

IDENTIFICATION OF MAIZE LANDRACES WITH HIGH LEVEL OF RESISTANCE TO STORAGE PESTS *Sitophilus zeamais* Motschulsky AND *Prostephanus truncatus* Horn IN LATIN AMERICA

IDENTIFICACIÓN DE VARIEDADES NATIVAS DE MAÍZ CON ALTA RESISTENCIA A LAS PLAGAS DE ALMACÉN *Sitophilus zeamais* Motschulsky Y *Prostephanus truncatus* Horn, EN LATINOAMÉRICA

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SUMMARY

The maize weevil (MW) (*Sitophilus zeamais*), and the larger grain borer (LGB) (*Prostephanus truncatus*) are major storage pests causing serious losses in maize (*Zea mays* L.) in developing countries of Latin America (LA). This study identified maize landraces with high levels of resistance to MW and LGB by screening 1171 genotypes collected from 24 LA countries in 38 sampling areas. Maize grain weight losses (GWL), total dust production (TDP) and number of adult progeny (NAP) were measured for LGB and MW attack in each genotype. Susceptibility traits to MW and LGB were related to specific geographical location. Range of resistance for MW was from 0.6 to 51 %, while for LGB from 0.1 to 66 %. Approximately 28 % of the analyzed genotypes showed high level of resistance to MW, with Antilles region offering the most resistant accessions with races of EarCar, Chande, Haitye, Nal-Tel, Tuson, and Canill. Resistance to LGB was observed in 22 % of genotypes analyzed with accessions from Southern México with races of Cónico, Nal-Tel, Vandeño, Elotes Occidentales, Cubano, Tuxpeño, and Tepecintle. Low correlation ($r = 0.28$; $P < 0.01$) between maize resistance to MW and LGB indicated a divergent adaptive response of maize grain to these two pest. Geographic data showed a negative correlation between latitude and longitude with MW resistance traits being significant only for longitude ($r = -0.253$; $P < 0.05$). Opposite trend of correlations, positive but not significant, was observed for LGB resistance traits. These results indicate an influence of geographic location in local varieties being selected for storage pest resistance over time. Genotypes with excellent postharvest insect resistance have now been identified for maize breeders to use in developing improved cultivars for use in LA.

Index words: *Zea mays*, landraces, maize weevil, larger grain borer, insect-resistance.

RESUMEN

El gorgojo del maíz (GM) (*Sitophilus zeamais*) y el barrenador grande del grano (BGG) (*Prostephanus truncatus*), son las principales plagas de los productos almacenados y causantes de graves pérdidas de maíz (*Zea mays* L.) en los países en desarrollo de América Latina (AL). Este estudio se realizó para identificar las razas nativas de maíz con alta resistencia al GM y BGG, en 1171 genotipos colectados en 38 áreas de muestreo de 24 países. Se midieron las pérdidas de grano de maíz (PGM), la producción total de polvo (PTP) y el número de insectos adultos (NPA), en bioensayos con GM y de BGG. Los valores de

susceptibilidad se asociaron con la referencia geográfica. El intervalo de resistencia al GM fue de 0.6 % a 51 %, y al BGG fue de 0.1 % a 66 %. Una fracción de 28 % de los genotipos mostró una alta resistencia a GM, provenientes de los territorios de las Antillas, y de las razas EarCar, Chande, Haitye, Nal-Tel, Tuson, y Canill. La resistencia al BGG fue de 22 % con genotipos asociados a áreas del sureste de México y con las razas Cónico, Nal-Tel, Vandeño, Elotes Occidentales, Cubano, Tuxpeño y Tepecintle. La correlación entre la resistencia de maíz al GM y al BGG fue baja ($r = 0.25$; $P < 0.001$). Los datos geográficos indicaron una correlación negativa entre la latitud y la longitud con los datos de resistencia al GM ($r = -0.253$; $P < 0.001$). Una tendencia opuesta de correlación, positiva pero no significativa, fue observada para los valores de resistencia al BGG. Estos resultados indican un efecto de la localización geográfica en el desarrollo y la dispersión de las respuestas naturales de resistencia a los insectos. Se identificaron genotipos con una alta resistencia a plagas poscosecha que podrían utilizar los mejoradores de maíz en el desarrollo de cultivares mejorados para AL.

Palabras clave: *Zea mays*, criollos, gorgojo del maíz, barrenador del grano, resistencia a insectos.

