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Agronomic and Biotechnological Strategies for Breeding Cultivated Garlic in Mexico

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1. Introduction

Garlic is an apomyctic diploid species (2n=2x=16) with vegetative reproduction that belongs to the *Allium* genus (Alliaceae), which includes onion (*Allium cepa*), leek (*A. ampeloprasum*) and shallot (*A. ascalonicum*) (Mc-Collum, 1987; Figliuolo et al., 2001; Ipek et al., 2003; 2005). The importance of garlic was recognized by humans at bronze era about 5000 years ago, and since these early times, has been used as food, condiment and medicine by Asians and Mediterranean (Ipek et al., 2005). World production of garlic is ranked 14th among vegetables with a total of 14.5 million ton (Trejo, 2006). In Mexico, its consumption is about 400 g *per capita* (Chávez, 2008), and the national production is considered low as compared to other countries such as China (80 % world production), India, Korea and the rest of the world (20 %) (FAOSTAT, 2011). Still, Mexico has a place as exporter of garlic produced mainly from the states: Zacatecas, Guanajuato, Aguascalientes, Baja California, Puebla and Sonora (Trejo, 2006). The areas in Mexico during winter 2008, dedicated to garlic were 5,085 ha with a total yield of 49,968 ton (SIAP, 2011).

Among other problems, Mexican garlic has a limited spectrum of high yielding cultivars for different environments and, at the same time, have good market qualities. Keeping in mind that kind of problems, this chapter was mainly focused on agriculture and biotechnology research done at four institutions. The first two sections include morphological, physiological and cytogenetic characterizations of the most common cultivars and related germplasm; the third section describes some advances on garlic micropropagation. The last

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section describes our strategy for obtaining garlic genotypes with higher yield capability and better bulb quality characteristics according to the market demand.

2. Origin and distribution

Garlic is native to India (Central Asia), where was considered a spice with mystical implications due to its medicinal attributes. Egyptian hieroglyphs and Roman texts refer to garlic as a source for health and strength required for physical work. During the Middle Ages was used to prevent cholera. Nowadays, it is known for its antiseptic, diuretic, vermifuge and vasodilator activities. It also stimulates bile and stomach secretions, and acts against atherosclerosis and thrombosis.

Spaniard conquerors carried with them garlic; first to Cuba and, later, to the rest of the American colonies. Early reports of garlic fields in Mexico appeared at the beginning of the twentieth century, and fifty years later, the central region of Mexico (Bajío) was the main area for garlic production. The time of harvest in that region made possible to start exporting surplus, since at that time of the year the world production is low.

Garlic species are widely distributed on boreal areas having temperate climates and mountainous areas from tropical regions. Most of the species diversity is found from Mediterranean countries to Central Asia. USA is considered as diversification center for *Allium* (Lagunes, 2009).

3. Plant description

Garlic (*Allium sativum* L.) is propagated asexually, but shows a high morphological diversity among cultivars. These cultivars have a range of adaptation to different environments (Paredes et al., 2008). Like onion, garlic plants have thin tape-shaped leaves about 30 cm long. Roots reach a 50 cm depth or little more. Heads or bulbs are white-skinned, divided into sections called cloves. Each head could have from 6 to 12 cloves, which are covered with a white or reddish papery layer or "skin". Bulbs are consumed fresh, totally or partially dried, and pickled. Although the bulb consumption is more common, tender shoots sometimes are a delicatessen for sophisticated cuisine. These shoots may be prepared like asparagus.

Each clove is capable to develop a new plant, since they have an apical shoot bud that can elongate even though if they are not sown. This shoot is apparent after three months after the harvest, depending on the genotype and conservation conditions. Flowers are white, and the stem of some species also produce small bulbils. These stems produce a strong odor from two compounds: alliin and diallylsulfide.

4. Mexican genotypes

The origin of present-day genotypes in Mexico was a group of cultivars: 'Perla', 'California', 'Chileno' and 'Taiwan', which were introduced from USA and China. A short description of each is included:

'Perla'. Late cultivar (240 d), with creamy-colored bulbs; 10 to 16 cloves per bulb covered with about seven outer layers. Plant height is 40-45 cm tall, having a pale-green open

canopy. Experimental yields from this genotype usually range from 16 to 18 ton/ha. Physiological disorders are common, such as brush-like plant growth with excessive number of thinner leaves; the more severe this problem, the more the plant opens its canopy of leaves with reduced sheath. Bulbs of brush-like plants lose their covering layers, producing naked cloves. This disorder is high temperature-dependent, having the highest temperature influence on March and April; therefore, it varies in severity from year to year. Experimental observation indicates that some other factors alone or combined may be related to the induction of brush-like plants such as: early planting, excessive nitrogen fertilization, and planting density. This disorder worsens when these factors appear combined.

'California'. Late cultivar (260 d); recently introduced to Aguascalientes (Mexico). Bulbs are white, containing 18-26 cloves. Experimental yields range from 18 to 20 ton/ha. Plants are 50 cm tall on the average; leaves are pale-green with open canopy.

'Chileno'. Early cultivar (160 d), with a yield average of 7 ton/ha. Bulbs are purplish with 5-6 covering layers; containing 11-22 cloves (average = 19). Plants are about 50 cm tall; with semi-compact canopy and dark green leaves.

