

Response of peanut (*Arachis hypogaea* L.) genotypes to smut (*Thecaphora frezii*) in the peanut growing region of Argentina

M. Kearney^{1*}, M. Zuza¹, M. Ibañez¹, V. Peralta¹, G. Peiretti¹, M. Alcalde¹, C. Mojica¹, and A. Rago^{2,1}

ABSTRACT

Genetic resistance is the most efficient tool in crop disease management. Peanut smut is currently one of most important peanut diseases, with its incidence increasing in terms of both damage level and crop area covered. The aim of this study was to assess the response of different genotypes obtained by the Facultad de Agronomía y Veterinaria of the Universidad Nacional de Río Cuarto (Argentina) to smut and their yield. During the 2016/17, 2017/18 and 2018/19 crop seasons, three experimental assays were conducted in General Deheza (Córdoba province, Argentina) to evaluate the varieties Uchaima, Utré and Mapu, the advanced lines LAX-1, LAX-2, LAX-3 and LAX-4, and the cultivar Granoleico, which was used as susceptible control. Final incidence and severity of peanut smut, as well as kernel yield, were evaluated. The variety Utré and the advanced line LAX-1 exhibited the best response to smut over the three crop seasons, without differences between them, but differing significantly from the remaining genotypes. Both genotypes showed incidence below 6.8% and severity below 0.21. In the 2016/17 crop season, LAX-1 had the highest kernel yield (3791.6 kg/ha). In the 2017/18 and 2018/19 crop seasons, Utré had the highest yield (1065 and 3975 kg/ha). Kernel yield of susceptible genotypes was below 2851.6 kg/ha in the 2016/17 and 2018/19 crop seasons, and below 805 kg/ha in the 2017/18 crop season. Genotypes LAX-1 and Utré are resistant to peanut smut. This is the first report of a peanut commercial variety developed in Argentina (Utré) with confirmed tolerance to smut.

Key Words: *Arachis hypogaea*, *Thecaphora frezii*, genotypes, resistance.

World production of oilseed crops, including peanut (*Arachis hypogaea* L.), has been very dynamic in the last years and has increased more rapidly than that of other important crops in the global agriculture. Argentina is the seventh pro-

ducer worldwide, contributing 3% of the world production; China is the first pod yield peanut producer, with 40% contribution. In the last years, Argentina has become the first world exporter of edible peanut and the second exporter of peanut oil, with its high quality being recognized worldwide (MAGyP, 2019). The peanut supply chain is a key factor in the national production matrix. Production is concentrated in the center/southwest region of Córdoba province (88%), with some contributions of San Luis, La Pampa and Buenos Aires provinces, whereas the complete industrial processing takes place in Córdoba. Both steps have an economic and social impact in this province, due to the generation not only of foreign currency revenues from exports but also of jobs in farms and industries (Fernández and Giayetto, 2017). According to the Bolsa de Cereales de Córdoba (2019), 324,600 ha were cultivated with peanut in the 2018/19 crop season, producing a total of 992,600 tons of grains, with a mean yield of 3.06 t/ha.

Fungal diseases are among the biotic factors affecting peanut production. A significant increase of peanut smut caused by *Thecaphora frezii* has been recorded in the last decade; this disease has become the soil-borne disease of highest prevalence and intensity (Paredes *et al.*, 2016). Peanut smut was first detected in Argentina in 1995, in commercial plots of the central-northern region of Córdoba province. This was the first record of the disease in cultivated peanut worldwide, and *Thecaphora frezii* was identified as the causal agent of smut (Marinelli *et al.*, 1995). Since then, peanut smut gradually increased, until reaching 100% prevalence in production fields in the 2011/12 crop season (Marinelli *et al.*, 2010; Rago, 2015; Paredes *et al.*, 2016; Rago *et al.*, 2017). The pathogen spread progressively via seed, machinery and wind, contaminating soils and infecting pods. However, the disease went practically unnoticed for several years, given that the aerial part of the plant does not exhibit symptoms. The infestation levels of the fields located near peanut processing plants and the high disease intensity detected demonstrate the potential risk of peanut smut (Rago, 2015).

