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Growth *of Zea mays* with endophytes of *Zea mays spp mexicana* (teocinte)

Abstract

The healthy growth of *Zea mays* demands nitrogenous and phosphorous fertilizers which, due to various problems in the soil, are not efficiently uptake by the roots of the plants. An alternative is the optimization of the NPF through the inoculation of the *Z. mays* seed with genera and species of endophytic fungi that promote plant growth of the type: *Aspergillus niger, Gliocladium virens and Rhizopus oligosporus*, which are effective in uptake of NPF reduced up to 50% for healthy plant growth. The objective of this research was to analyze the response *Z. mays to A. niger, G. virens and R. oligosporus* endophytesof teosinte with NPF reduced at 50%. The endophytic fungi were isolated from teosinte, and were individually inoculated in *Z. mays* with the NPF at 50%, through response variables: phenology and seedling biomass and flowering, the experimental data was analysed by ANOVA/Tukey ($P<0.05$). The results showed a positive response of *Z. mays* to *G. virens* registered 2.66g aerial dry weight (ADW), with *R. oligosporus* 1.70g of ADW and with *A. niger* 1.36g of ADW, values with a statistical difference with the 0.50g of ADW of *Z. mays* without the endophytic fungi fed with the NPF at 100% (relative control). The foregoing supports that endophytic fungi from teosinte were able to positively interact with the root system of *Z. mays*, which allowed healthy growth despite reducing the doses of NPF up to 50%.

Keywords: soil productivity, cereal, chemical fertilizers, endophytic interactions

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Introduction

In México, the healthy growth of Zea mays depends on an intensive production system, characterized by the continuous use of soil and the application of high doses of nitrogenous and phosphorous fertilizers (NPF), which causes deteriorarían of the soil, with risk additional contamination of surface and ground water; An alternative solution to avoid hyperfertilization is the optimization of NPF in *Z. mays* from seed inoculation with genera and species of endophytic fungi that promote plant growth,[1,2] which convert the exudates from the spermosphere of the seed and inside the roots into phytohormons which induce a denser root system,[3,4] for the optimization of NPF applied at doses lower than those recommended, without negatively affecting healthy plant growth. However, there is evidence that some endophytic fungy genera and species isolated from domestic plants do not cause the expected beneficial effect on commercially important cereals such as *Z. mays* when the dose of NPF is reduced by up to 50%, which could be attributed to the type and physicochemical conditions of the soil such as pH, salinity, moisture retention, etc., as well as the incompatibility of endophytic fungi with some varieties of *Z. mays,* the phytopathogenic agents. Therefore, a little explored option is to isolate and select endophytic fungi from wild plants such as *Zea mays spp mexicana* or teosinte due to the close genetic relationship with domestic *Z. mays*.[5,6] Specifically, because teosinte has managed to adapt to environments that are not conducive to healthy growth, such as nutritional and water stress, drastic changes in soil pH, and tolerance to a wide group of phytopathogenic agents.[2] These characteristics are encoded in teosinte DNA, which would make possible an effective selection of endophytic fungi that are competitive and efficient for the uptake and optimization of NPF reduced by up to 50%, compared to endophytic fungi genera and species recovered from domestic plants. Another biological factor that could favor the beneficial effect ofendophytic fungi from teosinte is the ability to invade the plant tissue of *Z. mays*, thus avoiding competition with other soil microorganisms, in addition to using energy to convert simple organic compounds derivatives of photosynthesis in phytohormons,[7] which improve the radical uptake capacity to maximize the NPF reduced to 50%, without causing problems in the healthy growth of *Z. mays,* while synthesizing acid and alkaline phosphatases to increase the disposal of PO_4^{-3} (phosphates) limiting for plant growth; as well as compounds in the roots that prevent dehydration in saline soils or with water problems.[8] Based on the above and the limited or scarce information related to genera and species of endophytic fungi of the type: *Aspergillus niger, Gliocladium virens, Rhizopus oligosporus* isolated from teosinte, which in *Z. mays* seeds ensure the effective optimization of the doses of the NPF up to 50%, without putting healthy plant growth at risk, the objective of this research was proposed: to analyze the response of *Z. mays to A. niger, G. virens* and *R. oligosporus* of teosinte with NPF reduced to 50%.

Materials and methods

The experiment was carried out in a 10x4m^2 greenhouse of the Environmental Microbiology Laboratory of the Biological Chemical Research Institute of the UMSNH, Morelia, Mich, Mexico. The average temperature was 23.2°C, luminosity 450µmol□m^{-2} s^{-1}, and relative humidity 67%.