'Taiwan'. Early cultivar (170 d); its yield average is 7 ton/ha. Bulbs are purple in color, with 7-13 cloves (average = 9). Plant height reaches 50 cm on the average, with semi-compact canopy and dark green leaves.

5. Field performance of promissory genotypes

Besides the previously described genotypes, some more garlic accessions from the germplasm bank of INIFAP-CAEPAB (Fig. 1) were tested for their performance on the field (Aguascalientes, Mexico). These accessions have features suitable for breeding, as described below.

5.1 Bulb size and number of cloves per head

Higher values for bulb perimeter were found in '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 cloves per bulb appeared to have greater clove weights. '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).

5.2 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 planting. Late cycle varieties showed greater bulb and clove weights [i.e., 'California' and 'Coreano' varieties, and 'Perla' cultivars (Table 1)]. Greatest bulb

Fig. 1. Garlic genotypes from the germplasm bank at Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias - Campo Experimental Pabellón Aguascalientes (INIFAP-CAEPAB).

weight varieties (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' 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). Correlation between bulb length and clove weight against bulb weight showed an $R^2 = 0.9668$, while clove weight against bulb weight had an $R^2 = 0.635$ (both highly significant, $p < 0.01$); in agreement with results of Heredia & Delgadillo (2002), these varieties which showed greater bulb weights also showed greater clove weights.

Genotype	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
Perla 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

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

5.3 Postharvest photosynthesis and respiratory activity of stored cloves

In order to understand some physiological events of stored garlic, analyses of six genotypes under storage were focused on the respiratory process and photosynthesis. To accomplish that goal, photosynthetic activity of stored cloves during 0, 30, 60 and 90 d were measured on three cloves selected at random from the container of each genotype. Measurements included: evaporation rate mM/s/m2/s (E), stomatal conductance mM/m2/s (G), net

photosynthesis assimilation μM/m2/s (A) and CO_2 internal concentration ppm (CI). From these measurements, it was found that for some genotypes like 'C-CN-9/2' evapotranspiration was the highest at 30 d, as opposed to 'Criollo Aguascalientes' and 'Chino Jaspeado' with the lowest value at 90 d. Stomatal conductance was high 'C-CN-9/25', mainly after 90 d. Most genotypes showed negative photosynthesis rate, and internal CO_2 showed no clear tendency within genotypes. Weight remained stable during the first 60 d, but after that period, it decreased about 1/3 of the initial values. The bulbs behavior at the final of postharvest period is show in the Fig. 2.

Fig. 2. View of stored garlic at 90 d. Sprouted heads are from short-shelf life cultivars. Heads with low number of sprouting bulbs are from 'Perla' (bottom left corner).

6. Isolation and culture of garlic protoplasts

Garlic genotypes derived from 'Perla' (C-37-1/8, C-3-1/25 and C-CN-95/2), 'Chino', 'Coreano' and 'Criollo Aguascalientes' were used for protoplast preparation. Cloves of these genotypes were peeled and disinfected (briefly: cloves were soaked in 70% ethanol 1 min and 20% commercial bleach 20 min, after three rinses with sterile distilled water the cloves were soaked again in 70% ethanol 1 min and rinsed again with sterile distilled water). Surface-sterilized cloves were cut into pieces 0.5 to 1.0 cm long, and were inoculated aseptically on MS (Murashige & Skoog, 1962) medium pH 5.8 containing 0.15 mg L^{-1} 2,4-D (Dichlorophenoxy acetic acid), 5.0 mg L^{-1} BA (Benzyl adenine), 50 g L^{-1} glucose and 3.5 g L^{-1} Phytagel. Incubation conditions were 28 ±2 °C, with a light intensity of 59 μEm²s.

Protoplasts were isolated from leaves and callus using a modification of the method from Spangenberg (1997). Briefly: 1 g of tissue was placed in petri dish together with 5 ml of enzyme mixture (2% Onozuka R-10, 1% macerozyme, 0.5% pectinase, 0.5% mannitol and 0.9% $CaCl_2$); dishes were incubated on orbital shaker at 5 rpm, about 4-6 h (Novák et al., 1983). Callus tissues were best for protoplast isolation within the range 10^6-10^8 counts/ml (Fig. 3). Protoplasts were isolated from the debris and inoculated on semi-solid MS medium. Unfortunately, whole plant regeneration remained elusive.