Surveys conducted in different zones of the production area in Córdoba in the 2015/16 crop season revealed a disease prevalence of 100%, with variable intensity, with the highest values being

¹Facultad de Agronomía y Veterinaria, Universidad Nacional de Río Cuarto, Córdoba, Argentina.

²Centro de Investigaciones Agropecuarias, Instituto Nacional de Tecnología Agropecuaria (INTA), Córdoba, Argentina.

*Corresponding author email: mkearney@ayv.unrc.edu.ar

recorded in the core area (Río Segundo, Tercero Arriba, General San Martín and Juárez Celman departments) and lower values to the south of the province (General Roca department). The highest disease incidence (52%) was recorded in the locality of General Cabrera (Juárez Celman department), with 35% loss of seed production. In the southern zone, which is the largest cultivated area, severity index and incidence values were lowest (0.04 and 1.32%, respectively), with production loss of 0.74%, representing a negative impact of more than 1 million dollars, whereas in the northern area, losses would be above 11 million dollars (Paredes *et al.*, 2016).

In the last 10 years, different management tools have been evaluated, such as chemical control, rotation and tillage systems; results have been variable and no significant disease control level has been achieved. During that period, obsolete and modern commercial varieties have been tested, with no significant differences in tolerance or resistance to the disease having been found (Oddino *et al.*, 2015).

The most widely cultivated variety at present is highly susceptible to peanut smut, and cultural or chemical management strategies have failed to achieve the expected control level (Rago, 2015). Although there are a few registered cultivars with some tolerance to smut (e.g. Pepe Asem INTA and Manigran, with 30% incidence reduction), all of the currently cultivated cultivars are highly susceptible, which has contributed to the rapid spread of *T. frezii* across the entire crop region in Córdoba (Cignetti *et al.*, 2010). Therefore, the greatest management efforts should be focused on providing the peanut production sector with resistant varieties. While there are reports of germplasm sources and crosses showing good performance, obtaining peanut smut resistant or tolerant varieties with desirable agronomic traits requires several years of work in breeding programs (Faustinelli *et al.*, 2015; Rago, 2015). The peanut crop has limited genetic variability; hence, wild peanuts have received great attention as potential sources of genetic variability to increase disease resistance (Simpson, 2001; Chopra *et al.*, 2016). These germplasms are valuable resources that can provide genes for incorporation into commercial cultivars. Most of the wild species, such as *A. cardenasii*, *A. correntina*, and *A. villosa*, have exhibited resistance to *T. frezii* (Astiz Gassó *et al.*, 2011; de Blas *et al.*, 2019). However, the main difficulty involved in the use of wild materials is that most of the species of section *Arachis* are diploid, whereas cultivated peanut is allotetraploid. Genetic breeding with the aim of transferring resistance from wild materials

appears as one of the most promising strategies for a long-term sustainable control of this disease (Rago *et al.*, 2017; de Blas *et al.*, 2019; Bressano *et al.*, 2019).

Several factors support the importance of genetic resistance as one of the main tools in integrated disease management. Given the high levels of *T. frezii* infestation in soils of the peanut production area in Córdoba, only resistant varieties can produce acceptable yields and limit inoculum multiplication. In addition, *T. frezii* has been found to have a high genetic variability, which might affect maintenance of resistance over time. Therefore, the study and development of new cultivars should be a continuous effort (Rago *et al.*, 2017). Oddino *et al.* (2015) observed that the commercial peanut variety, EC-98 (*high oleic*) exhibited a significantly lower incidence of peanut smut than Granoleico, which was consistent throughout the years of analysis. During the 2016/17 crop season, advanced lines and varieties from the Facultad de Agronomía y Veterinaria of the Universidad Nacional de Río Cuarto (FAV-UNRC) and the Instituto Nacional de Tecnología Agropecuaria (INTA) were evaluated in the field in two different environments of the peanut region (General Deheza and Las Acequias). The varieties Utré (FAV-UNRC) and Ascasubi Hispano (INTA), as well as some advanced breeding lines, stood out for their excellent performance towards peanut smut (Ibañez *et al.*, 2017). On the other hand, Ibañez *et al.* (2018) evaluated the behavior of 18 peanut genotypes in three localities of the peanut region in the 2017/18 crop season, and identified materials of high tolerance to peanut smut and with good stability. Several of these genotypes showed high potential for their use as new varieties or as a source of tolerance to the disease in genetic breeding programs.

