

Performance and seed quality of ‘pearl millet’ with the use of mycorrhizae *Glomus intraradices* and chemical fertilizers

María Guadalupe López-Ortega¹
Mauricio Velázquez-Martínez²
Filogonio Jesús Hernández-Guzmán^{3§}
Miguel Ángel Mata-Espinosa¹
Adelaido Rafael Rojas-García⁴

¹Autonomous University Chapingo-University Regional Unit of Arid Zones. Highway Gómez Palacio-Ciudad Juárez km 40, Bermejillo, Durango, Mexico. Tel. 01 (872) 7760160. (lupitauach@gmail.com; mamata@chapingo.uruza.edu.mx). ²Highway San Luis Potosí-Matehuala km 14.5, Ejido Palma de la Cruz, Soledad de Graciano Sánchez, San Luis Potosí. Mexico. Tel. 01 (444) 8524303 (velazquez.martinez@inifap.gob.mx). ³Polytechnic University of Francisco I. Madero. Domicile known s/n, Tepatepec, Francisco I. Madero, Hidalgo. Tel. 01 (738) 7241174. CP. 42660. ⁴Autonomous University of Guerrero-Faculty of Veterinary Medicine and Animal Husbandry no. 2, Cuajinicuilapa, Guerrero, Mexico. CP. 41940. (rogarcia-05@hotmail.com).

§Corresponding author: fjhernandez@upfim.edu.mx.

Abstract

The objective of this research was to determine the effect of mycorrhizae, chemical fertilization and its combination on foliar area, morphological composition, production and seed quality of pearl millet (*Pennisetum glaucum* Br.). The evaluation was carried out in spring-summer 2013 in the San Luis Experimental Field of INIFAP, INIFAP and Azo-Fer mycorrhizae were used, as well as 120-60-00 and combination of mycorrhizae with 60-30-00 and a control (without any fertilizer). The variables evaluated were plant height, leaf area index, morphological composition, seed production, weight of one thousand seeds, hectoliter weight, purity, moisture content, number of seeds per kg and efficiency in water use. A completely randomized experimental design was used (Tukey, 0.05). No differences were observed ($p > 0.05$) in the index of plant height, leaf area, purity, humidity, hectoliter weight and weight of a thousand seeds. Seed production was similar ($p > 0.05$, kg ha⁻¹) with the use of biofertilizers and fertilizers (mycorrhiza INIFAP= 2 221, mycorrhiza INIFAP + 60-30-00= 1 811, mycorrhiza Azo-Fer= 1 783, mycorrhiza Azo-Fer + 60-30-00= 1 754) and mineral nutrition (2 685); however, different from the control ($p \leq 0.05$; 1 631). In morphological composition in both anthesis and milk grain state, there was no difference in stem biomass between the sources of nutrition ($p \leq 0.05$) and they were different from the control.

Keywords: foliar area, biofertilization, morphological composition.

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The domestication of Pearl millet began 4 500 years ago, and originated in the North and Central African Sahelian area (Clotault *et al.*, 2012). In Africa and Asia an area of 14 million hectares is planted and the world production of the grain exceeds 10 million tons per year, with India being the largest contributor of global production with 50%, of this grain depend on 500 million people in the world (DAFF, 2011).

The pearl millet adult plants can grow from 50 cm to 4 m in height and each plant produces more than four stems and from which emerges a panicle that can measure up to 35 cm long and 3.5 cm in diameter. Pearl millet caryopses mature after 25 days of anthesis initiation and weigh about 8 mg in addition, this plant is characterized by thriving in areas of low rainfall with green forage yields of 13 to 23 t ha⁻¹ and 2 a 4 t ha⁻¹ of grain (Hernández and Zavala, 2009); however, millet forage pearl millet in the state of embed, flowering, milk grain and senescent, contains: 11.3, 9.68, 6.93 and 7.77% of PC, as well as NDF of 62.6, 65.2, 67.9 and 66.8%, respectively (Urrutia *et al.*, 2011). On the other hand, Urrutia *et al.* (2014), in pearl millet, 12.2% of PC and 65% of NDF were found in flowering stage.