Genotype		Clove weight (g)		Evaporation rate (mM/s/m²/s)		Stomatal conductance (mM/m²/s)		Net photosynthesis assimilation (µM/m²/s)		CI (ppm)	
	days	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
'C-CN-9/2'	0	65.30	8.21	69	21	45	15.5	-2.1	0.5	267	49.5
	30	64.6	9.7	0.3	0	14	1	-1.6	2.9	327	16.9
	60	64.0	8.3	0.5	0.7	40	59	-5.6	7.1	1332	1938.2
	90	19.4	30.4	7.6	0.8	6618.3	2929.3	-1.6	0.4	192.7	6
'C-3-1/25'	0	68.50	7.27	0.5	0	23.7	4	-2.5	1.1	306.3	56.9
	30	61.1	4.4	0.3	0.1	13.3	3.8	0.3	3.7	247.7	223.1
	60	67.2	7.0	0.4	0.1	28.3	3.2	-3.8	1.3	372.7	66
	90	15.6	31.8	1.6	1.3	122.7	138.5	-3.5	1.3	313.7	120.5
'C-37-1/8'	0	66.35	17.22	1.3	0.6	75.7	27.1	-1.6	0.6	288.7	15.2
	30	63.2	4.3	0.2	0.2	8.7	7.6	-14.4	17.2	3849	5328
	60	65.1	16.4	41	23.4	27.7	1.5	-4.7	3.4	356	289.2
	90	21.8	28.7	4.6	3.6	1064.7	1210	7.5	15.5	295	119.8
'Chino Jaspeado'	0	89.93	12.51	0.3	0.1	20.7	10.8	-3	0.5	492.7	309.5
	30	92.3	15.7	0.6	0.3	30.3	13.5	-2.9	1.3	346.3	46.2
	60	88.5	12.0	1.1	0.2	78.3	14.6	4.4	7.9	156.3	50.3
	90	28.6	42.2	0.1	0.1	6.3	5.5	-1	2.7	0	0
'Coreano Jaspeado'	0	94.38	11.73	0.2	0.1	18.3	10.5	-1	2.1	333.3	140.8
	30	92.2	8.7	0.4	0.2	16.7	7.1	-0.2	2.6	233.7	212.7
	60	93.1	11.4	2.1	1.1	152.3	96	-8.3	2.6	311.3	131.5
	90	25.1	45.0	4.1	5.4	3371	5740	-3.8	6.1	315	103.6
'Criollo Aguascalientes'	0	48.83	7.77	0.4	0.1	23.7	5.7	-1.5	0.3	309.3	68.2
	30	51.0	7.2	0.2	0.2	7.7	6.7	-2.3	2.5	3678.7	5477.2
	60	48.0	7.5	0.4	0.2	26	12.5	-8.2	9.3	471.7	40.1
	90	15.9	23.0	0.1	0	5.3	2.9	-9.6	4.1	1899.3	1388.7

Table 2. Photosynthetic and respiration rate of cloves from six garlic genotypes after 90 d storage.

Fig. 3. Protoplast isolation from P-C-3 1/25 genotype: A,B) Bulb ('Perla' type), C) *In vitro* callus culture and D) Protoplasts at 40x magnification.

7. Karyotyping Mexican garlic genotypes

Karyotypes of C-CN-95/2, C-37 1/8, C-3 1/25 (all these 'Perla' type genotype), 'Chino', 'Coreano' y 'Criollo' were obtained from root tips. Cloves of these genotypes were placed inside petri dishes containing wet cotton wool in order to induce roots 1-2 cm long. Roots were removed and soaked with 0.05% w/v colchicine and placed in the darkness for 3:30 h at 25°C. These roots were fixed with Farmer´s solution (ethanol and glacial acetic acid, 3:1 v/v); then, they were hydrolyzed with 1N HCl at 60 °C for 10 min. Feulgen stain was applied to fixed roots before maceration with an enzymatic solution (2% pectinase, 5% celulase and citrate buffer pH 4.5) for 30 to 60 min. Roots tips were placed on microscope glass slides with a drop of 2% propionic orcein, and sandwiched with a cover glass. The slides were heated for few seconds with an alcohol burner with a very soft press so that single cells could be freed from the tissue. Then the cover glasses were gently tapped with a pencil in order to squeeze single cells for releasing and spreading the chromosomes. Observation of microphotography 100x25" allowed the following counts and measurements: chromosome number, short arms (p), long arms (q) total length, relative size of the chromosome and arm relationship (García, 1990). All of these observations were useful to classify each garlic genotype according to karyotype nomenclature and formule from Levan et al., (1964).

According to the observations, all of the genotypes tested have a chromosome number $2n$=8x=16 (Fig. 4) in agreement with other reports (Battaglia, 1963; Verma & Mital, 1978; Koul et al., 1979). The karyotype characteristics found for centromer position were as follows: 'P-CN-95/2' (1M+4m+3sm), 'P-37-1/8' (1M+6m+1st), 'P-C-3-1/25' (7m+1sm), 'Chino' (1M+3m+3sm+1st), 'Coreano' (6m+2sm) and 'Criollo' (5m+3sm); Code: M or m=metacentric, sm= submetacentric and st=subtelocentric (Table 3). The nuclear content (2C value), which is considered one of the highest among the cultivated plants (Ipek et al., 2005), is 32.7 pg. Additionally, garlic has a high karyotype variability that may be attributed to repetitive ADN (Kirk et al., 1970).