Considering the importance of genetic resistance as a disease management tool, the aim of this work was to evaluate the response of different commercial and pre-commercial peanut genotypes obtained by FAV-UNRC towards peanut smut caused by *Thecaphora frezii*, over three crop seasons.

Materials and Methods

The assays were conducted in a field located in General Deheza, province of Córdoba (Argentina), during the 2016/17, 2017/18 and 2018/19 crop seasons. The crop rotation was as follows: the first year on *Glycine max*, the second year on *Zea mays* and the third year on *Glycine max*. The soil in the

Table 1. Main characteristics of the evaluated genotypes.

Genotypes	Market type	Growth habit	Maturity ^a	Oleic acid	Other characteristics ^b
			—days—		
Uchaima	virginia	decumbent	140	Low	High YP, high 100-SW
Utré	spanish	bunch	125	Low	AGLT, high YP, high 100-SW
Mapu	virginia	prostrate	145	Low	High YP, high 100-SW
Granoleico	runner	prostrate	150	High	Good adaptability and grain yield
LAX-1	virginia	decumbent	125	Low	High YP, high 100-SW
LAX-2	spanish	bunch	120	Low	High 100-SW
LAX-3	virginia	decumbent	130	Low	High 100-SW
LAX-4	virginia	prostrate	140	Low	High YP

^aMean value of three crop seasons (2016/17, 2017/18 and 2018/19).

^bYP: yield potential; 100-SW: weight of 100 seeds; AGLT: ability to germinate at low temperatures.

area is typical Haplustoll. The climate is tropical monsoon; annual precipitation amounts to 800 mm, with 80% of rainfall being concentrated between October and March. The temperature regime is characterized by a mean of 16.9 °C, with a mean of 23.8 °C in the warmest month (January) and 10 °C in the coldest month (July). Precipitation records for the crop cycle during the three crop seasons were obtained from the weather station of General Cabrera Agricultural Engineer Center (INTA General Cabrera, 2019).

The field selected for the trial had an inoculum density of 4800 *T. frezii* teliospores per gram of soil in the 2015/16 crop season; this density was high enough to cause a peanut smut incidence above 50% in susceptible cultivars (Oddino *et al.*, 2010; Paredes *et al.*, 2019). The following peanut genotypes were evaluated: the varieties Uchaima, Utré and Mapu, and the advanced lines LAX-1, LAX-2, LAX-3 and LAX-4 (see more details on Table 1); all of them were developed at FAV-UNRC. The commercial cultivar Granoleico was used as the disease susceptible control (Table 1). The experiment followed a randomized complete block design, with three replications per genotype. Each genotype was planted in three plots, with each plot consisting of three 5-m long rows. Sowing was performed manually with a density of 15 seeds/m and a distance of 0.52 m between rows on November 15, 2016, November 13, 2017, and November 08, 2018.

Sanitary management of the crop consisted of fungicide seed treatment (Fludioxonil 2.5% plus Metalaxyl 3.5%) of all genotypes with the aim of reducing problems such as seed and/or seedling rot caused by soil-borne pathogens. This fungicide does not have any effect on *T. frezii* since the infections of this pathogen occur in the period of nailing the peanuts under the soil on the gynophores of the crop. In addition, the entire trial had two foliar applications of Pyraclostrobin 13.3% +

Epoxiconazole 5% at a dose of 750 cm³/ha for the control of late leaf spot (*Cercosporidium personatum*) that occurs every year with an epidemic character in the peanut region of the province of Córdoba, Argentina. Weed control and peanut leaf spot was conducted following the phytosanitary plan of a commercial field.