INTRODUCTION

Maize (*Zea mays* L.) is a staple crop for food and feed in developing world. Post-harvest losses of maize due to storage insect pests, such as maize weevil (MW) *Sitophilus zeamais* (Motschulsky, Coleoptera: Curculionidae) and the larger grain borer (LGB) *Prostephanus truncatus* (Horn, Coleoptera: Bostrichidae), are an increasingly important constraint of food security worldwide (FAO, 2009). Subsistence farmers of developing countries of Latin America (LA) and Africa often experience grain damage exceeding 30 % during on-farm storage due to storage pests (Tigar *et al.*, 1994; Bergvinson and García-Lara, 2004).

The MW is one of the major insect pests of stored maize throughout the world (Pingali and Pandey, 2001). This insect is considered a primary pest, infesting maize both before and after harvest. The MW occurs in over 60 % of

the field and stored maize in México and LA, causing the most damage in humid areas (Tigar *et al.*, 1994; Bergvinson, 2001). The LGB is a woodborer and an invasive post-harvest insect pest native from Mesoamerica that has acquired the status of serious pest in several of North and LA countries (Markham *et al.*, 1994; Tigar *et al.*, 1994; Kumar, 2002). Studies in LA have shown that subsistence farmers of tropical and subtropical agroecologies experience from 10 to 45 % maize losses and from 10 to 80 % of damages caused by LGB attack in storage (Tigar *et al.*, 1994; Bergvinson, 2001).

To diminish insect attack, host plant resistance has become an important component of integrative pest management practices (Markham *et al.*, 1994). This strategy has been associated to the discovery, development and use of insect resistant varieties (Dobie, 1977) to reduce post-harvest losses and maintenance of grain quality (García-Lara and Bergvinson, 2007). This effort should be accomplished by the study and access to global genetic resources. Fortunately, maize landraces collected by International Maize and Wheat Improvement Center (CIMMYT) during the past 40 years in collaboration with national and international institutions, represent worldwide diversity of 23 409 accessions of maize from México, the Caribbean, Central, and South America (Taba *et al.*, 1998, 1999; Ortiz *et al.*, 2010).

Using this important genetic resource, several maize landraces such as Sinaloa-35 of Chapalote race, México-55 of Palomero-Toluqueño, and Yucatán-7 of race Nal-Tel have been identified and characterized as sources of resistance to MW (Dobie, 1977; Widstrom *et al.*, 1983; Giga and Mazarura, 1991; Arnason *et al.*, 1994) and LGB (Arnason *et al.*, 1997; Kumar, 2002). Maize resistant varieties have been found to suffer only 13 to 50 % as much grain weight loss compared to susceptible counterparts (García-Lara *et al.*, 2007). Mechanisms of resistance include at least two biochemical processes (Arnason *et al.*, 1997): 1) Mechanical fortification or strengthening of the pericarp cell walls that act as physical barrier (Bergvinson and García-Lara, 2004; García-Lara *et al.*, 2004), and 2) Antibiosis, the toxic effects of compounds localized in the aleurone layer (García-Lara *et al.*, 2007; Winkler and García-Lara, 2010).

In the last decade, important efforts have been made to develop resistant maize populations against LGB and MW using Caribbean accessions (Bergvinson, 2001; Kumar, 2002). Unfortunately, few inbred lines derived from these landraces have been incorporated into the breeding programs (Bergvinson and García-Lara, 2003), in part due to lack of interest in postharvest problems and to the fact that the major agronomic priority is still yield, highlighting the necessity to explore more diversity in the germplasm bank for novel sources of insect resistance adapted to Latin American environments. Due to the climatic change, more

problems with seed supply for small farmers (Bellon *et al.*, 2011) and postharvest pest are expected, especially in maize varieties available in the market that have never been selected for this trait.

The purpose of the present study was to evaluate the variability of postharvest insect resistance among 1171 native maize races from México (455) and LA (716), within the context of MW and LGB attack. The objectives were: (1) To determine resistance traits for MW and LGB under bioassay in whole grain of 1171 maize genotypes; (2) To identify maize landraces with high resistance to MW and LGB in a germplasm bank collection; and (3) To correlate resistance parameters of both insects with geographical information.

MATERIALS AND METHODS

Passport data of maize landraces from Latin America

One thousand one hundred seventy one (1171) maize landraces available at the CIMMYT germplasm bank were used in the current study (Table 1). Criteria of selection were based on previous studies where landraces were detected as sources of insect resistance. Selected landraces originated from 38 sampling locations in 24 Latin America countries were related to groups of maizes such as “cónicos” (G1 group), “tropicales precoces” (G5 group), “dentados tropicales” (G6 group), and “maduración tardía” (G7 group). Each accession was scored for country, state, geographic coordinates (latitude and longitude), altitude and collection origin, based on germplasm bank of CIMMYT (Figure 1).