8. Genetic profile of Mexican garlic

Genetic markers are efficient tools for genetic analysis of populations and individuals. According to this concept, molecular characterization of garlic around the world has been performed either through RAPDs (Bradley & Collins, 1996; Eom & Lee, 1999; Shasany et al.,

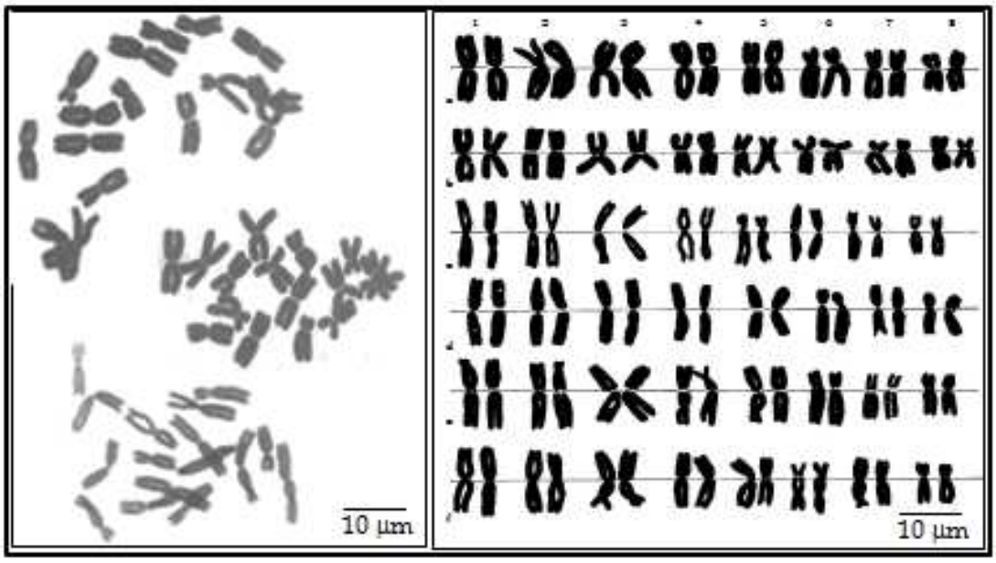

Fig. 4. Chromosomes from somatic cells (*n*=8x=16) from garlic genotypes: 'C-CN-95/2', 'C-37- 1/8', C-3 1/25, 'Chino', 'Coreano' and 'Criollo'.

2000; Peiwen et al., 2001; Ipek et al., 2003; Paredes et al., 2008; Pardo et al., 2009) or through AFLP (Rosales & Molina, 2007). Our version of this kind of analysis with 20 Mexican genotypes was as follows: Twenty garlic genotypes were subjected to RAPDs in order to construct a distance tree using clustering with the Unweighted Pair Group Method with Erithmetic Mean (UPGMA). DNA was extracted according to Doyle and Doyle (1990); DNA samples were run on agarose gel 0.8% 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. Amplification products were analyzed by electrophoresis in a 1.2% agarose gel. It was run at 100 V for 4 h, 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 & 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. The results obtained were compared with others studies realized by different authors, and were discusses as following: Six decamer OPB primers showing

Chrom. no.	*LBL*	*LBC*	*LT*	*LR*	*r*	*N*	*Chrom. no.*	*LBL*	*LBC*	*LT*	*LR*	*r*	*N*
'P-CN-95/2'							**Chino'**						
1	6.75	2.5	9.25	0.12	2.7	sm	1	6.75	4	10.75	0.13	1.7	sm
2	6.5	2.5	9	0.11	2.6	sm	2	6.5	6.25	12.75	0.16	1.04	m
3	6	6	12	0.15	1	M	3	6.5	2	8.5	0.10	3.25	st
4	6	5.5	11.5	0.15	1.09	m	4	6	6	12	0.15	1	M
5	6	5	11	0.14	1.2	m	5	6	5.5	11.5	0.14	1.09	m
6	5.5	4	9.5	0.12	1.3	m	6	5.5	4.75	10.25	0.13	1.2	m
7	5	4.5	9.5	0.12	1.1	m	7	5.5	3.5	9	0.11	1.6	sm
8	4.5	2.5	7	0.09	1.8	sm	8	4.5	2.5	7	0.08	1.8	sm
Formula: 1M+4m+3sm							Formula: 1M+3m+3sm+1st						
'P-37-1/8'							**'Coreano'**						
1	6.75	2	8.75	0.14	3.4	st	1	7	5.5	12.5	0.15	1.2	m
2	5	4.5	9.5	0.15	1.1	m	2	7.5	4	11.5	0.14	1.8	sm
3	5	4	9	0.14	1.2	m	3	7	2.5	9.5	0.11	2.8	sm
4	4	4	8	0.13	1	M	4	6.5	6	12.5	0.15	1.08	m
5	4.5	2.75	7.25	0.12	1.6	m	5	6.5	5	11.5	0.14	1.3	m
6	4	3.25	7.25	0.12	1.6	m	6	5	4.5	9.5	0.11	1.1	m
7	4	3	7	0.11	1.3	m	7	5	4.5	9.5	0.11	1.1	m
8	3.5	2.25	5.75	0.09	1.5	m	8	4.5	3	7.5	0.09	1.5	m
Formula: 1M+6m+1st							Formula: 6m+2sm						
'P-C-3-1/25'							**'Criollo'**						
1	6.75	4	10.7	0.14	1.7	m	1	7	5.5	12.5	0.15	1.2	m
2	6.5	5.5	12	0.15	1.2	m	2	7.5	4	11.5	0.13	1.8	sm
3	6	5	11	0.14	1.2	m	3	6.5	6	12.5	0.15	1.0	m
4	5.75	5.5	11.25	0.15	1.04	m	4	6.5	6	12.5	0.15	1.0	m
5	5.75	2.75	8.5	0.11	2.0	sm	5	6.5	4.5	11	0.13	1.4	m
6	5	4	9	0.12	1.3	m	6	6.5	2.5	9	0.10	2.6	sm
7	5	3.5	8.5	0.11	1.4	m	7	5.5	3.25	8.75	0.10	1.7	sm
8	4	2.5	6.5	0.08	1.6	m	8	4.5	3.5	8	0.09	1.3	m
Formula: 7m+1sm							Formula: 5m+3sm						

Table 3. Chromosome morphological description from Mexican garlic genotypes. LBL=Long arm, LBC=Short arm, LT=Total length, LR=Relative length, r=Arm relationship and N=Centromere nomenclature.