Isolation of *Aspergillus niger*, *Gliocladium virens*, *Rhizopusoligosporus* teosinte endophytic fungi

The plant growth-promoting endophytic fungi used in this trial were isolated from *Z. mays* sp var mexicana or teosinte, from which the roots, stem and leaves were disinfected with Clorox® 5% 5min, washed five times with sterile water, then with 70% alcohol/5min, rinsed five times with sterile water, cut into 5cm pieces, macerated in a mortar with a saline solution (NaCl 0.85%) Roma™ detergent 0.01% (SSD), then 1.0mL was taken and grown in Sabouraud Agar with the following chemical composition (g.L^{-1}): polypeptone, 10.0; glucose, 40.0; agar, 18.0; with a mixture of broad-spectrum antibiotics with the following concentration: 1.0mL/100mL of

chloramphenicol, cefotaxine, tetracycline, ciprifloxacin, trimethoprim with sulfametazole; a pH adjusted to 5.6, after sterilizing at 120ºC/15minutes. The teosinte tissues grown in Sabouraud Agar were incubated at 35°C/7 days. [9] During this time, colonies were detected that were similar to the genera by microscopic lactophenol blue staining. of *Aspergillus, Gliocladium and Rhizopus*, which according to the literature belong to the genera and species of *A. niger, G. virens, R. oligosporus*.[10,11]

Inoculation of *Zea mays* with *Aspergillus niger, Gliocladium virens, Rhizopus oligosporus t*eosinte endophytes.

In *Z. mays* seeds were disinfected with the same procedure described in section a); for every 10 seeds they were inoculated with 1 mL of *A. niger, G. virens, R. oligosporus* at a density of 1.5 x 10^{-8} PFU (propagule-forming units) /mL calculated based on a viable plate count in Agar Sabouraud. Subsequently, in the upper part of the Leonard jars (Figure 1), 4 seeds of *Z. mays* with or without *A. niger, G. virens, R. oligosporus* were sown as described in the randomized block experimental design with two controls, three treatments and six repetitions (Table 1): *Z. mays* without endophytic fungi irrigated only with water or absolute control (AC); *Z. mays* untreated with endophytic fungi, *R. oligosporus* fed with 100% NPF or relative control (RC); *Z. mays* inoculated with *A. niger, G. virens* or *R. oligosporus* with 50% NPF in a mineral solution with the following chemical composition (g.L^{-1}): NH_4NO_3 12.0, KH_2PO_4 3.0, K_2HPO_4 3.5, $MgSO_4$ 1.5, $CaCl_2$ 0.1, $FeSO_4$ 0.5mL/L, and 1.0mL/L of the trace element solution, the pH was adjusted to 7.0. The response variables were based on phenology: plant height (PH) and root length (RL); in the biomass: aerial fresh weight (AFW) and radical (RFR); for the aerial dry weight (ADW) and radical (RDW).[9] The experimental data obtained were analyzed by ANOVA and Tukey (P≤0.05), to establish the minimum difference.[12]

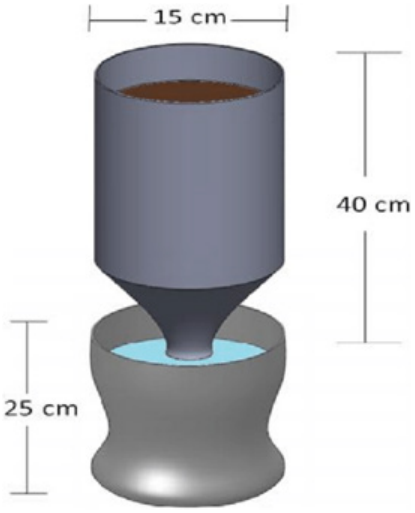

Figure 1 Leonard's Jar Diagram.

Recovery of *Aspergillus niger, Gliocladium virens, Rhizopus oligosporus* from plant tissues *of Zea mays.*

To determine that the healthy growth of *Z. mays* both at the seedling and flowering level was due to the beneficial effect of *A. niger, G. virens* and *R. oligosporus*, the roots, stem and leaves were disinfected as in the subsection a) for teosinte, then they were crushed; they were suspended in 9.0mL of SSD at 0.85%, then 1.0mL was grown in Sabouraud Agar with a mixture of antibiotics of the type: chloramphenicol, cefotaxine, tetracycline, ciprifloxacin, trimethoprim with sulfametazole; the pH was adjusted to 5.6, the Sabouraud Agar was incubated at 35°C/7 days.[9] At the end of the incubation time, morphologies similar to the genera and species of *A. niger, G. virens, R. oligosporus* appeared, which were compared with the endophytic fungi isolated from the plant tissues of teosinte, as well as with resistance to antibiotics used for isolation. While in *Z. mays* without *A. niger, G. virens* and *R. oligosporus,* fed with the doses of the NPF at 100% or relative control, no fungus was detected, especially those previously described. In contrast, if they were detected in *Z. mays* when inoculated with *A. niger, G. virens, R. oligosporus*.