Geographic area of this study was selected between 1.2° to 27.2° N and 51.6 to 111.0° E, and was mainly constituted by territories of México, Caribbean and Antilleans Islands under 220 meters above sea level, masl (Figure 1). Landraces were selected based on passport data related to their agronomic performance and use by the local farmers. Selected landraces were fixed at 1171 genotypes for this specific study, representing 5 % of CIMMYT total accessions.

Seed increase of maize landraces

Seed was increased during 2004 at the experimental station of CIMMYT at Tlaltzapán, Morelos, México (18°41' N, 940 masl). Plots were managed following the standard agronomic recommendations for that region. Self-pollinated ears were handled separately for their use within insect bioassays. Ears were sun dried for 2 d in an insecticide-free environment, air dried at 35 °C using a forced air dryer for 3 d, shelled and stored at 13 % grain moisture and 4 °C until used.

Table 1. Passport data and number of maize accessions of tropical maize landraces collected by CIMMYT germplasm bank from Latin America.

ID	Sampling area		Average			Accessions s/location
	Location	Country	Lat (N)	Long (W)	Altitude (m)	
1	Sonora	México	27.2	111.0	14	2
2	Sinaloa	México	24.5	107.2	85	5
3	Nayarit	México	21.6	105.2	6	44
4	Colima	México	18.5	103.5	45	7
5	Guerrero	México	17.4	101.3	75	46
7	Veracruz	México	20.6	97.2	15	153
8	Oaxaca	México	16.2	95.1	44	28
9	Tabasco	México	17.6	93.2	23	4
10	Chiapas	México	15.3	92.5	79	27
11	Campeche	México	19.1	90.2	28	33
12	Yucatán	México	20.6	89.0	13	104
13	Quintana Roo	México	19.3	88.0	18	2
14	Guatemala	Guatemala	15.4	88.3	36	38
15	Salvador	El Salvador	13.3	88.1	162	30
16	Honduras	Honduras	15.5	87.5	29	42
17	Nicaragua	Nicaragua	12.1	85.2	98	12
18	Costa Rica	Costa Rica	10.1	83.3	218	211
19	Panamá	Panamá	9.2	79.5	9	58
20	Cuba	Cuba	21.3	78.1	60	39
21	Jamaica	Jamaica	18.3	77.5	46	5
22	Magdalena	Colombia	10.3	75.3	50	2
23	Atlántico	Colombia	10.5	75.1	52	6
24	Córdova	Colombia	8.4	75.5	27	2
25	Haití	Haití	19.3	72.4	146	22
26	Rep. Dominicana	Rep. Dominicana	19.5	70.4	78	52
27	Puerto Rico	Puerto Rico	18.1	67.1	30	27
28	San Cristóbal	San Cristóbal	17.2	62.5	0	11
29	San Vicente	San Vicente	13.1	61.1	19	23
30	Santa Lucía	Santa Lucía	13.5	60.6	78	2
31	Antigua	Antigua	17.0	61.5	49	8
32	Grenada	Granada	12.0	61.4	160	26
33	Guadalupe	Guadalupe	15.6	61.4	31	31
34	Trinidad y Tobago	Trinidad y Tobago	10.4	61.3	65	37
35	Barbados	Barbados	13.1	59.4	70	15
36	Guyana	Guyana	6.5	58.1	3	2
37	Guyana	Guyana Francesa	5.5	55.1	5	4
38	Amapa	Brazil	1.2	51.6	135	11
		Mín	1.2	51.6	0	2.0
		Máx	27.2	111.0	218	211.0