It has been documented that the application of biofertilizers such as *Glomus* spp, form symbiotic associations with grass roots and increase water capture capacity and solubilize soil phosphates, making them available to plants (Mena *et al.*, 2013). the above, in corn, Díaz-Franco *et al.* (2008a) found no differences in three years of grain production between mycorrhizae and chemical fertilization and Díaz-Franco *et al.* (2008b) in sorghum, reported higher grain production when using mycorrhiza in conditions of restricted humidity compared to chemical fertilization, and in the same way, Plana *et al.* (2008), in wheat, reported higher yield with the use of mycorrhizae, followed by mineral nutrition, as a greater leaf area; better nutrition, grain filling and, therefore, higher yield (Dell'Amico *et al.* (2002); Lopez-Castañeda (2006). On the other hand, Ali *et al.* (2010) in corn, found that seedlings infected with *Glomus mosseae* they did not exceeded the control (with chemical fertilization) in the first 10 weeks after sowing in dry matter, plant height, stem thickness and root length, arguing that more time is needed for fungi to colonize roots.

The objective of the study was to evaluate the effect of mycorrhizae *Glomus intraradices* from two commercial houses (INIFAP and Azo-Fer), chemical fertilization 120-60-00, as well as the combination of mycorrhizae and 50% fertilization, in response to the foliar area, morphological composition and quantity and quality of seeds of millet pearl variety MF13, under irrigation conditions in the Plateau of San Luis Potosi.

Development of the experiment

The experiment was carried out in the spring-summer 2013 agricultural cycle at the San Luis Experimental Field of INIFAP, in the state of San Luis Potosi, located at an altitude of 1 835 m. The soil classification corresponds to a Calcisol and clay texture (FAO-UNESCO-ISRIC, 1988), with dry-temperate climate (BSk), average annual temperature of 16.2 °C and 306 mm of precipitation (INEGI, 2014).

The preparation of land consisted of fallow, as well as two steps of harrow, it was irrigated in a rounded way and then another tracing was carried out and it was planted (May 16). The seeds used for MF13 pearl millet for sowing came from the same San Luis Experimental Field of INIFAP, San Luis Potosi, Mexico, from the spring-summer 2012 sowing cycle, for this, they were

characterized for germination before sowing, when using four repetitions of 100 seeds in sand with soil moisture at field capacity 2 cm deep in the shade and covered with dark plastic and the emergence was counted 15 days after sowing (dds).

Planting was carried out in a mechanized way when using a Lucatero[®] brand planter, for which a density of 5 kg ha⁻¹ of germinable pure seed was used for each treatment (Hernández *et al.*, 2013), in addition, the seed lot, was inspected under the regulations of the National Service of Inspection and Certification of Seeds (SNICS). Each plot consisted of 15 rows at 80 cm distance with 70 m in length and three repetitions were assigned at random and these were isolated with reinforced boards to avoid that the irrigation water had contact between plots; in total, there were 18 plots, besides, within each plot three experimental units of two furrows were marked by 6 m in length. The treatments consisted of (1) chemical fertilization 120-60-00 (TF100), (2) mycorrhiza INIFAP[®] (TM- INIFAP[®]), (3) mycorrhiza Azo-Fer[®] (TM- Azo-Fer[®]), (4) mycorrhiza INIFAP[®] + 60-30-00 (TM-INIFAP + F50), (5) Mycorrhiza Azo-Fer[®] + 60-30-00 (TM-Azo-Fer + F50) and (6) control (T).

The source of mycorrhizae *Glomus intraradices* to pearl millet seeds came from INIFAP[®] and Azo Fer[®] mycorrhizae (Biofabricas siglo XXI[®]). To adhere the fungi to the seeds, 1 mL of adherent (adherent INIFAP[®]) dissolved in 100 mL of tap water was used; later, the prepared solution was sprinkled with a manual atomizer to the seeds and finally the mycorrhizae were added, uniformizing the application manually, so that they were all covered. The mycorrhiza dose was 0.5 kg ha⁻¹ (100 000 mycorrhiza propagules in 1 kg of seed for each treatment). After 20 dds, a relief irrigation was applied and 48 h later the first weeding was done and the second weeding was performed at 42 dds. The fertilization was carried out at the time of the weeding, manually, 10 cm away from the base of the plants with the formula 120-60-00, which is optimal for the type of soil and for this crop (Hernández and Zavala, 2009). When the fertilizer was combined with mycorrhizae, the formula used was 60-30-00. The sources of the chemical fertilizers used were urea (46-00-00) and ammonium diphosphate (18-46-00).

The irrigation was rolled and each consisted of a 13 cm sheet, which was monitored and registered (m³) with the use of a McCrometer[®] brand fluxometer on six occasions. The irrigations started from 18 dds, taking place when the crop had symptoms of wilt, until the stage of seed filling. When there was rain, irrigation was suspended. To calculate the total amount of water used, rainfall was added to the amount of water irrigated. There was no presence of weeds that interfered with the development of the crop. To control the worm infestation (*Spodoptera frugiperda*), the commercial product Lorsban 480E[®] was used at a dose of 1.0 L ha⁻¹ and applied on August 15, 2013, with the help of a 15 L capacity sprayer. To prevent the predation of caryopses by birds during seed filling (30 days), low-noise caves were used.

