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# Germination of bean cultivars with contrasting physical characteristics under osmotic stress conditions

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## Abstract

The cultivation of common bean (*Phaseolus vulgaris* L.) in Mexico occupies the third place by established surface, although the production reports annual variations due to the heterogeneity of the climatic conditions in which it is produced. Given the cultural and nutritional importance of this legume, farmers and geneticists have selected cultivars with desirable culinary characteristics and adaptations to restrictive environments. The objective was to characterize the germination of bean cultivars collected in the Central Valleys of Oaxaca and the Potosino Altiplano, regions with contrasting environments and culture. In the Phytochemistry laboratory of the Desert Areas Research Institute, during the summer of 2015, five traditional cultivars and ten improved bean cultivars were evaluated. The percentage of germination was quantified with the procedures of the standard test described by ISTA (2005) and the seeds that showed lower incidence of fungal contamination and higher percentage of germination were selected. With four improved cultivars and four traditional cultivars, the average weight of a sample of 100 seeds and the proportion of seminal structures were determined. The effect of three osmotic potentials on germination was also recorded (Ψo: 0, -0.5 and -1.1MPa). Differences ($p \leq 0.05$) were observed in the weight of 100 seeds, the percentage of the testa and the embryonic axis. In Ψo: 0.0 Mpa and -0.5 Mpa, differences were obtained ($p \leq 0.05$) among the cultivars evaluated. In Ψo: -1.1 Mpa germination was null in all cultivars.

**Keywords:** creole, Mesoamerican, races.

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# Introduction

The common bean (*Phaseolus vulgaris* L.) is the most important legume for human consumption in Mexico, since prehispanic times it has had great nutritional, social and economic importance (Anaya *et al*., 2015; Espinosa *et al*., 2015; Saburido and Herrera, 2015). At present, beans are present in practically all the soil and climatic conditions of Mexico (Aguilar *et al*., 2014; Magaña *et al*., 2014, Delgado and Gama, 2015), and a wide variety of wild, traditional forms are found (variants selected and cultivated with empirical procedures) and improved (Espinosa *et al*., 2015; Delgado and Gama, 2015).

In the Central Valleys of Oaxaca, in its cultivated form (wild varieties have been recorded that are harvested), the black beans of the Mesoamerican breed have a high demand for regional consumption (Lara, 2015), and in response to this, the local farmers with small cultivation areas have selected the traditional cultivars that best adapt to the edaphoclimatic conditions of the different microenvironments of the region, to their cultivation systems that are often polycultures and to the culinary needs of their consumers. For these farmers, improved seeds are not a viable option because of the economic cost, because they do not adapt to their growing conditions and are not fully accepted by consumers (Aguilar *et al*., 2014).

In the Potosino Altiplano region, light beans (races Durango and Jalisco) have wide acceptance in the market (Lara, 2015). Traditional cultivars have been displaced by improved cultivars with high yield and tolerance to drought and fungal diseases associated with *Rhizoctonia* and *Fusarium* and common blight associated with *Xanthomonas* (Saburido and Herrera, 2015). According to direct information from producers, they generally receive seed of improved cultivars through government programs; however, the use of improved seed is not done year after year, for them it is common to use recycled seed (seed that the producer it conserves of its harvest to seed in the following cycle) and they sell the seed that receive of the programs, and even many times they consume it in spite of being treated with chemical products.

In Mexico 80% of the area planted with beans is under the temporary regime, therefore, in production systems for self-consumption and commercial drought is a meteorological phenomenon that causes partial or total loss of crops (Ambachew *et al*., 2015). Although any stage of the crop cycle can be affected by drought, germination is one of the most important stages in the life of plants, since it determines the efficient use of nutrients and the water resources available from them (Aguilar *et al*., 2014), so that the varieties with greater germination in conditions of moisture restriction should receive special importance.