The plants were harvested at two different times with an interval of 15 days (genotypes Utré, LAX-1, LAX-2 and LAX-3 at 135 days after planting (DAP) and genotypes Uchaima, Mapu, Granoleico and LAX-4 at 150 DAP). When all the genotypes reached harvest maturity (R8), plants were harvested manually; all the mature pods were collected from a 2-m² area on the central row of each plot and the disease was quantified for each treatment. Final incidence and severity of peanut smut were evaluated. Incidence was determined as the percentage of infected pods. Final severity was quantified using a diagrammatic five-score scale (0: healthy pods/with no smut; 1: normal pod, one kernel with a small sorus; 2: deformed or normal pod, one half affected kernel; 3: deformed pod and a completed smutted kernel; 4: deformed pod and two smutted kernels); severity index (SI) (Marinelli *et al.*, 2010) was obtained using the following formula:

$$SI : \sum (X_0.Y_0) + (X_1.Y_1) + (X_2.Y_2) + (X_3.Y_3) + (X_4.Y_4) \quad [1]$$

where X_0 , X_1 , and X_n , is the value of severity level, whereas Y_0 , Y_1 , and Y_n , is the proportion of pods affected by the corresponding severity level.

A Pearson correlation analysis was conducted to determine phenotypic relationships among the variables: incidence, severity, in-shell yield and kernel yield in each crop season. To test variability in tolerance to peanut smut, phenotypic values of incidence and severity were squared-root trans-

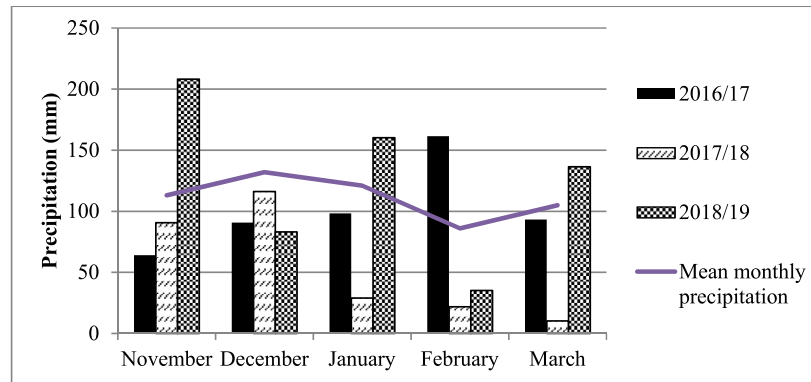


Fig. 1. Monthly precipitation (mm) recorded in each crop season (2016/17, 2017/18, 2018/19) and mean monthly precipitation (mm). General Deheza, Córdoba.

formed and evaluated using linear mixed models by modeling variance heterogeneity among genotypes. In-shell values and kernel values were evaluated using linear mixed models, including variance heterogeneity for each genotype level. The effects of genotype, year, genotype \times year interaction, and blocks nested within the factor year were considered fixed effects. The statistical model was selected using the likelihood ratio and the Akaike information criteria (AIC) and the Bayesian information criteria (BIC). Means were compared using the DGC comparison test of non-overlapping groups of means ($\alpha = 0.05$). All the statistical analyses were performed using Infostat software (Di Rienzo *et al.*, 2020).

Results and Discussion

Monthly accumulated precipitation during the peanut growth cycle (November to March) for the 2016/17, 2017/18 and 2018/19 crop seasons is shown in Fig. 1. The lowest value was recorded in 2017/18 (268 mm), being 507 mm for 2016/17 and 623 mm for 2018/19; the highest water deficit was recorded during the pod formation-grain filling period.

Results of the assessment of peanut smut in the three crop seasons show that Utré variety and LAX-1 advanced line were the genotypes with best performance. Considering the three crop seasons, both genotypes had an incidence below 6.8% and severity below 0.21 (Table 2). For the 2016/17 crop season, the lines LAX-3 and LAX-4 exhibited intermediate values of incidence (27.33–29.87%) and severity (0.85–0.97). Furthermore, cultivar Granoleico, the varieties Uchaima and Mapu, and the line LAX-2 had the highest incidence (35.56–50.69%) and severity (1.2–1.84) values. In the 2017/18 crop season, besides the previously mentioned genotypes, LAX-4 had high values, with

all of them together showing the highest incidence (45.65–75.74%) and severity (1.70–2.70) values of the three study years. In the same crop season, LAX-3 had intermediate incidence (32.72%) and severity (1.06) values. In 2018/19, LAX-3 also had intermediate intensity (24.48% incidence and 0.76 severity), followed by LAX-4 and LAX-2, with incidence values of 37.52 and 33.3%, and severity values of 1.22 and 1.14, respectively. Finally, Mapu, Granoleico and Uchaima were the most susceptible genotypes, with incidence values of 52.7, 52.00 and 46.55%, and severity values of 1.78, 1.72 and 1.55, respectively.

