteristics and yields (Table 1) In agreement with results of Heredia & Delgadillo (2002), varieties which showed greater bulb weights also showed greater clove weights.

Genetic characterization. Six decamer OPB primers showing distinct polymorphic fingerprint were selected to reveal the genetic variation among the garlic samples. In almost all varieties, it was possible to identify around 10 bands. A dendrogram was generated from the binary matrix of measured data (Fig. 2), and two groups were identified. The first group was formed by eight varieties (Durango, Nicaragua, Cortazar, Hermosillo, Massone, Pepita, Sonora and Napuri) that are characterized by a lower production (smaller clove weight and/or greater number of cloves: Table 1), require more days to dormancy (6 months) and need fewer days (150) to harvest (data not shown). The second group was constituted by white, colored and marbled garlic (Coreano, Positas, Perla's cultivars, Criollo Aguascalientes, Español, Chileno, Ixmiquilpan, California, Chino, Pata de Perro and Guatemala). These are characterized by better bulb and clove weights, lower clove numbers/bulb, fewer days to dormancy (5-6 months), and between 180-210 days to harvest. In general, garlic varieties were clustered according to yield level, clove and bulb weights, number of cloves/bulb and dormancy period (Table 1). These results agree with those of García et al. (2003) using the AFLP technique. The most productive variety (California) has the inconvenient of having a larger number of cloves/bulb and requires lower temperatures to form complete bulbs.

Fig. 1. Perla garlic cultivar C-37-1/8. Lateral and transversal views

Fig. 1. *Cultivar de ajo Perla C-37-1/8. Vistas lateral y transversal.*

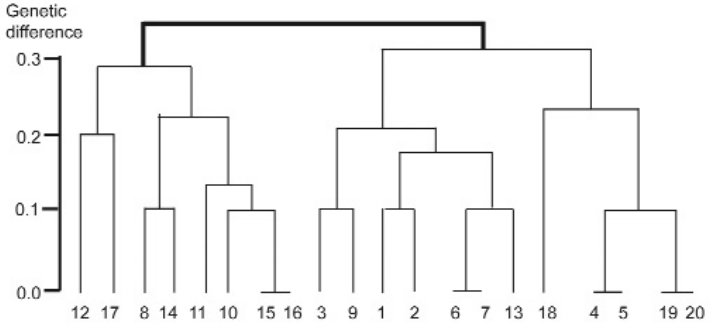
Dissimilarity among the two groups was 0.33. The lowest dissimilarity (0.0) corresponded to the most related varieties (Sonora-Napuri, Criollo Aguascalientes-Español, California-Chino and Pata de Perro-Guatemala). The highest dissimilarity (0.70) was between the variety Cortazar and the varieties Ixmiquilpan, Pata de Perro, and Guatemala. Choi et al. (2003) reported a dissimilarity of 0.4 between two large groups from a total of 75 garlic varieties using the "M" or affinity coefficient. Using the Jaccard coefficient, Ipek et al. (2003) obtained a lowest dissimilarity of 0.0 and a highest dissimilarity of 0.75 between two large groups of garlic. These results are similar to those of Al-Zaim et al. (1997). Evaluating diversity and genetic relationships among the progenitor *A. longicuspis* and 27 garlic varieties collected from different regions of the world, these authors found a dissimilarity of 0 between two samples, and a highest dissimilarity of 0.82 between two large groups. Through the RAPD technique used in this work, the two Perla cultivars were grouped with the best production varieties, where the two selections presented a dissimilarity of 0.1. However, the Perla C-3-1/25 cultivar showed a band of 2100 bp, and could thus be identified as a possible molecular marker.

Our results allow to identify highly related garlic varieties (Sonora-Napuri, Criollo Aguascalientes-Español, California-Chileno and Pata de Perro-Guatemala), and separate them from varieties that are characterized by a lower yield (i.e., Pata de Perro and Napuri), and from mixed garlic that has been generated from introduced commercial varieties (Criollo Aguascalientes); these have lost their potential yield because they have not been subjected to an appropriate selection process. Because we do not know the source of most garlic varieties and cultivars used in this work, we cannot establish relationships among their origins.

A likely ancestor of current, cultivated garlic plants could be *A. longicuspis* (L.). Domestication of garlic followed a different path than that of onion (*A. cepa* L.) and leek (*A. porrum* L.); they produce large quantities of true seeds for their propagation (Koul & Gohil, 1991). Garlic propagation is exclusively done by bulbils, and during this process it is not known how much variability has been selected. This is because *A. sativum* or its ancestor still multiply sexually (FAO, 1991). Some recent garlic cultivars form flowers intermingled with bulbils, but they never produce viable seeds (FAO, 1991). In Mexico, there are two garlic phenotypes: *A. sativum vulgare*

Fig. 2. Dendrogram obtained by the RAPD technique and general description according to physiological, morphological and genetic characteristics in garlic (*Allium sativum* L.) varieties cultivated in the Central-North Region of Mexico. 1.Perla C-3-1/25, 2. Perla C-37-1/8, 3. Coreano, 4. California, 5. Chino, 6. Criollo Aguascalientes, 7. Español, 8. Cortazar, 9. Positas, 10. Pepita, 11. Massone, 12. Durango, 13. Chileno, 14. Hermosillo, 15. Sonora, 16. Napuri, 17. Nicaragua, 18. Ixmiquilpan, 19. Pata de Perro 20. Guatemala.

Fig. 2. *Dendrograma obtenido por la técnica RAPD, y descripción general de acuerdo a características fisiológicas, morfológicas y genéticas en variedades de ajo (Allium sativum* L.*) cultivadas en la Región Norte-Central de México. 1.Perla C-3-1/25, 2. Perla C-37-1/8, 3. Coreano, 4. California, 5. Chino, 6. Criollo Aguascalientes, 7. Español, 8. Cortazar, 9. Positas, 10. Pepita, 11. Massone, 12. Durango, 13. Chileno, 14. Hermosillo, 15. Sonora, 16. Napuri, 17. Nicaragua, 18. Ixmiquilpan, 19. Pata de Perro, 20. Guatemala.*



Kunz and *A. sativum sagitarum* Kunz. The first phenotype does not produce inflorescences. The second phenotype flowers at the terminal part of the pseudo stem, reaching heights of 1.0-1.2 m when the plant reaches maturity (García, 1998; Aljaro, 1991). Burba (1992) mentioned that yield increased with larger garlic plants, which was confirmed in this work by using the individual selection method. Analysis of potential yield and genetic diversity of cultivated varieties will increase knowledge of the genetic bases of garlic. This will open the way for introduc-

ing new sources of genetic variation, and the possibility for studying factors that control agronomic characteristics of interest.

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