The objective of the research was to determine the correspondence between physical characteristics, seminal structures and the germination of seeds of cultivars of common bean (*Phaseolus vulgaris* L.) of Mesoamericana races, Durango and Jalisco, simulating moisture restriction with osmotic stress conditions.

## Materials and methods

The first phase of the research was carried out at the end of the autumn-winter cycle of 2014. Seeds were collected from traditional black bean cultivars in the Central Valley region of Oaxaca. This region has a dominant climate BS0hw(w) (semi-warm dry), a temperature of 16 to 22 °C and precipitation of 600 to 800 mm. During the same period, improved cultivars were collected from the Potosino Altiplano, where a Bs1K climate prevails (temperate Semi-dry), temperature of 7 to 30 °C and precipitation of 250 to 400 mm.

In Oaxaca, the seeds of cultivars used by the same producer or his family were collected for 30 years or more, in the areas identified as the Ojuelo (16° 58’ 21.4” North latitude and 96° 46’ 16.9” West longitude, 1 557 masl), Rancho Juárez 16° 56’ 06.4” North latitude and 96° 44’ 37.6” West longitude, 1 507 masl), Villa de Zaachila 1 (16° 56’ 28.4” North latitude and 96° 44’ 16.7” West longitude, 1 512 masl), Villa de Zaachila 2 (16° 57’ 28.3” North latitude and 96° 44’ 21.3” West longitude, 1 513 masl) and Cuilapam (16° 59’ 50” North latitude and 96° 46’ 54” West longitude, 1 560 masl).

The characteristics by which the producers conserve these cultivars are: color of the seed, short time for cooking, flavor and thick consistency of the broth. In the state of San Luis Potosi, improved clear cultivar seeds were collected in the communities of La Herradura (23° 02’ 83” North latitude and 101° 74’ 75” West longitude, 2 140 masl), San Pablo (22° 48’ 26” North latitude and 101° 54’ 80” West longitude, 2 180 masl), El Zacaton (23° 43’ 61” North latitude and 102° 20’ 9” West longitude, 2 024 masl) in the municipality of Villa de Ramos (23° 02’ 83” North latitude and 101° 74’ 75” West longitude, 2 140 masl) and in the municipality of Salinas de Hidalgo (22° 37’ 39” North latitude and 101° 42’ 52” West longitude, 2 075 masl) (Figure 1).