Mean pod yield values ranged between 778 and 5203 kg/ha, depending on the genotype and crop season, whereas kernel yield ranged between 247 and 3975 kg/ha (Table 2). The correlation between pod yield and kernel yield was highly significant and positive ($r=0.96$ and $P < 0.01$, respectively) (Table 3).

Regarding kernel yield (Table 1), line LAX-1 reached the highest value (3792 kg/ha) in the 2016/17 crop season, with precipitations close to the annual mean (Fig. 1). In the 2017/18 and 2018/19 crop seasons, characterized by low and normal precipitations, respectively, Utré was the variety with highest kernel yield (1065 and 3975 kg/ha, respectively). Kernel yield values of smut-susceptible genotypes were below 2852 kg/ha in 2016/17, below 805 kg/ha in 2017/18, and below 2850 kg/ha in 2018/19. Thus, the marked effect of water deficit on peanut crop in the 2017/18 crop season is clearly observed (Fig. 1), which is evident by yields below 1100 kg/ha in all the genotypes.

The tests evaluating response to the disease, both within and between crop seasons, showed a wide phenotypic variation, with mean incidence values ranging between 0.30 and 75.74%, and mean severity values between 0.01 and 2.70 (Table 2). A strong and significant positive correlation

Table 2. Incidence (%), severity (0-4), in-shell and kernel yield, and standard error (SE) of eight genotypes in three environments (2016/17, 2017/18, 2018/19 crop seasons). General Deheza, Córdoba, Argentine.

Season	Genotype	Incidence	Severity	In-shell yield	Kernel yield
		—%—	0 to 4 scale	—kg/ha—	—kg/ha—
2016/17	Uchaima	41.35 (± 8.16)	1.41 (±0.30)	3128 (±293)	1683 (±329)
	Utré	2.46 (± 1.02)	0.08 (±0.03)	4992 (±562)	3678 (±459)
	Mapu	35.56 (± 5.01)	1.20 (±0.19)	5072 (±459)	2852 (±187)
	Granoleico	41.84 (± 1.29)	1.32 (±0.07)	4722 (±194)	2775 (±156)
	LAX-1	1.76 (± 0.42)	0.06 (±0.02)	5203 (±254)	3792 (±204)
	LAX-2	50.69 (± 4.79)	1.84 (±0.20)	3648 (±137)	1378 (±135)
	LAX-3	27.33 (± 1.62)	0.85 (±0.06)	4402 (±322)	2643 (±306)
	LAX-4	29.87 (± 3.30)	0.97 (±0.11)	4427 (±523)	2837 (±465)
2017/18	Uchaima	75.74 (±2.51)	2.70 (±0.06)	1070 (±191)	388 (±80)
	Utré	0.94 (±0.32)	0.03 (±0.01)	1452 (±341)	1065 (±264)
	Mapu	70.06 (±2.83)	2.49 (±0.15)	1347 (±282)	500 (±178)
	Granoleico	59.30 (±1.21)	1.97 (±0.08)	1522 (±181)	805 (±87)
	LAX-1	3.74 (±3.24)	0.12 (±0.11)	802 (±100)	533 (±64)
	LAX-2	45.65 (±3.47)	1.70 (±0.13)	778 (±46)	247 (±19)
	LAX-3	32.72 (±1.56)	1.06 (±0.06)	862 (±86)	432 (±61)
	LAX-4	54.88 (±1.03)	1.79 (±0.06)	920 (±135)	462 (±74)
2018/19	Uchaima	46.55 (±2.06)	1.55 (±0.07)	3712 (±362)	2263 (±264)
	Utré	0.30 (±0.30)	0.01 (±0.01)	5038 (±127)	3975 (±108)
	Mapu	52.72 (±2.65)	1.78 (±0.09)	4260 (±272)	2608 (±241)
	Granoleico	52.00 (±3.30)	1.72 (±0.13)	3107 (±542)	2053 (±316)
	LAX-1	6.76 (±2.76)	0.21 (±0.09)	5148 (±321)	3645 (±147)
	LAX-2	33.30 (±3.62)	1.14 (±0.13)	3950 (±960)	2162 (±456)
	LAX-3	24.48 (±3.31)	0.76 (±0.09)	4077 (±375)	2850 (±284)
	LAX-4	37.52 (±3.39)	1.22 (±0.11)	3803 (±332)	2598 (±264)

($r=1.00$, $P<0.01$) between incidence and severity was also confirmed (Table 3).