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. 5), 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: Fig. 6), required 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', and 'Perla' 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 achieve complete bulb formation. 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

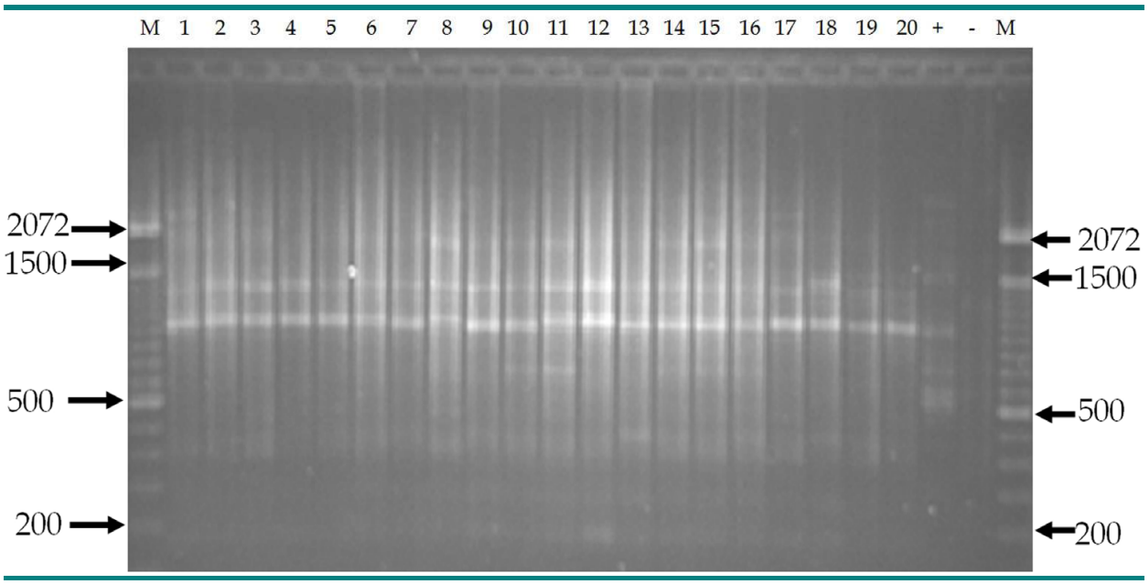

Fig. 5. RAPD from Mexican genotypes. Lanes: 1: C-3-1/25, 2: C-37-1/8, 3: 'Coreano', 4: 'California', 5: 'Chino', 6: 'Criollo Aguascalientes', 7: 'Español', 8: 'Cortazar', 9: 'Positas', 10: 'Pepita', 11: 'Massone', 11: 'Durango', 13: 'Chileno', 14: 'Hermosillo', 15: 'Sonora', 16: 'Nápuri', 17: 'Nicaragua', 18: 'Ixmiquilpan', 19: 'Pata de perro', 20: 'Guatemala', (+): positive control, (-): negative control and M: Molecular weight marker.

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 allowed 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