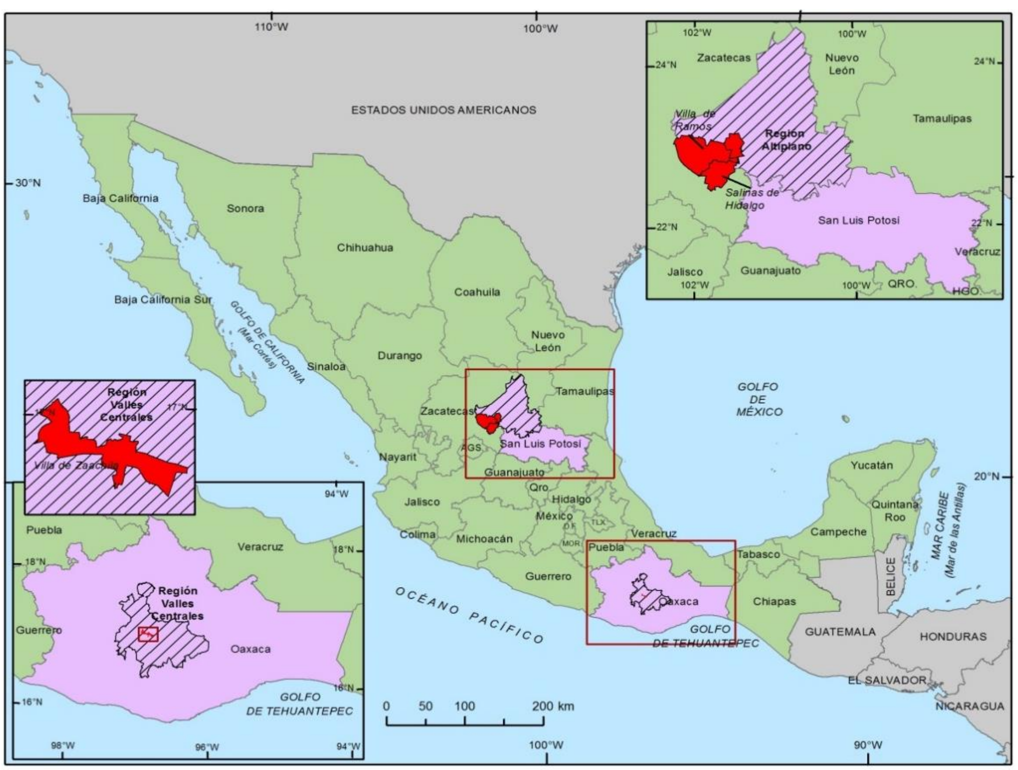


**Figure 1. Location of harvest areas of cultivars of *Phaseolus vulgaris* L. Mexico.**

The producers provided us with recycled seed of recent harvest and said that they decide to cultivate to sow in dependence on what the intermediary that buys them asks for them. A total of 15 cultivars were obtained, which were labeled as shown in Table 1. The same sample number was not obtained per region, since in Oaxaca we should be fully sure of collecting traditional cultivars, with the condition previously described.

**Table 1. Description of labeling for the harvests of cultivars of *Phaseolus vulgaris* L. obtained in the Central Valleys of Oaxaca and Potosino Altiplano, Mexico.**

| Cultivars with black testa (Oaxaca) | Cultivars with light colored testa (San Luis Potosi) |
|---|---|
| Negro delgado el Ojuelo (NDOJ) | Pinto Saltillo (PS) |
| Negro delgado Rancho Juárez (NDRJ) | Flor de Junio (FJ) |
| Negro delgado Villa de Zaachila 1 (NDVZ1) | Bayo la Herradura (BLH) |
| Negro delgado Villa de Zaachila 2 (NDVZ2) | Flor de Mayo (FM) |
| Blanco Cuilapam (BDC) | Rosa de Castilla La Herradura (RCH) |
| | Bayo Salinas (BS) |
| | Rosa de Castilla Salinas (RCS) |
| | Bayo Villa de Ramos (BVR) |
| | Bayo San Pablo (BSP) |
| | Bayo Zacaton (BZ) |

The second phase of the research was conducted at the Desert Areas Research Institute of the Autonomous University of San Luis Potosí. To partially know the quality of the seeds, each cultivar was previously disinfected in a solution of sodium hypochlorite (5% NaCl of active chlorine) for five minutes and then washed until the rest of sodium hypochlorite was removed with distilled water.

Seeds disinfected with standard germination test described by ISTA (2005), the adequacy of the sample size, considering 25 instead of 100 seeds for growing, with four replications was performed. The seeds were placed in a germinating chamber under dark conditions, at a temperature of 25 ±1 °C and 45% humidity for 168 h. A spray irrigation was applied with 15 ml of distilled water every third day to keep the substrate moist. On the third and seventh days after the experiment was established, germinated, ungerminated seeds and the presence of pathogens were quantified.

After the germination test, the harvests whose seeds showed a lower incidence of fungal contamination and higher percentage of germination were selected, leaving eight bean cultivars: four traditional cultivars (NDOJ, NDRJ, NDVZ1 and NDVZ2) and four improved cultivars (PS, FJ, BLH and FM). In the eight cultivars selected, the individual size (mass in grams) of a sample of 100 seeds was quantified on an Ohatus Adventure TM AR1530 digital scale. Five repetitions were made of this procedure.