Given the significant and positive phenotypic correlation observed between both disease parameters (incidence and severity) and production traits (in-shell and kernel yields), subsequent analyses were performed using a single variable of each group, since the behavior of one variable is directly reflected in the behavior of the other.

Incidence and severity were negatively and significantly correlated with in-shell yield, with correlation coefficients ranging between -0.57 and -0.62, except for the 2017/18 crop season, when there was no correlation between characters ($P>0.05$) (data not shown). The same pattern was observed for kernel yield. Correlation coefficients were high, ranging between -0.81 and -0.84 during

the 2016/17 and 2018/19 crop seasons, whereas in 2017/18 there was no correlation between characters ($P>0.05$) (data not shown).

Regarding the analysis of variability for tolerance to peanut smut among genotypes, the selected statistical model (linear mixed model with heterogeneous residual variances according to the different levels of the factor genotype) was the one with the best fit among the alternative models, which allowed us to statistically differentiate non-overlapping groups of genotypes over the three crop seasons. The analysis of the genotypes across environments (years) showed statistically significant differences ($P<0.0001$) for the main effects of genotypes, environments and genotype \times environment ($G \times E$) interaction in all the evaluated traits. These results demonstrate that genotypes have a differential response in the tested environments. However, Utré and LAX-1 differed significantly from the other genotypes in that they had the lowest incidence values and the highest mean kernel yield values across environments, showing a high stability in their response (Fig. 2 and 3). The 2017/18 crop season was the environment with highest peanut smut incidence value and the lowest mean kernel yield value.

The possibility of identifying groups of genotypes according to their differential response to

Table 3. Pearson correlation coefficients and statistical significance for eight genotype during three cropping seasons.

Variable	Incidence	Severity	In-shell yield
	—%—	0 to 4 scale	—kg/ha—
Severity	1.00**	—	—
In-shell yield	-0.40**	-0.42**	—
Kernel yield	-0.55**	-0.57**	0.96**

** , significantly different from zero ($P = 0.01$)

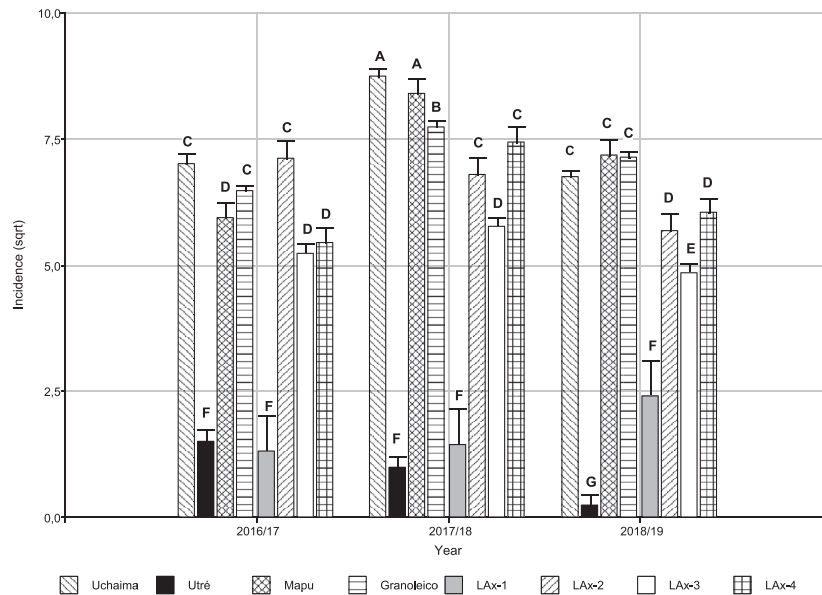


Fig. 2. Groups of genotypes evaluated during three crop seasons (years) for mean values of incidence, defined by the algorithm of DGC test ($\alpha = 0.05$).

smut is consistent with results obtained by Oddino *et al.* (2015), who identified highly smut-resistant materials; most of them are advanced lines, with incidence below 10%. Some of these genotypes exhibited good agronomic characteristics and are therefore being used to develop varieties with good yield and smut resistance.