Results and discusion

Table 2 Response of *Zea mays to Aspergillus niger, Gliocladium virens and Rhizopus oligosporu*s to seedlings stage with 50% nitrogen and phosphorus fertilizer.

Table 2 shows the response of *Z. mays* 30days after sowing inoculated with R. oligosporus and the NPF reduced to 50%, which registered 30.80cm of PH and 16.80cm of RL both numerical values without statistical difference compared to those recorded in *Z. mays* with *A. niger or G. virens*. This supports that once these teosinte endophytic fungi survived on the surface of the *Z. mays* seed, they converted the exudates from the spermosphere into phytohormons, to end embryo dormancy and accelerate germination sedes,[13,14] to later colonize the interior of the root system where they transformed some simple carbon compounds derived from photosynthesis into phytohormons, for rapid and better uptake of the NPF reduced to 50%.[15,16] The values of the parameters of the phenology of *Z. mays* with the endophytic fungi, were statistically different in comparison with the 23.60cm of pH and the 13.20cm of *Z. mays* without *A. niger, G. virens and R. oligosporus* fed with the 100% NPF or relative control. Depending on the biomass, the positive effect of *A. niger* on the growth of *Z. mays* with the NPF at 50%, was recorded: 0.95g of AFW, 0.43g of RFW, 0.21g of ADW and 0.05RDW, all of these numerical values were statistically different from those recorded in *Z. mays* with *G. virens* and *R. oligosporus* with 50% NPF, as well as with 0.69g of AFW, 0.17g of RFW, 0.05g of ADW and 0.02 of *Z. mays* without the endophytic fungifed with the NPF at 100% or RC. The positive response of *Z. mays* to *A. niger, G. virens and R. oligosporus* according to the data shown, indicates that by colonizing the interior of the root primordium and normal roots, they converted some organic compounds such as amino acids from plant metabolism, in phytohormons that promoted the multiplication of the roots, which increased the exploration capacity in the soil, while improving the uptake and optimization of the NPF dose reduced to 50% for healthy growth of *Z. mays*.[17,18]

In Table 3, at flowering, the positive response of *Z. mays* inoculated with A. niger plus the NPF at 50% is shown, which registered 96cm of PH and 25.20cm of RL, statistically different numerical values with the 67cm of PH. and the 20cm RL of *Z. mays* untreated with the endophytic fungi alone with the NPF at 100 or relative control, as well as with those obtained in *Z. mays* with *G. virens and R. oligosporus*. The positive response of *Z. mays* to these endophytic fungi confirms that they were able to colonize the interior of the root system, where they transformed carbon compounds derived from photosynthesis into phytohormons, which stimulated mitosis and karyokinesis of the root system tissue for optimization of NPF reduced up to 50%.[1,7] Regarding the fresh biomass, a beneficial effect of *R. oligosporus was recorded in Z. mays* and the NPF at 50%, where it reached 46g of AFW and 11.84g of RFW, both values without statistical difference from those obtained in *Z. mays with A. niger and G. virens*, however, when comparing the previous numerical values with the 12.65g of AFW and the 1.60g of RFW of *Z. mays* not treated with the

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endophytic fungi, but if fed with the NPF at 100% or RC if there was a statistical difference, while for the dry weight *Z. mays* with *G. virens* registered 5.64g of ADW and 2.66g of RDW, numerical values with the statistical difference compared to 1.90g of ADW and 0.50g of RDW of *Z. mays* without inoculating with *A. niger, G. virens or R. oligosporus* fed exclusively with the NPF at 100% or RC. The parameters according to the fresh and dry weight of *Z. mays* with ***endophytic fungi*** indirectly demonstrate the ability of these fungi isolated from teosinte to invade and colonize the interior of the roots of *Z. mays* where they transformed photosynthesis metabolites into phytohormons to optimize the maximum radical uptake capacity of NPF reduced up to 50%, without compromising healthy plant growth.[19-21] This indicates that *A. niger, G. virens or R. oligosporus*, by locating inside the root system, avoided external competition with other microorganisms: bacteria, fungi and actinomycetesfrom the rhizosphere, which limits the optimization of the NPF in *Z. mays*. In addition, the genetic specificity that exists between the endophytic fungi genera and species of teosinte and the organs of *Z. mays* also contributed, which favored the endophytic fungi to interact positively with *Z. mays*.[22-25]