The proportion of weight of the seminal structures was made taking for each cultivar groups of five seeds with four repetitions. On an analytical balance Scientech® RSA 120 (precision 0.0001 g) were weighed and seeds were placed in 100 ml of distilled water at room temperature until imbibition. The seminal structures were separated manually and individually with the help of a scalpel. The cotyledons, embryonic axis and testas were dehydrated at 70 ºC for three days, then their weight was recorded on a dry basis.

To perform the germination tests under osmotic stress, with osmotic stress values reported in the literature and preliminary tests. The seeds were disinfected. Subsequently, 25 seeds of each cultivar were placed in Petri dishes (100x15 mm.), using filter paper moistened with 5 ml of distilled water (control) or aqueous solutions of polyethylene glycol (PEG 8000; Sigma-Aldrich®) as substrate with Ψo of -0.5 and -1.1 Mpa. With a completely randomized experimental design, with a factorial arrangement of treatments 8x3, with three repetitions. Seeds under these conditions were maintained for 156 h in a germination chamber under dark conditions at 25 ±1 °C, with a relative humidity of 45%. The boxes were weighed every 60 h to replace the evaporated water.

Every 12 hours the germinated seeds were quantified. The data obtained were analyzed with the Test of Shapiro Wilk and the variables were transformed with the square root of the arc-sinus when the assumption was not fulfilled. The variance analysis and principal components were performed using the statistical package SAS version 9. The Tukey test ($p \leq 0.05$) was used for multiple comparison of means.

The graphs and adjustment models for the germination curves were processed with the SigmaPlot program from Jandel Scientific® (Version 12). A cluster classification analysis was performed by hierarchical clustering, by the method of inter-linkage groups and measurement interval Euclidean squared by SPSS Software V. 20 for IOS.

## Results and discussion

### Weight and standard germination of seeds

Differences ($p \leq 0.05$) were recorded in the standard germination test for the 15 initial bean cultivars evaluated and the presence and development of fungal and bacterial colonies in the seeds of the cultivars with the lowest germination was observed. Because it was not the objective of this work to evaluate sanitary quality, the fungi present were not identified, only the appearance of structures with cottony or powdery appearance with aerial or vegetative mycelium of white color or pink pigment, greenish yellow or yellow diffusible was visually recorded.

Given the high incidence in some cultivars, it is speculated that these microorganisms affected the germination of the seed or limited the growth of the radicle in this test. Based on the above, it was decided to select four traditional cultivars and four improved cultivars, which on average had germination greater than 80% and showed less presence of fungi.

Of the eight cultivars selected differences ($p \leq 0.05$) were obtained in the weight of 100 seeds, resulting, in general terms, that the seeds of black beans were less heavy than the seeds of light beans, and the difference in weight was 118% between the less heavy variety NDVZ1 and the less heavy FM (Table 2, Figure 2).

**Table 2. Weight of 100 seeds of traditional and improved bean cultivars (*Phaseolus vulgaris* L.).**

| Cultivate | Weight of 100 seeds (g) |
|---|---|
| Negro Delgado El Ojuelo | 12.946 [g] |
| Negro Delgado Rancho Juárez | 15.564 [f] |
| Negro Delgado Villa de Zaachila 1 | 13.59 [g] |
| Negro Delgado Villa de Zaachila 2 | 17.034 [e] |
| Pinto Saltillo | 29.706 [b] |
| Flor de Junio | 24.832 [d] |
| Bayo | 32.8 [a] |
| Flor de mayo | 28.244 [c] |

Values in the same column with different letter are statistically different (Tukey, $p \leq 0.05$).


**Figure 2. General aspect of the improved (clear) and traditional (black) cultivars of beans (*Phaseolus vulgaris* L.) evaluated.**

Espinosa *et al*. (2015), found that the seeds of the cultivars that correspond to the Mesoamerican race (black testa) are of lower weight compared to the seeds of the cultivars of the Jalisco race. For the Mesoamerican race, the authors mentioned report a weight range between 22.4 to 57.5 g/100 seeds, while the range obtained in this study was 12.9 to 17.03 g/100 seeds.