Faustinelli *et al.* (2015) observed that the variety Granoleico was the genotype most susceptible to *T. frezii*. Moreover, the authors confirmed the presence of peanut genotypes obtained from crosses, with variable levels of tolerance to peanut smut. In addition, Ibañez *et al.* (2018) identified genotypes with good performance and stability against smut (varieties obtained by FAV-UNRC and INTA, and

advanced lines of FAV-UNRC). Several of these genotypes have great potential to be used as new varieties or as source of peanut smut tolerance in genetic breeding programs.

Considering the genetic variability of wild peanuts, Bressano *et al.* (2019) and de Blas *et al.* (2019) studied for the first time the genetic resistance to peanut smut in landraces and their introgression into elite cultivars. These studies show the benefits of wild varieties as source and means to enhance the genetic basis of resistance to smut. Thus, after several years of experiment, they developed a “runner” peanut cultivar with resistance to smut and high-oleic trait. This cultivar meets the market requirements, since that trait

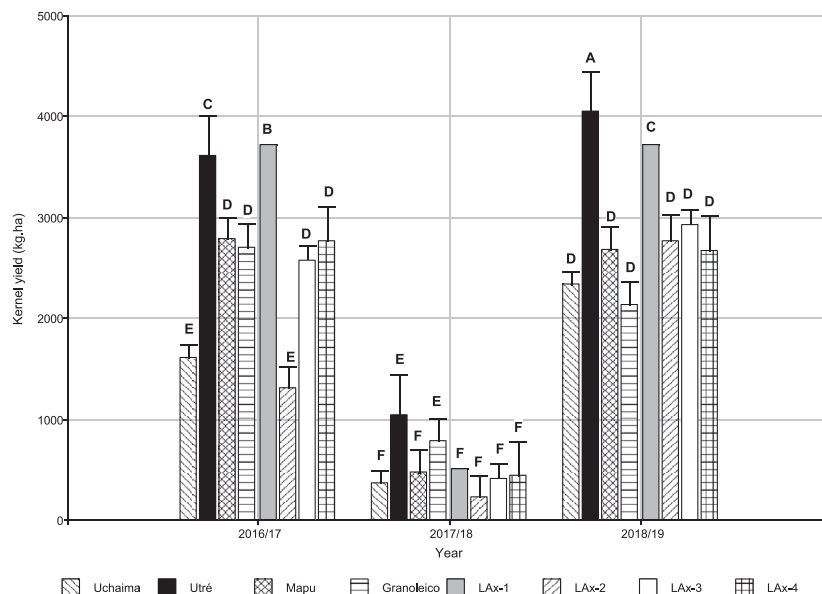


Fig. 3. Groups of genotypes evaluated during three crop seasons (years) for mean values of kernel yield, defined by the algorithm of DGC test ($\alpha = 0.05$).

confers stability to peanut products (Fernández and Giayetto, 2017).

Conclusions

This is the first report of genetic resistance associated with production traits of commercial and pre-commercial peanut varieties derived from the FAV-UNRC breeding program. The assessment of the three registered varieties and four advanced lines in an environment with high inoculum pressure and during three consecutive crop seasons shows a wide phenotypic variability in terms of tolerance to peanut smut and production-related traits. Some genotypes have shown a significantly superior response to the disease and performance in terms of kernel yield compared to the commercial genotype used as control (Granoleico), which has been found to be more susceptible. Disease pressure allowed us to detect genotypes with high tolerance level and yield stability during three crop seasons, whose agronomic performance can exceed the currently cultivated varieties.

Some of those genotypes have potential for use as new varieties or, rather, as source of tolerance to the disease in order to enhance the genetic basis of resistance to peanut smut in genetic breeding programs.

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