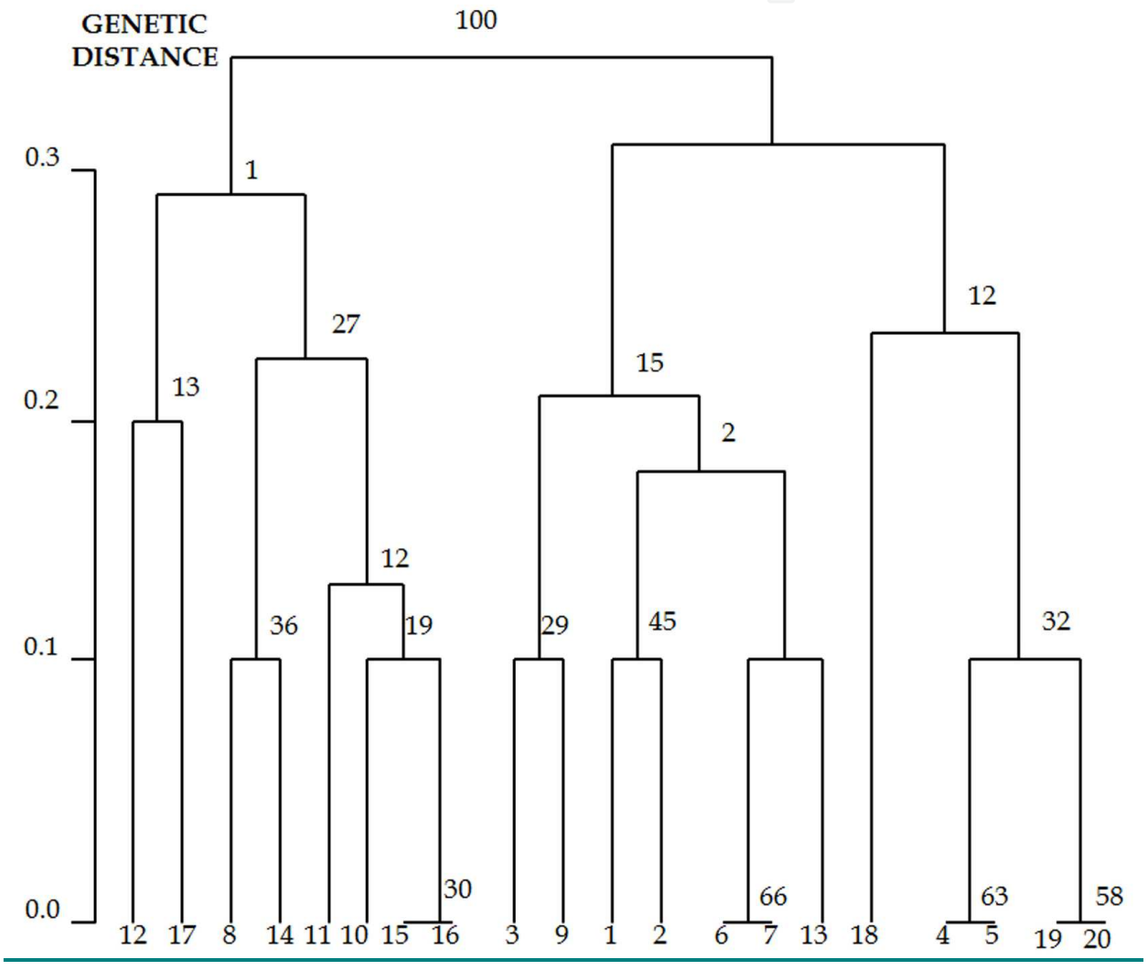

Fig. 6. Dendrogram obtained by the RAPD technique and general description according to physiological, morphological and genetic characteristics in garlic (*Allium sativum* L.) varieties cultivated in the Central Region of Mexico. Arms or branches of the dendrogram: 1. 'Perla' C-3-1/25, 2. 'Perla' C-37-1/8, 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'.

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.

9. *In vitro* propagation

Most Mexican garlic cultivars are somewhat susceptible to pests and diseases. Furthermore, garlic is a seedless plant that could carry diseases to the next generation through vegetative propagation. "Seed cloves" per hectare range from 200,000 to 250,000 for typical plant density; therefore, totally clean vegetative material may be too difficult to be generated. The solution for this kind of problems is considered under biotechnological view such as *in vitro* culture so that we can obtain homogeneous healthy plants. *In vitro* bulbils after four years may produce, depending on the cultivar, from 1,400 to 10,700 bulbils ready to be used as "seed" (Burba, 1993). A recent report mentioned plants produced from cloves cultivated *in vitro* with 1 mg L^{-1} TDZ-1 (Thidiazuron), 1 mg L^{-1} GA_3 (Gibberelic acid) and coconut milk (Lagunes, 2009). *In vitro* garlic plants were also obtained on MS supplemented with 2.0 mg L^{-1} 2iP (2-isopentenyl adenine), 0.1 mg L^{-1} NAA (Naphthalene acetic acid) and 30 g L^{-1} sucrose (Mujica et al., 2008). Basal plate from cloves has the highest callus production as compared to leaves, stem segments, pedicels and aerial bulbils (Rabinowitch & Brewster, 1990). Another report mentioned that callus formation from 'Rojo de Cuenca' was the best on media having BA and NAA; furthermore, high BA concentration promoted adventitious shoot formation, but did not show influence on callus formation (Barandiaran et al., 1999). Callus was also obtained from leaves exposed to 0.3 a 0.5 mg L^{-1} 2,4-D (Fereol et al., 2002).

Our own results showed that explants about 5 mm^2 of clove sections were enough to regenerate *in vitro* whole plants from 'C-3-1/8' and 'C-37-1/25', 'Chino' and 'Coreano' garlic genotypes. The first step began with explants for root production, that were placed into MS medium supplemented with combinations of 0.15 mg L^{-1} 2,4-D, 5 mg L^{-1} adenine, 1.4 mg L^{-1} 4-amino-3,5,6-trichloropicolinic acid (Pichloram) and 1 mg L^{-1} 6-(γ,γ-dimetilamino) purine (2iP) (Table 4). MS medium also contained 30-50 g sucrose and 3.5 g L^{-1} Phytagel. Cloves were soaked in 70% ethanol 1min; after that, were transferred to 20% commercial bleach for 20 min. Then, cloves were rinsed thrice with dH_2O, placed back to 70% ethanol 1 min and rinsed again. Incubation conditions were: 28 ±2 °C and 16/8 h photoperiod. Adventitious roots were used to produce protocorms and protocorm-like bodies. These protocorms were cultured for 30 weeks on MS supplemented with 1 mg L^{-1} IAA and 5 mg L^{-1} adenine.