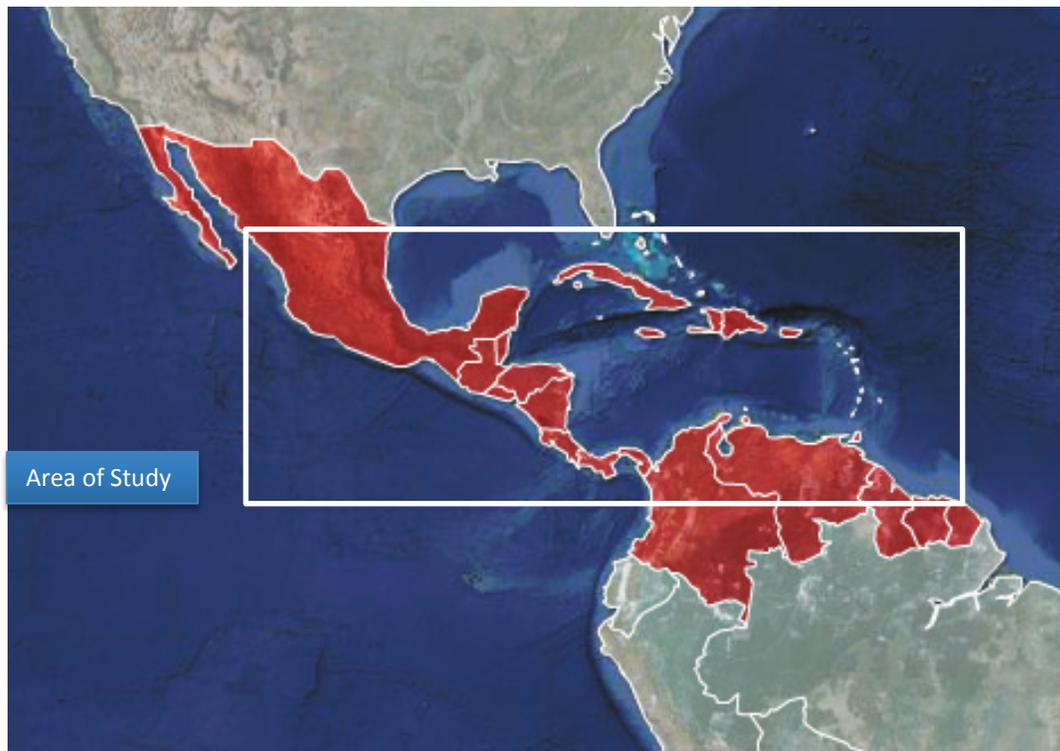


Figure 1. Selected area of study for discovery of new sources of maize landraces resistant to storage pest.

Maize weevil resistance bioassay

Cultures were developed and maintained using the methods described by García-Lara *et al.* (2009). Briefly, a MW colony was collected from Poza Rica, Veracruz and cultured on the hybrid CML244 x CML349 for four cycles at 27 ± 1 °C and 70 ± 5 % RH. Grain was equilibrated for 30 d at 27 ± 1 °C and 70 ± 5 % of relative humidity prior to infestation. For insect bioassays, three separated replicates were used in the evaluation. Each replicate contained 30 g of maize grain and was infested with 25 adult weevils 0 to 7 days-old. The adults were removed after 1 week. After 12 weeks, mesh sieves (#10 and #16) were used to separate grain, adult weevils and dust. Grain weight loss, adult progeny, and total dust production were recorded. Resistant (Pop. 84) and susceptible (CML244X256) checks were included for comparison.

Large grain borer resistance bioassay

The LGB colony was collected in Oaxaca, México, and renewed every 8 months. Insects were cultured on maize hybrid CML460 x CML461, a single- cross dent highland hybrid, for four cycles at 27 ± 1 °C, 70 ± 5 % relative humidity (RH) and 12:12 h light and dark (L:D). Cultures were maintained and renewed using the methods described by Bergvinson *et al.* (2001). Briefly, *P. truncatus* was reared in

0.5 L glass jars with vented lids that were filled with 400 g of equilibrated maize (30 d at 27 ± 1 °C, 70 ± 5 % of RH) covered with 10 g of maize flour and infested with 250 unsexed adults. Adults progenies were collected after 6 to 8 weeks.

P. truncatus adults were obtained by sieving (#10 and #16; USA Standard Testing Sieve E-11™, (Seedburo Equipment Company, Chicago IL, USA) maize containing grain damage greater than 50 %. For bioassay, three replicates were conducted on a jar with 30 g samples of maize, which were allowed to equilibrate at 13 % of grain humidity for 3 weeks prior to infestation with LGB. Each jar was infested with 25 unsexed adults of LGB (0 to 7 d old). After 8 weeks the grain was sieved using mesh sieves (#10 and #16) to separate grain, dust, and adult insects. Grain weight loss, dust production, adult emergence were recorded.

Statistical analysis

Resistance data were subjected to analysis of variance using the statistical software Statistix v.7 (Analytical Software, Tallahassee, FL) and differences among means were compared by LSD test at $P < 0.05$. To summarize the data, means were calculated per region or country. Best accessions were elected using a simple sort analysis based on an index (all traits were converted to maximum of 1 and then the total average was calculated) which included all resistance traits

for each pest. Statistix also calculated Pearson correlations among resistance traits and geographic data.