The harvest was done manually and was placed panicles in polyethylene sacks under plastic roof and anti-bird mesh with air flow for 30 days and then manually shelled, later, to remove the residues of floral appendages was used a fan and placed the seeds in poly bags. Before placing seeds in bags, the humidity of the seeds was determined by the direct stove method (Moreno, 1996), and once the desired humidity was reached, the sacks were stamped and sealed.

The variables to be evaluated

Height of plant (cm)

For this, four plants were taken at random from each plot and measured from the soil to the longest leaf apex and panicle apex. This activity was carried out every 10 days from the first weeding until presenting anthesis.

Index of leaf area (cm²)

It was determined in the period of anthesis and in milk grain state in each experimental unit, for this, a complete plant was extracted and the leaf area of each leaf, present in each stem, was measured, using the following formula: leaf area (long x width of each foliar leaf of the plant) x 0.75 (Carrillo and Ruiz, 2004).

Morphological composition (g MS pl⁻¹)

From the plants to measure the leaf area, they were separated into leaf blades, stems, panicles and dead material, then the material was placed in paper bags for drying in a stove (CIDERTA[®]) of forced air at 55 °C for 72 h and weighed on a scale (Lecsa[®]; g or kg).

Seed production ha⁻¹ (kg)

It was obtained by weighing the totality of caryopses of each experimental unit and by a rule of three, it was related to 1 ha.

Weight of a thousand seeds (mg)

Eight repetitions of 100 caryopses taken at random from each experimental unit were counted and weighed using an analytical scale (Ohaus[®]) and the weight of 1000 caryopses was obtained by multiplying by ten the arithmetic mean of the eight repetitions (ISTA, 2012).

Test weight (kg 100 L⁻¹)

A volume of one liter of seeds was weighed four times for each of the 36 experimental units and was related to 100 L.

Purity (%)

From the seed obtained from each experimental unit, four repetitions of 25 g of each were weighed and separated into three components: seed of the species of interest, other seeds and inert material (stones, soil particles and fragments of plants). According to the ISTA (2012) it considers as seeds those that are complete or intact and also those that are smaller than normal, wrinkled, diseased or germinated, as long as they can be identified as belonging to the species analyzed. To obtain the purity, the following formula was applied: purity = weight of pure seed (g) x 100/total weight of the sample.

Moisture content (%)

The direct stove method was used, which consisted of weighing a sample of 24 g, obtained from the batch of seeds harvested in each experimental unit, which was separated into four subsamples of 6 g and each was placed in a metal container with lid, which were weighed before and after entering the stove. The stove was calibrated at 103 ± 1 °C and the samples were maintained for 6 h. The moisture content was determined by the difference in seed weight before drying and after drying (Moreno, 1996).

Number of seeds per kg

In each experimental unit four repetitions of five grams were weighed and the number of caryopses was counted and subsequently, by rule of three, the respective content per kg was calculated.

Water efficiency It was obtained by dividing the amount of seed of each treatment between the water consumed in the irrigations (m^3) plus the accumulation of rainfall.

Statistical analysis

A completely randomized experimental design was used and the data obtained were analyzed by the GLM procedure of SAS (2009). The means of the evaluated variables were compared by the Tukey test ($\alpha= 0.05$).

Discussions

The characterization of pearl millet MF13 seed before planting (Table 1), shows good germination with an adequate moisture content (Moreno, 1996), as well as good germination.

Table 1. Initial characterization of MF13 seed of pearl millet.

Tradename	Name ICRISAT*	Purity (%)	Humidity (%)	Weight 1 000 seeds (g)	Hectoliter weight (g)	Number of seeds kg^{-1}	Germination (%)
MF13**	IP-19586	98	11.5	1000	88140	100 000	87.2

*= International Crops Research Institute for the semi-arid-tropic; **= variety of pearl millet with a tendency to forage; INIFAP (2013).

Plant height

Only differences in height were observed at the second sampling date ($p \leq 0.01$, Table 2), in the control treatment the lowest height (70 cm) was recorded and in the remaining dates, as well as no differences were observed on average ($p > 0.05$). Therefore, the use of mycorrhizae or chemical fertilizers, as well as their combination, under irrigation does not affect the plant height between fertilizers and control, which differs with Plana *et al.* (2008); Díaz-Franco *et al.* (2008b) who report that mycorrhizal inocula promote the development of cereals.