9.1 Protocorm formation

Protocorms 0.5-1 cm merged from root tips after 8 weeks on MS supplemented with 1.5 mg L^{-1} 2,4-D and 5 mg L^{-1} adenine (Table 4). Then they were placed into basal MS during 3 weeks, before protocorms were inoculated into four regeneration media at 18 ±4 °C and 16/8 h photoperiod during six weeks (Capote et al., 2000; Robledo-Paz et al., 2000; Quintana-Sierra et al., 2005). Protocorm and protocorm-like structures were both light and dark-green colored, compact and easily detachable (Fig. 7a, b and c), somehow similar to organogenic callus from *A. cepa* (Van der Valk et al., 1992; Zheng et al., 1998). 'Chino' and 'Coreano' protocorms were even more easily detached, dark-green colored as compared to 'Perla' derived genotypes; Novák et al., (1986) also found differential pigmentation among genotypes. Media supplemented with Pi (1.4 Mg/L^{-1}) and 2iP (1 Mg/L^{-1}) only promoted long roots.

9.2 Microbulbil formation

The treatment supplemented with 2,4-D showed the highest number of protocorm formation (Table 4); when the treatment included IAA and adenine, 'Chino' and 'Coreano' doubled to genotypes 'C-3-1/8' 'C-37-1/25'. Other treatments induced root formation. This different varietal response was found by Capote et al., (2000) and Quintana-Sierra et al., (2005) for *Allium cepa* and would be related to differences in sensibility to growth regulators (Fehér et al., 2003). Microbulbils placed, during three weeks, on basal MS (no regulators) increased their size (Fig. 7d), but after 30 weeks they developed into bulbils 1 cm diameter (Fig. 7e and 7f) and finally grew into whole plants.

Media (%)	Growth regulators ($mg/^{-1}$) Auxins	Cytokinins	Protocorm induction (%)	Number of observed structures ´C-3-1/8´	C-37-1/25´	Chino´	´Coreano´
Protocorms							
MS 100	2,4-D (1.5)	Adenine (5)	90	4	4	8	8
MS 100	Pi (1.4)	2iP (1)	0	----	----	----	----
Microbulbils							
MS 100	0	0	20	----	----	----	----
MS 100	IAA (1)	Adenine (5)	80	2-4	2-4	4-8	4-8
MS 100	NAA (1)	Kin (2)	0	----	----	----	----
MS 100	IAA (1)	Kin (2)	0	----	----	----	----
MS 100	Pi (1.4)	2ip (1)	0	----	----	----	----

Table 4. Effect of growth regulators on protocorm and microbulbil formation of four garlic 12 genotypes. IAA: Indole acetic acid, NAA: Naphthalene acetic acid, Kin: Kinetin, 2,4-D: 2,4-13 Dichlorophenoxyacetic acid, Pic: Pichloram, 2iP: 2-isopentenyl adenine.

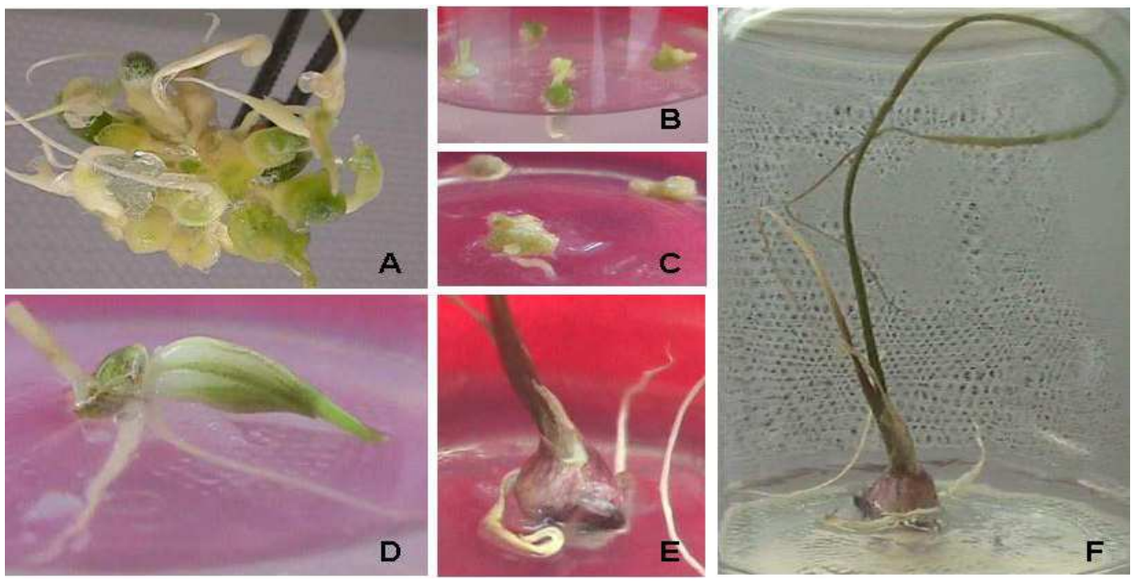

Fig. 7. *In vitro* regeneration of garlic plants (*A. sativum* L.). A) Protocorms regenerated from root tips, B) 'Chino' protocorms, C) 'Perla' protocorms, D) Microbulbil, E) and F) 'Chino' whole plant.