RESULTS AND DISCUSSION

Resistance of maize landraces to maize weevil

Geographic area of this study (Figure 1) has been used before to find and study sources of maize postharvest insect resistance with high success (Arnason *et al.*, 1994; Kumar, 2002). Selected landraces represented 5 % of CIMMYT total accessions (Table 1). Considerable variation in MW resistance traits was observed among 1171 maize landraces accessions (Figure 2). Significant differences between genotypes were observed for grain weight losses (GWL), total dust production (TDP) and number of adult progeny (NAP). Range of response to GWL, TDP and NAP were observed from 0.6 to 51 %, 0.3 to 4.9 g, and 6 to 197 adults, respectively. A fraction of 28 % of the 1175 genotypes analyzed showed a high level of resistance to MW. Compared with previous studies (Serratos *et al.*, 1987; Giga and Mazarura, 1991; García-Lara *et al.*, 2003; Abebe *et al.*, 2009), high incidence of resistance response was observed in the germplasm fraction used in this study.

Resistance of maize landraces to large grain borer

Comparative results based on MW were observed in LGB resistance traits among 1171 maize landraces accessions (Figure 3). Significant differences between genotypes were also observed for GWL, TDP and NAP after 8 weeks of infestation. Compared to MW, LGB required less time in bioassay because of its voracity (Nansen and Meikle, 2002). Range of response to GWL, TDP and NAP were observed from 0.1 to 66 %, 0.1 to 13 g, and 20 to 231 adults, respectively. In general, more GWL (22 %), TDP (65 %) and NAP (14 %) were found for LGB compared with MW. This damage is related to LGB biology, which is characterized by 35 weeks longevity, high flight activity, and reproduction under wide spectrum of conditions (Farrell, 2000; Golob, 2002; Nansen and Meikle, 2002). Present results confirm the fact that LGB causes major losses compared to MW, as formerly proposed by Meikle *et al.* (1998). Levels of resistance were higher compared with previous studies (Bergvinson, 2001; Kumar, 2002; Mwololo *et al.*, 2012).

Highly resistance maize landraces

The discovery and identification of new sources with higher resistance to MW and LGB in maize germplasm of LA was achieved in this study (Table 2). Arnason *et al.* (1994) reported important levels of MW resistance in native maize races collected in Belize area. In this study the novel germplasm fraction of MW resistance was associated to

wet lowland areas of Antilles islands with maize landraces collected from Guadalupe, Rep. Dominicana, Haiti, Puerto Rico and Yucatán, México. Novel high resistant landraces include EarCar, Chande, Haitye, Nal-Tel, Tuson, and Canill (Table 2). Maize varieties resistant to LGB have been recognized from African and Caribbean accessions (Arnason *et al.*, 1994; Kumar, 2002). However, this study showed novel sources of resistance for LGB identified from Southern México, including states such as Nayarit, Guerrero, Oaxaca, Campeche, Veracruz, and Yucatán. Novel higher resistant landraces included Cónico, Nal-Tel, Vandeño, Elotes Occidentales, Cubano, Tuxpeño, and Tepecintle (Table 2).

Relationship between MW resistance, LGB resistance, and geographic data

Maize weevil GWL, TDP and NAP were correlated to each other ($r > 0.89$; $P < 0.001$). Similar statistical responses were observed for LGB resistance traits. Although MW and LGB have different biology, evolutionary history, agro-ecologies, and habitats (Dobie, 1977; Longstaff, 1981; Nansen and Meikle, 2002), resistance traits were compared between response of maize against MW and LGB attack (Table 3). As expected, the relationship between MW and LGB traits was low indicating a divergent adaptive response of maize grain to insects. This result highlights the importance of performing divergent selection of postharvest insect resistance germplasm in the breeding programs.

Geographic data were also correlated with MW and LGB traits (Table 3). Geographic coordinates showed a negative correlation between latitude and longitude with GWL, TDP and NAP for the maize weevil ($r = -0.177$, $r = -0.253$, $r = -0.251$, at $P < 0.05$, respectively). Opposite trend of correlations, positive but not significant, was observed for LGB resistant traits LGB. Latitudinal trend (low north latitude) is related to high humidity, temperature and insect diversity (Zhang *et al.*, 2011), conditions which allow better conditions for insect development and more insect-plant interactions for natural development of resistance.