Table 2. Plant height of the MF13 variety of pearl millet by sampling date.

Treatment	14-june	24-june	04-july	15-july	25-july	05-augo	\bar{X}
Control	35a †	70 b	92 a	148 a	202 a	249 a	199
Fertilization 100%	36 a	73 ab	96 a	153 a	214 a	254 a	162
Mycorrhiza INIFAP®	36 a	74 ab	98 a	154 a	214 a	254 a	185
Mycorrhiza INIFAP® + 50% F*	36 a	75 ab	94 a	147 a	208 a	262 a	196
Mycorrhiza Azo Fer®	36 a	76 a	99 a	154 a	207 a	244 a	169
Mycorrhiza Azo Fer® + 50% F*	36 a	72 ab	91 a	141 a	196 a	244 a	201
DMSH	1.73	5.6	10.2	16	19.2	25.3	9.25

†= Equal volumes per column are statistically equal averages ($p > 0.05$); *= 0-30-00 fertilization; DMSH= honest significant minimum difference.

Leaf area

No differences were observed in the length and width of the leaves with the assigned treatments ($p > 0.05$, Table 3), so the use of mycorrhizae and chemical fertilizers, as well as their combination, do not have an impact on the greater leaf area of the plants. pearl millet, which differs with Dell'Ámico *et al.* (2002) who reported 20% more leaf area when using mycorrhizae compared to the control (without inoculation). In the morphological composition in physiological state of anthesis of the plant (Table 3), significant differences were observed only in biomass of MS of stems ($p \leq 0.01$), the highest value was result of fertilization 120-60-00 (368 g MS pl^{-1}) and the minor the control (167 g MS pl^{-1}); however, no differences were found in leaves, panicles, dead material or in total ($p > 0.05$). When fertilizing with both sources, higher biomass of dry matter was obtained per plant, therefore, the use of mycorrhizae and fertilizers, as well as their combination was important to obtain heavier stems and in case that it is decided to provide the green milled cattle, it covers protein requirements in cattle of 350 kg (12%, NRC, 2001; Urrutia *et al.*, 2014).

Table 3. Foliar area (cm^2) and biomass production of the morphological components of pearl millet plants in anthesis (g MS pl^{-1}) with the use of mycorrhizae, chemical fertilizers and their combination, under irrigation conditions

Treatment	Leaf area	Stems	Leaves	Panicles	MM	Total
Control	3 814 a †	167 b	23 a	94 a	5 a	288 a
Fertilization 120-60-00	4 533 a	368 a	32 a	91 a	16 a	507 a
Mycorrhiza INIFAP®	4 805 a	240 ab	25 a	97 a	9 a	371 a
Mycorrhiza INIFAP® + 50% F*	4 805 a	252 ab	30 a	84 a	11 a	376 a
Mycorrhiza Azo Fer®	3 744 a	249 ab	25 a	106 a	12 a	392 a
Mycorrhiza Azo Fer® + 50% F*	3 594 a	264 ab	32 a	110 a	4 a	410 a
DMSH	1 290	195**	17	53	14	237

†= Equal literals, in column, are statistically equal averages; *= fertilization (60-30-00); MM= dead material; **= $p \leq 0.01$; DMSH= honest significant minimum difference.

Morphological composition (g MS pl⁻¹)

The milk grain state of the pearl millet plants only showed difference in the weight of stems (Table 4, $p \leq 0.01$) and the control resulted in lower weight of stems and was different to biofertilization and chemical fertilization and their combinations ($p \leq 0.01$), which agrees with Díaz-Franco *et al.* (2008a); Díaz-Franco *et al.* (2008b); Plana *et al.* (2008). It is worth mentioning that, on average, stems contribute 65%, panicles 25%, leaves 7% and dead material 2%, so in this crop, the application of mycorrhizae or combinations with mineral nutrition or with mycorrhizae will have an impact on the production of green matter; for the foregoing, Urrutia *et al.* (2014) in pearl millet mention that the protein content for the stages of the seventh leaf, flag leaf, booting and flowering were: 15.4, 13, 12.7, 12.2, respectively, while for the *in vitro* digestibility of the dry matter (DIVMS) were: 81.7, 72.5, 74.5 and 69%, respectively, and metabolizable energy (ME) was not affected (1.9 Mcal kg⁻¹), therefore, Pearl millet is a good forage alternative for the semiarid zone of Mexico.

Table 4. Biomass production of the morphological components of millet pearl in milk grain state (g MS pl⁻¹) with the use of mycorrhizae, chemical fertilizers and their combination, under irrigation conditions.