The Jalisco race (Flor de Mayo and Flor de Junio) showed a seed size greater than Mesoamerican, with weights between 24.8 and 28.2 g/100 seeds. These data agree with those presented by Barrios *et al*. (2014), who registered weights of 20.2 to 28.7 g/100 seeds in different cultivars of the same race.

The outstanding race by weight was the Durango race (Pinto Saltillo and Bayo), registering an average weight greater than 28.9 g/100 seeds, a fact that coincides with that found by Barrios *et al*. (2014).

The results presented in this study show that the weight of the seed is inherent characteristic of the breed and variety and has a genetic character as cited by Mondal *et al*. (2012). In addition, it has been documented that the seed size of some legumes responds to climatic adaptations, finding that small seeds correspond to lineages better adapted to tropical conditions (Mugnisjah *et al*., 1987). There are previous reports that small seeds of tropical origin have higher germination than medium and large seeds (Pearson and Miklas, 1992), which may indicate that the selection is directed to a superior root system and not to a larger size of seed (Kuruvadi *et al*., 1998).

Otherwise, the weight differences found in this study, in relation to previous records for the Mesoamerican race (Espinoza *et al*., 2015), may be the result of factors such as the period of seed filling and the growth rate of the seed, which is known to have a positive correlation with the size of it and are influenced by environmental conditions (Egli, 1999).

**Seminal structures**

In relation to the proportion of seminal structures, differences were observed ($p \leq 0.05$) in the percentage of testa and embryonic axis, while, in the proportion of cotyledons, which was recorded between 87.65 and 90.55%, no significant differences were obtained ($p \geq 0.05$) among cultivars evaluated (Figure 3). Barrios *et al*. (2014) and Celis *et al*. (2008) showed that the cotyledons represented more than 90% of the embryo biomass, also without presenting significant differences between cultivars and races.

The cultivar that presented the highest percentage of testa ($p \leq 0.05$) was Negro Delgado Villa de Zaachila 1 (10.04%), while the cultivars Pinto Saltillo (8.30%) and Bayo la Herradura (8.47%) were the ones that showed the lowest proportion of testa. Barrios *et al*. (2014), report that the percentage of seed for cultivars of race Durango was 8.3%, coinciding with the present work. Celis *et al*. (2010), found a significant difference in the percentage of testa of beans with different degrees of domestication, observing that the lowest values correspond to the improved cultivars and the highest to the wild cultivars, this agrees with the results presented here, since that the Negro Delgado Villa de Zaachila 1 corresponds to a traditional cultivar.