But in contrast, longitude has no established biological interpretation. Because in this study locations were located in tropical environments, starting in longitude of 51.6° S (Amapa, Brazil) and finishing at 111° N (Sonora, México), it is possible that this correlation is due to temperature range. This is also supported by the fact that latitude was significantly correlated with longitude for this area ($r = 0.356$, $P < 0.001$). In fact, this indicates the important influence of geographic location for developing maize resistance to postharvest pests.

This can be related to human practices in agriculture, where through years of human selection, properties of storage have

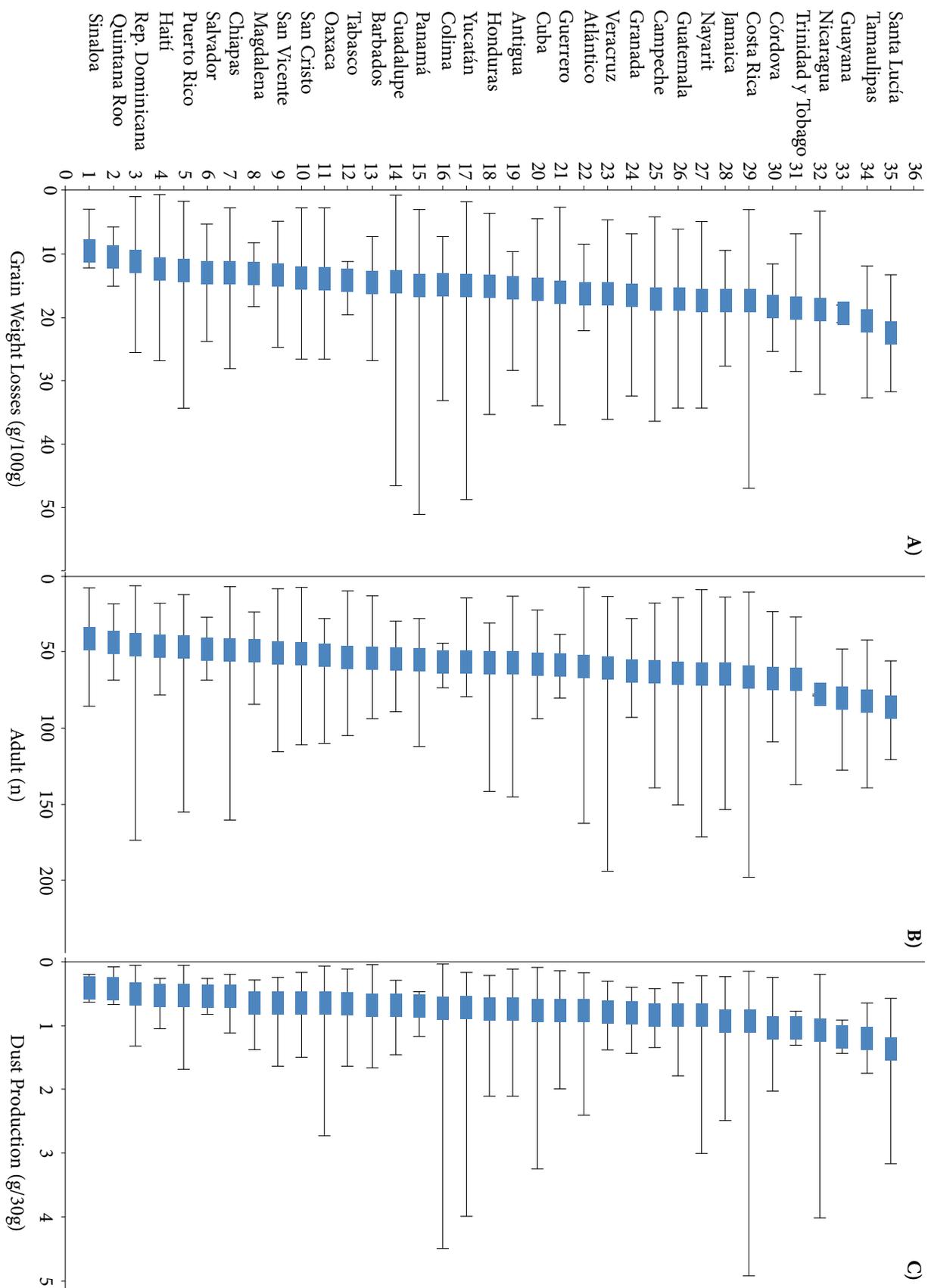


Figure 2. Mean, minimal and maximal values for grain weight losses (A), adult progeny (B), and dust production (C), after 12 weeks of artificial infestation of 1171 Latin American maize landraces with the maize weevil (*Sitophilus zeamais*).