Treatment	Stems	Leaves	Panicles	MM	Total
Control	167 b †	23 a	94 a	5 a	288 a
Fertilization 120-60-00	368 a	32 a	91 a	16 a	507 a
Mycorrhiza INIFAP [®]	240 ab	25 a	97 a	9 a	371 a
Mycorrhiza INIFAP [®] + 50% F*	252 ab	30 a	84 a	11 a	376 a
Mycorrhiza Azo Fer [®]	249 ab	25 a	106 a	12 a	392 a
Mycorrhiza Azo Fer [®] + 50% F*	264 ab	32 a	110 a	4 a	410 a
DMSH	195**	17	53	14	237

†= Literals in equal column, are statistically equal averages; * = fertilization (60-30-00); MM= dead material; ** = $p < 0.01$.

Seed production ha⁻¹ and water use efficiency

Significant differences were observed in seed production ($p \leq 0.05$, Table 5) and it is in the range of production reported by Hernández *et al.* (2013) and the highest yield was obtained when fertilizing with 120-60-00, which was similar to mycorrhizae INIFAP ($p > 0.05$), which coincides with Díaz-Franco *et al.* (2008a); Díaz-Franco *et al.* (2008b); Plana *et al.* (2008); Mena *et al.* (2013) and on the other hand the production of Azo Fer[®] mycorrhizal seeds, combinations and control were similar ($p > 0.055$). Therefore, with adequate plant nutrition, increased seed production (Villanueva *et al.*, 2001; López-Castañeda, 2006) and therefore greater use of water.

Table 5. Production of pearl millet seed (kg ha⁻¹) and water use efficiency of the pearl millet variety MF13.

Treatment	Yield (kg ha ⁻¹)	Use of water efficiency (€)
Control	1631 b †	96 b
Fertilization 120-60-00	2685 a	152 a
Mycorrhiza INIFAP [®]	2221 ab	131 ab
Mycorrhiza INIFAP [®] + 50% F*	1811 b	107 b
Mycorrhiza Azo Fer [®]	1783 b	105 b
Mycorrhiza Azo Fer [®] + 50% F*	1754 b	103 b
DMSH	774	45.3**

†= Literals in equal column, are statistically equal averages; * = fertilization (60-30-00); €= total water used (precipitation + irrigation) in the experimental unit; ** = $p < 0.01$.

Physical analysis of the seed produced

No differences were observed in the physical analysis variables of seeds from chemical fertilizers, mycorrhizae, combinations and control ($p > 0.05$, Table 6) and the biological or chemical fertilization influences yield, but not in hectoliter weight and weight of a thousand seeds; however, Noda (2009), in wheat, reports increases of 20 to 50% in the number of grains per spike. It should be mentioned that the hectolitre weight of the treatments was 3% lower than that found in the characterization of seeds before sowing, while the weight of one thousand seeds of the control was 20% higher compared to that found in initial characterization and, on the other hand, comparing the amount of seeds per kg, this was greater during the initial characterization of the seed since the caryopses were smaller compared to that obtained with the evaluated treatments.

Table 6. Physical analysis of MF13 pearl millet seeds after harvest.

Treatment	Hectoliter weight (g)	Weight of 1 000 seeds (g)	Number seeds kg ⁻¹	Purity (%)	Humidity (%)
Control	84667 a †	1202.7 a	44094 a	96.2 a	11.9 a
Fertilization 120-60-00	86667 a	2685.2 a	86667 a	96.1 a	11.9 a
Mycorrhiza INIFAP	85700 a	2220.7 a	85700 a	96.3 a	12.2 a
Mycorrhiza INIFAP + 50% F*	85450 a	1811.5 a	85450 a	96.1 a	11.6 a
Mycorrhiza AzoFer	85617 a	1782.8 a	85617 a	96 a	12.6 a
Mycorrhiza AzoFer + 50% F*	84383 a	1753.7 a	84383 a	96.2 a	12.6 a
DMSH	3522	1.47	15070	0.6	0.97

* = fertilization (60-30-00); † = equal lowercase letters per column, are statistically similar averages ($p > 0.05$).

Conclusions

The use of mycorrhizal biofertilizers and chemical fertilizers had a similar effect on the production of biomass of stems in the period of anthesis and state beds-mass, while in seed production (kg ha⁻¹), mycorrhizae INIFAP and chemical fertilizers were similar and much greater water efficiency. The foliar area, plant height and seed quality did not depend on the use of fertilizers or biofertilizers.

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