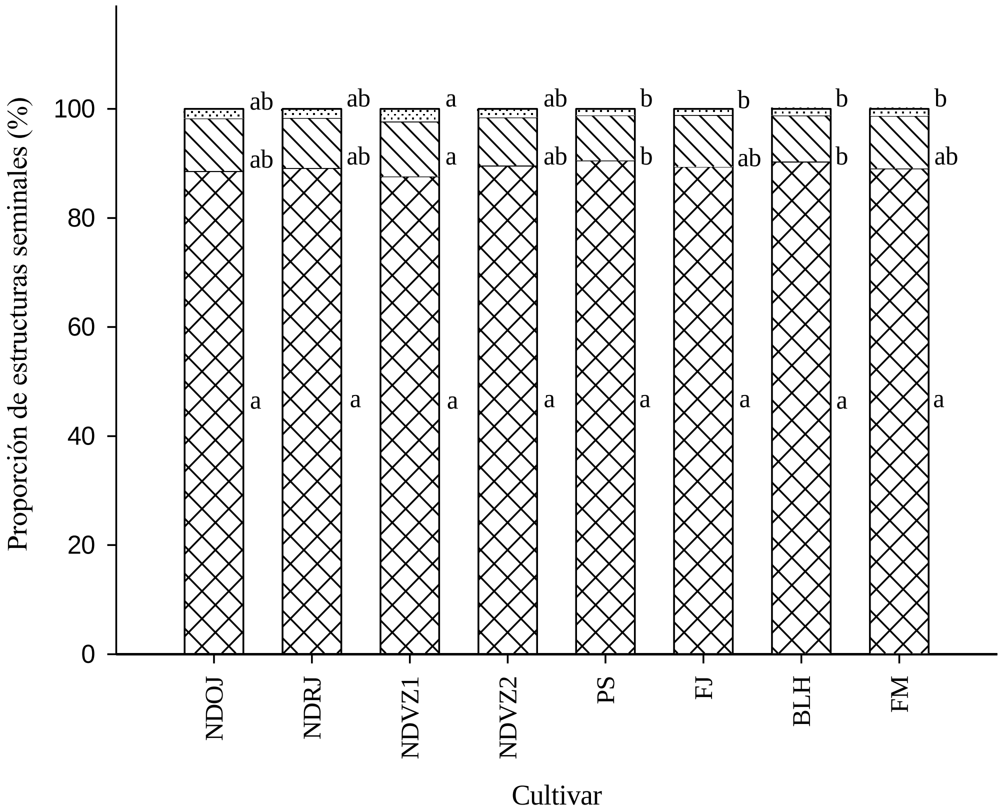


**Figure 3. Proportion of seed structures of *Phaseolus vulgaris* L. seeds of the cultivars evaluated. Cotyledons ( ), testa ( ), embryonic axis ( ).** Values in segments of the bars with different letters are statistically different (Tukey, $p \leq 0.05$).

In addition, it has been reported that a higher proportion of testa compared to the total weight of seed is considered an attribute related to its color, where dark colored testas are thicker, so both attributes can be favorable to ensure physical quality and seed health, since a thick testa is less susceptible to cracking and protects the other structures from damage by rapid inhibition and can be a factor that ensures a greater longevity of the seed; in addition, some authors mention that dark testas contain fungicidal properties (Tully *et al*., 1981, De Souza and Marcos-Filho, 1998, 2001). The latter may partially explain why improved light-colored cultivars are more susceptible to mechanical damage to the testa and fungal attack.

In the present investigation, the cultivar with the highest proportion of the embryonic axis was Negro delgado Villa de Zaachila 1 (2.30%) and the cultivars with smaller proportions were Pinto Saltillo (1.13%), Flor de Junio (1.11%), Bayo la Herradura (1.17) %) and Flor de Mayo (1.25%). According to Pearson and Miklas (1992), the larger the size of the seed, the smaller the embryo axis and the emergence is slower compared to small seeds. The foregoing would indicate a vigorous emergence in small seeds and a greater development of organs conferred by the greater biomass of the embryonic axis, which could prioritize root development, in addition to the fact that small seeds require less moisture for emergence (Barrios *et al*., 2014), as observed with the results obtained in the germination in different osmotic potentials.

**Germination in different osmotic potentials**

Differences ($p\leq 0.05$) were obtained among the cultivars evaluated in percentage of germination, in Ψo: = 0 and -0.5 Mpa the black beans exceeded ($p< 0.05$) the clearings and in the Ψo: -1.1 Mpa there was no germination (Table 3).