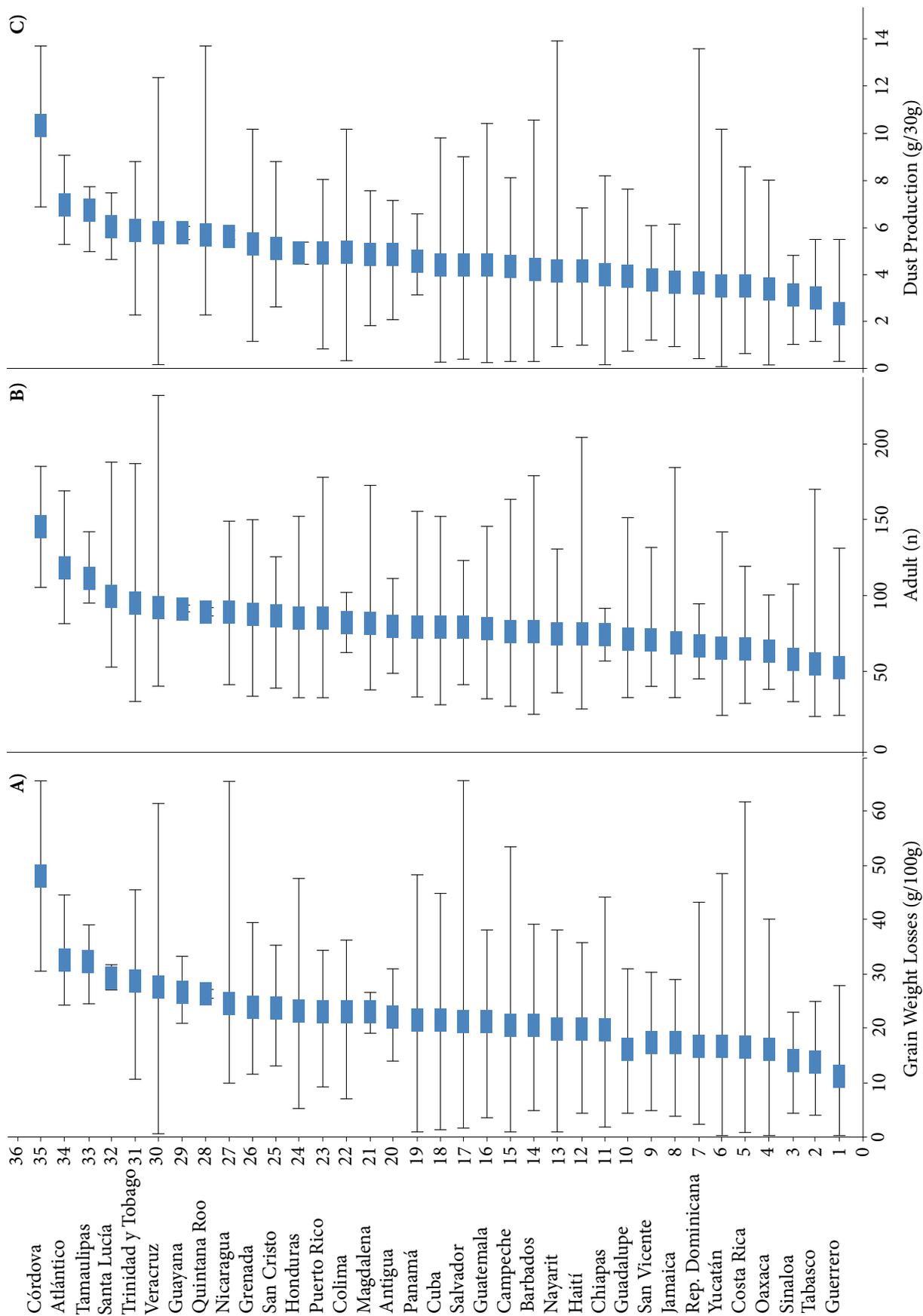


Figure 3. Mean, minimal and maximal values for grain weight losses (A), adult progeny (B), and dust production (C), after 8 weeks of artificial infestation of 1171 Latin American maize landraces with the large grain borer (*Prostephanus truncatus*).

Table 2. Maize genotypes identified with best postharvest insect resistance from 1171 Latin American maize landraces accession at CIMMYT germplasm bank.