Table 1 Experimental design to analyze the response of *Zea mays to Aspergillus niger, Gliocladium virens and Rhizopus oligosporus* endophytes of teosinte

*Zea mays	Aspergillusniger	Gliocladiumvirens	Rhizopusoligosporus	water	Nitrogen and Phosphorus fertilizer (NPF)
Absolute control (AC)	-	-	-	+	-
Relative Control (RC)	-	-	-	-	100%
Treatment-1	+	-	-	-	50%
Treatment- 2	-	+	-	-	50%
Treatment 3	-	-	+	-	50%

*number of repetitions (n), 6; applied (+); not applied (-); nitrogenous and phosphorous fertilizer (NPF)

Table 2 Response of *Zea mays to Aspergillus niger, Gliocladium virens and Rhizopus oligosporus* to seedlings stage with 50% nitrogen and phosphorus fertilizer

*Zea mays	Plant height (cm)	Radical length(cm)	Fresh weight (g)		Dry weight(g)	
			aerial	radical	aerial	radical
Irrigated only water or control absoluto (AC)	21.20^{c*}	14.80^{b}	0.63^{c}	0.20^{bc}	0.05^{b}	0.02^{b}
Fedwith NPFat 100% orrelative control (CR)	23.60^{b}	13.20^{c}	0.69^{b}	0.17^{b}	0.05^{b}	0.02^{b}
Aspergillus niger and NPFat 50% (T1)	28.20^{a}	18.20^{a}	0.95^{a}	0.43^{a}	0.21^{a}	0.05^{a}
Gliocladiumvirens NPFat 50% (T2)	27.00^{a}	16.00^{b}	0.75^{b}	0.14^{c}	0.06^{b}	0.03^{b}
Rhizopusoligosporus NPFat 50% (T3)	30.80^{a}	16.80^{b}	1.07^{a}	0.31^{ab}	0.08^{b}	0.02^{b}

*n, 6; NPF, Nitrogenous and phosphorous fertilizers; **values with statistical difference (P<0.05) according ANOVA/Tukey.

Table 3 Response of *Zea mays to Aspergillus niger, Gliocladium virens and Rhizopus oligosporus* to flowering with 50% nitrogen and phosphorus fertilizers

*Zea mays	Plant height (cm)	Radical length(cm)	Fresh weight (g)		Dry weight(g)	
			aerial	radical	aerial	radical
Irrigated only water or control absoluto (AC)	21.20^{c*}	67.00^{c**}	30.00^{a}	6.00^{d}	1.30^{c}	0.90^{d}
Fedwith NPFat 100% orrelative control (CR)	23.60^{b}	71.00^{c}	20.00^{c}	12.65^{c}	1.60^{c}	1.90^{c}
Aspergillus niger and NPFat 50% (T1)	28.20^{a}	96.00^{a}	25.20^{b}	36.25^{b}	8.89^{b}	4.30^{b}
Gliocladiumvirens NPFat 50% (T2)	27.00^{a}	75.20^{b}	19.80^{c}	37.52^{a}	10.10^{a}	5.64^{a}
Rhizopusoligosporus NPFat 50% (T3)	30.80^{a}	84.20^{b}	18.00^{c}	46.00^{a}	11.84^{a}	5.80^{a}

n, 6; NPF, Nitrogenous and phosphorous fertilizers; **values with a statistical difference (P<0.05) according to ANOVA/Tukey.

Conclusions

Teosinte is a source of plant growth-promoting fungi such as *A. niger, G. virens and R. oligosporus*, which are able to be competitive and effective in optimizing the reduced dose of NPF up to 50% for healthy growth regarding that the intensive production system used to crop *Z. mays*. To maintain the production of this cereal, both the NPF, when they are not uptake by *Z. mays*, cause deterioration not only of the soil and water, but also contribute to global warming. In this sense, the use of endophytic fungus isolated from teosinte for the production of *Z. mays* is an option to avoid these problems.

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Conflicts of interest

The authors declared no have conflict interest for the study.

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