10. Individual selection for breeding Mexican garlic cultivars

Garlic in some cases may produce inflorescences but infertile seed; hence, crosses are not possible. Sometimes, bulbs o bulbils develop onto inflorescences (Brewster, 1994). Individual selection on best plants (yield or quality) has been used for breeding (Heredia and Heredia, 2000; González, 2006; Con, 1997). The CAEPAB group worked with individual selection from 'Perla' clones and 'Chileno' having heads with fewer cloves than the average for the original cultivar taking into account also: head size, vigor (hardiness) and plant healthiness. This initial work led to obtain two garlic cultivars: 'San Marqueño' from line 'C-37-1/8' (Macías et al., 2009) and 'Diamante' from line 'CAL-RN-11-1-1-2-4' derived from an Aguascalientes-Zacatecas collection (Macías & Maciel, 2003). In brief methodology for garlic breeding:

1. Bulb collection of promissory plants from the fields of outstanding growers of Aguascalientes and Zacatecas (May 1999).
2. Bulbs were planted in the experimental fields at CAEPAB, in order to check all of the collected material under the same growing conditions.
3. Evaluation and selection of garlic plants during 6-8 years (Table 6).
4. Storage of best clones at the germplasm bank (CAEPAB).
5. "Seed" production, enough to be transferred to growers for commercial validation.
6. After validation, best genotypes, having consistent yield results through time, are released to farmers.

Clones obtained through this kind of breeding are grown by farmers from Aguascalientes and Zacatecas (Macías et al., 2009). Nowadays, 'San Marqueño' and 'Diamante' garlic are demanded in Europe because of their high quality that makes them suitable to be exported (Fig. 8). These clones have their optimal conditions at 2000 meters over the sea level, on loamy soils, well drained, without salinity or pedregosity. Lab test are encouraged to check for soil pathogens that may reduce yield.

Evaluation	San Marqueño		Diamante	
Year	Yield (kg/ha)	SD	Yield (kg/ha)	SD
1	16,072	1,975	18,285	3,470
2	19,296	3,115	21,785	3,828
3	16,148	2,778	22,254	4,144
4	15,689	2,537	22,323	3,188
5	17,735	3,510	20,321	3,999
6	17,064	2,571	20,731	3,304
7			16,405	3,383
8			24,043	4,336

Table 6. Evaluation of 'San Marqueño' and 'Diamante' garlic cultivars.

Fig. 8. Garlic cultivars from the Mexican breeding program (CAEPAB): A) 'San Marqueño' ('Perla type'), B) 'Diamante' ('California' type) and C) Packaging box for exportation.

11. Conclusion

Horticulturists around the world look for answers from experimental stations to problems such as low yields, pests, diseases and quality defects. Similarly, garlic growers from Central Mexico have been in contact with institutions such as CAEPAB, PRODUCE-Ags and ITEL, in order to agree on agronomic and biotechnological research that may be applied to their fields. Original garlic genotypes from these growers and some other introduced to Mexico were the source for new cultivars and promissory genotypes developed by CAEPAB. Some of these that were analyzed showed a good correlation between bulb sizes and clove weight against bulb weight. It was also found that Mexican genotypes have a wide variety of on clove size and number that reflects a good genetic pool for breeding through individual selection for this seedless plant. Some other characteristics are qualitative that may have positive impact for worldwide market demands. For example, it was found for late cultivars the longest storage life.

Garlic biotechnology was also directed to characterize of Mexican cultivars. Molecular and cytogenetic characterizations for these cultivars may help to identify and to register, unambiguously, cultivated varieties. Molecular analysis such as AFLP´s or RAPDs is required in order to establish genetic uniqueness or relatedness. So far, only RAPDs have been performed but this kind of work is not concluded yet. Another biotechnological application is to produce *in vitro* pathogen-free vegetative material for massive propagation that may be released to growers. Protocorm and protocorm-like bodies were produced *in vitro* before they grew into bulbils and whole plant. Nevertheless, massive propagation of garlic has not been achieved as desired. Therefore, garlic biotechnology is going at slow pace. Finally, breeding through individual selection allowed releasing cultivars appropriate for national and international demands.

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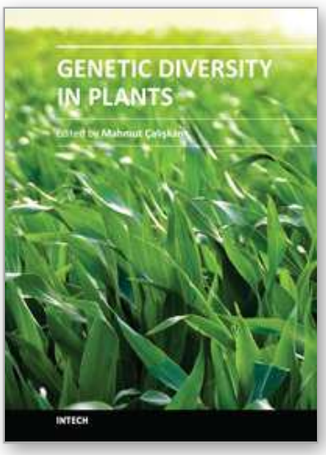

Genetic Diversity in Plants
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Genetic diversity is of fundamental importance in the continuity of a species as it provides the necessary adaptation to the prevailing biotic and abiotic environmental conditions, and enables change in the genetic composition to cope with changes in the environment. Genetic Diversity in Plants presents chapters revealing the magnitude of genetic variation existing in plant populations. The increasing availability of PCR-based molecular markers allows the detailed analyses and evaluation of genetic diversity in plants and also, the detection of genes influencing economically important traits. The purpose of the book is to provide a glimpse into the dynamic process of genetic variation by presenting the thoughts of scientists who are engaged in the generation of new ideas and techniques employed for the assessment of genetic diversity, often from very different perspectives. The book should prove useful to students, researchers, and experts in the area of conservation biology, genetic diversity, and molecular biology.

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