No.	CIMMYT bank accession [†]	Pedigree	Race	Group ^{††}	Index
Maize weevil					
1	CIMMYTMA-003891	Guadalupe 16	EarCar	G6	0.08
2	CIMMYTMA-001322	Rep. Dominicana 259	Chande	nd [‡]	0.09
3	CIMMYTMA-001307	Haití 20	Haitye	nd	0.1
4	CIMMYTMA-002358	Yucatán 151	Nal-Tel	G6	0.11
5	CIMMYTMA-001333	Puerto Rico 16	Tuson	G6	0.12
6	CIMMYTMA-003931	Rep. Dominicana 246	Canill	G6	0.13
7	CIMMYTMA-001261	Rep. Dominicana 287	Chande	G6	0.16
8	CIMMYTMA-001056	Guadalupe 11	EarCar	G6	0.17
9	CIMMYTMA-003948	Rep. Dominicana 267	Chande	nd	0.18
10	CIMMYTMA-003947	Rep. Dominicana 266	Chande	nd	0.19
Large grain borer					
11	CIMMYTMA-000103	Oaxaca 19	Cónico	G1	0.15
12	CIMMYTMA-000725	Campeche 4	Nal-Tel	G6	0.18
13	CIMMYTMA-000188	Guerrero 210	Vandeno	G6	0.18
14	CIMMYTMA-000195	Guerrero 153	Vandeno	G6	0.2
15	CIMMYTMA-002557	Yucatán 15	Nal-Tel	G6	0.23
16	CIMMYTMA-000362	Nayarit 29	Elotes Occid.	G3	0.23
17	CIMMYTMA-000984	Panamá 65	Cubano	G6	0.24
18	CIMMYTMA-000473	Veracruz 187	Tuxpeño	G6	0.27
19	CIMMYTMA-003414	Costa Rica 206	Tepecintle	G6	0.27
20	CIMMYTMA-001791	Yucatán 30	Nal-Tel	G6	0.36

[†]Data provided by CIMMYT germplasm bank. ^{††}** Based on CONABIO data (www.conabio.gob.mx). [‡] nd, not determined.

Table 3. Pearson correlations between susceptibility traits to maize weevil MW (*Sitophilus zeamais*) and to large grain borer LGB (*Prostephanus truncates*) with geographic data from 1171 Latin American maize landraces.

Trait [†]	Maize weevil (MW)			Larger grain borer (LGB)		
	GWL	TDP	NAP	GWL	TDP	NAP
MW-TDP	0.893***					
MW-NAP	0.849***	0.827***				
LGB-GWL	0.289*	0.271*	0.179*			
LGB TDP	0.285*	0.288*	0.199*	0.957***		
LGB-NAP	0.136	0.142	0.044	0.876***	0.864***	
Latitude	-0.102	-0.106	-0.036	0.152	0.131	0.147
Longitude	-0.177*	-0.253*	-0.251*	0.017	0.014	0.079
Altitude	0.069	0.057	0.076	0.056	0.050	0.024

*, *** Significant at P < 0.05 and P < 0.001, respectively; [†]GWL = grain weight losses; TDP = total dust production; NAP = number of adult progeny.

been selected to ensure food security. Examples of this selection has been reported by Taba *et al.* (2006) for the Zapalote Chico maize race, where the conservation of this traditional landraces grown by farmers in Latin America has contributed to food security by dispersion and adoption of the new generation of local “criollos”. Selection for storage resistance is mainly the role of women (Parvathi *et al.*, 2000), whose decision of maize storage properties include easy shelling and grain quality for tortillas (Keleman and Hellin, 2009).

Tropical areas are the most severely impacted by MW and LGB damage (Bellon *et al.*, 2005); however, the use and adoption of identified landraces or improved varieties (generated with novel sources of landraces resistance) could help reduce losses and maintain maize diversity in LA. Finally, the application of this study could lead to new insights into the patterns of landraces genetic diversity within LA maize accessions (Prasanna, 2012), helping to track migration routes of maize from the centers of origin, and understanding the genetic diversity during maize domestication.

CONCLUSIONS

This study identified novel maize landraces with high levels of resistance to MW and LGB from Latin American accessions from 24 countries. The best sources for MW resistance were associated to areas of Antilles, while for LGB the best sources were collected from Southern México. Genotypes with high postharvest insect resistance have now been identified for maize breeders to use in developing improved cultivars in Latin American, for reducing postharvest losses and increase food security.

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