**Table 3. Percentage of germination of seeds of cultivars *Phaseolus vulgaris* L. evaluated under conditions of osmotic stress.**

| Cultivate | Germination percentage in different Ψo (Mpa) | | |
|---|---|---|---|
| | 0 | -0.5 | -1.1 |
| Negro Delgado El Ojuelo | 90 [a] | 41.48 [a] | 0 [a] |
| Negro Delgado Rancho Juárez | 79.04 [a] | 30.82 [ab] | 0 [a] |
| Negro Delgado Villa de Zaachila 1 | 78.46 [a] | 31.82 [ab] | 0 [a] |
| Negro Delgado Villa de Zaachila 2 | 79.39 [a] | 24.1 [b] | 3.84 [a] |
| Pinto Saltillo | 3.84 [b] | 0 [c] | 0 [a] |
| Flor de Junio | 18.63 [b] | 0 [c] | 0 [a] |
| Bayo | 10.6 [b] | 0 [c] | 0 [a] |
| Flor de Mayo | 21.47 [b] | 0 [c] | 0 [a] |

Values in the same column with different letter are statistically different (Tukey, $p\leq 0.05$).

The results with the Ψo= 0 suggest that to germinate the seeds of the clear cultivars, more moisture is required than for the black testa seed. Aguilar *et al*. (2014), Reyes *et al*. (2014) and Polon *et al*. (2014) found similar results when experimenting with creole and improved bean cultivars, under conditions of water stress, differential responses between species and harvests were observed, evidencing superior germination in traditional cultivars.

It is notorious in the results with the potential of -0.5 Mpa, that the seeds with greater weight of the traditional cultivars registered lower germination than the rest, which indicates that the small seeds in this study required a smaller amount of water for their germination.

In this study the three representative races of Mexico were used: Durango, Jalisco and Mesoamericana and clearly the Mesoamerican race has a better response regarding germination under osmotic stress. Beebe *et al*. (2013), identified the Durango and Mesoamerican races, with greater tolerance to drought, while the genotypes of the Jalisco race were susceptible. Thome *et al*. (1995), using a set of 20 genotypes, mostly from the Durango race, conducted a test of eight trials in three different regions in Mexico, the cultivars that showed wide adaptation in all regions were neutral to the photoperiod; characteristic found in Mesoamerican race lines, in addition to superior performance in drought conditions (Beebe *et al*., 2008).

The correlation analysis shows negative correlations between seed weight and proportion of testa and germination, while germination correlated positively with the proportion of testa (Table 4).

**Table 4. Correlation coefficients between variables of seminal structures and germination of *Phaseolus vulgaris* L.**

| | Weight | Cotyledons | Testa | Germination |
|---|---|---|---|---|
| Weight | 1 | | | |
| Cotyledons | 0.0576 | 1 | | |
| Testa | -0.6945 | 0.3907 | 1 | |
| Germination | -0.9594 | 0.0486 | 0.618 | 1 |

These data strengthen what was previously discussed in relation to the fact that the larger seeds had a lower proportion of testa, smaller seeds germinated in a higher percentage, and a higher proportion of testa in the seed could have an effect on greater germination.

With the variables weight and proportion of seminal structures (testa, cotyledons), the dendogram was obtained (Figure 4) in which there is a clear separation between light and black beans. In light beans, the cultivars Flor de Mayo and Pinto Saltillo are more similar to each other with respect to Bayo Herradura and Flor de Junio. In black beans, Zaachila 1 and 2 are not grouped in spite of being harvested in the same locality, while Rancho Juárez and Negro Ojuelos, harvested in Cuilapam de Guerrero, if they are related.

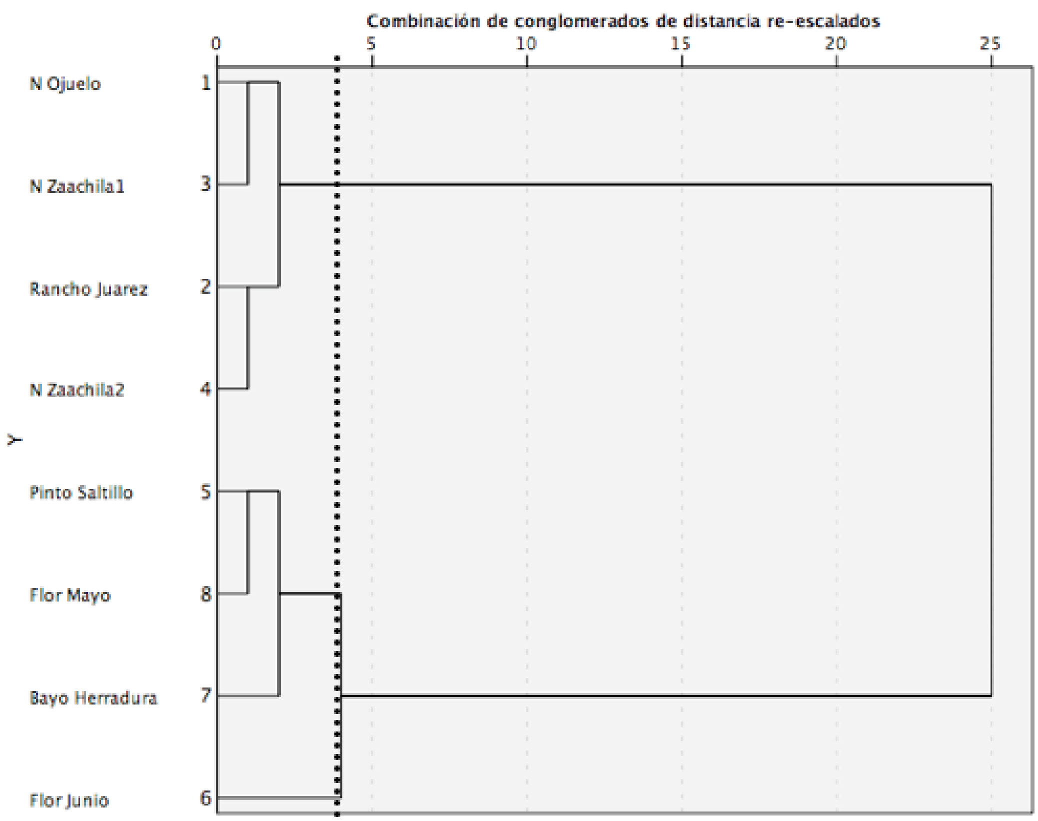


**Figure 4. Mean linkage dendogram (between groups) with the variables weight and seminal structures.**

In this investigation, no phenotypic characteristics of the cultivars collected were recorded, so the discussion of the results focuses on the information provided by the producers and the records to characterize the seeds evaluated. During the stage of harvesting the seeds, the producers of Oaxaca indicated that the selection of their seed is based mainly on culinary criteria such as flavor, consistency of the broth and cooking time, which are factors that determine consumer acceptance, also agronomic criteria as tolerance to drought or attack of weevil and yield are variables that incline them to select their seed, but a large seed is not an important criterion.

In counterpart, the producers of the Potosino Altiplano sow the seeds of cultivars that they know they will be able to sell easily and recycle seeds of improved cultivars to lower production costs, for them the size of seed is important because the hoarders have screens to classify their product and based in it to establish the price, a bigger and more homogeneous grain is better paid.

Evidently, the selection criteria in each study region seek to respond to the different needs of the farmers and the destination market, fulfilling for each context with particular objectives. The results highlight the variation among black cultivars that producers and consumers clearly identify as ‘criollo delgado’ and this variation can be attributed to the different management forms, selection micro-environments and selection criteria. This result is striking to urge the need to continue efforts to systematize knowledge of the variability of traditional germplasm and promote *in situ* conservation of the special attributes that small farmers have achieved, in terms of food security and biocultural heritage.

## Conclusions

The variation within the traditional cultivars in seed size characteristics, proportion of seminal structures and germination under low humidity was evidenced, which makes evident the need to continue generating knowledge about the genetic potential of these cultivars and the selection criteria of the producers that conserve them.

The seeds of improved cultivars were the largest, did not present differences among them in the proportion of seminal structures and only a low percentage germinated in conditions suitable for small seeds of traditional cultivars.

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