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# *Xanthobacter autotrophicus* and endophytic yeasts preventing greenhouse gases in the growth of *Phaseolus vulgaris*

## Abstract

Currently, in agricultural production, to ensure that it is not a source of greenhouse gases, without affecting the healthy growth of *Phaseolus vulgaris*, it is necessary to apply $NH_4NO_3$ at 70% since previous studies indicate that values of 100 to 80% are uptake by the plant (data not showed) and generate $N_2O$ in addition to contaminating surface and underground water. An alternative solution is to apply $NH_4NO_3$ 70% and inoculate the seeds with microbial consortia that optimize this nitrogen fertilizer. The objective of this research was to analyze the response of *P. vulgaris* to *Pichia norvegensis, Saccharomyces cervesiae* and *Xanthobacter autotrophicus* $NH_4NO_3$ at 70%. The experiment was carried out with a randomized block design; the response variables: germination percentage, days of emergence, phenology: plant height (PH), root length (RL) and biomass: aerial/radical fresh weight (AFW/RFW) aerial and radical dry weight (ADW/RDW) of *P. vulgaris*. All the experimental data were analyzed by ANOVA/Tukey HSD ($P<0.05$).

The results showed a positive effect of *P. norvegensis and X. autotrophicus* with 94% germination of *P. vulgaris* seeds; at seedling stage registered 37.48 cm of PH, 18 cm of RL, 1.96 g of FAW, 1.55 g of RFW, 0.24 g ADW and 0.14 g RDW, all this numerical values statistically were different, compared to 70.7% germination, 28.8 cm PH, 10.66 RL, 0.82 g AFW, 0.29 g RFW, 0.12 g ADW and 0.03 g RDW in *P. vulgaris* not inoculated with *P. norvegensis* or *X. autotrophicus* fed with 100% $NH_4NO_3$ , used as relative control (RC). These results support that it is feasible to use interactions between yeasts and endophytic bacteria, such as *P. norvegensis* and *X. autotrophicus* to activate and improve the physiological capacity of *P. vulgaris* root to increase $NH_4NO_3$ 70% uptake, which prevents the release of greenhouse gases associated with global warming, loss of fertility and contamination of surface and groundwater.

**Keywords:** soil, chemical fertilizer, endophytic microbes promoting plant growth, climatic change

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## Introduction

Healthy growth of *Phaseolus vulgaris* depends on nitrogen fertilizers such as $NH_4NO_3$ in excess generates a loss of soil productivity,[1–3] by unbalancing the carbon: nitrogen ratio, it causes changes in microbial diversity, affecting biogeochemical cycles necessary for plants,[4,5] excess nitrogen fertilizer generates greenhouse gases[6] surface and groundwater pollution.[7] An ecological alternative, to regulate nitrogen fertilizer application, is to decrease $NH_4NO_3$ to 70% and inoculate seeds of *P. vulgaris*, with multiple natural endophytes such as: *Pichia norvegensis, Saccharomyces cervesiae* and the nitrogen-fixing prokaryote *Xanthobacter autotrophicus*, capable of invading the root tissue, to transform intermediate compounds of plant metabolism, into phytohormones to enhance root hairs for maximum exploration area, in the soil to optimize $NH_4NO_3$ to 70%.[8–10] The application of bacteria and fungi is common, but not yeasts and endophytic bacteria.[11,12] In this regard, Agamy & Alamri,[13] analyzed the positive response of *Beta vulgaris* beer to *Sccaromycopsis cataegensis*, at a restricted dose of nitrogen fertilizer, with values of aerial dry weight statistically different compared to ADW and RDW in the non-inoculated *B. vulgaris*. This form of plant biomass enhancement is reported by Ignatova et al, 2015 as they demonstrated that *Rhodotorula mucilaginosa*, synthesizes phytohormones to induce root formation, and optimize nitrogen fertilizer uptake, that can be enhanced with *X. autotrophicus* endophyte due to the wide interaction with domestic and forest plant species, so the objective of this research was to analyze the response of *P. vulgaris* to *P. norvegensis, S. cervesiae* and *X. autotrophicus* and $NH_4NO_3$ 70% preventing global warming due to greenhouse gases.

## Materials and methods

This research was conducted in the greenhouse of the Environmental Microbiology Laboratory, Research Institute in Chemistry and Biology, Universidad Michoacana de San Nicolás de Hidalgo (UMSNH), Morelia, Michoacán, Mexico, in a greenhouse under the following average microclimatic conditions: 23.2 °C, luminosity of 450 µmol m-2s -1 and 67 % relative humidity. In a non-sterile soil collected in the municipality of Salvador Escalante, Michoacán, Mexico with a silty clay loam texture with 10.44% organic matter, a moderately acid pH of 5.75, the soil was classified according to NOM-021.RECNAT-2000, the soil was sieved with No. 20 mesh, solarized at 70°C/48 h to minimize pests and diseases.

### Origin of endophytic yeasts

The isolation of plant growth promoting endophytic yeasts was from the root, stem and leaf of *Amarylis belladonna* (summer lily), *Clivia miniata* (clivia), *Spanthodea campanulata* (african tulip). For this, plant tissues were disinfected with a 3% sodium hypochlorite solution/3 min, washed 6 times with sterile water, then disinfected with 75% ethanol/3 min and washed 6 times with sterile water. The plant organs were cut into 5 cm pieces, macerated with a 0.85% saline solution *Roma*$^{MR}$ 0.01% detergent (SSD), from there 1.0 mL was taken in yeast malt agar (YMA) (g/L): 3.0 yeast extract, 3.0 malt extract, 10.0

 

sucrose, 5.0 peptone of casein, 18.0 of bacteriological agar, adjusted to pH 5.5 and 10 mL of ciprofloxacin at 50 mg/mL to eliminate any bacteria, then incubated at 30°C/36 h. of 5.5 to 6; Malt Yeast Extract Agar or MYEA (g/L): malt extract, 3.0; yeast extract, 3.0; peptone, 5.0; glucose, 10.0; 18.0 agar, pH 5.0; Malt Yeast Broth or MYB (g/L): malt extract, 3.0; yeast extract, 3.0; peptone, 5.0; glucose, 10.0, pH at 5.0; Wickerham-1 (g/L): glucose 10, yeast extract, 4.5; peptone, 7.5 and pH 6.5;and Wickerham-2 (g/L): $KH_2PO_4$, 0.15; $MgSO_4H2O$, 0.5; NaCI, 0.1; CaCI, 0.1;dextrose, 10.0; $NH_4CI$, 0.5, pH 6.5, 1 mL of ciprofloxacin was added to each culture medium at a concentration of 50 mg/mL (Sánchez-Yañez, 2007).

## Identification of plant growth promoting endophytic yeast

Subsequently, all the culture media were inoculated with 500 and 1000 µL, then incubated until growth was observed at 27°C and performed the following biochemical tests: catalase, fermentation of glucose and sucrose, Simmons citrate, *P. norvegensis,* and *S. cerevisiae* on yeast malt agar (YMA) with the following chemical composition (g/L): 3 yeast extract, 3 malt extract, 10 sucrose, 5 casein peptone, 17 bacteriological agar, 10 mL of ciprofloxacin was added at a concentration of 50 mg/mL pH 5.5.

*X autotrophicus* was kindly donation from Department of Chemical and Biology, Harvard University, Cambridge, Ma, USA, *X. autotrophicus* grew on non-sucrose or nitrates agar with the following chemical composition (g/L): $K_2HPO_4$ 3.0, $MgSO_4$ .$7H_2O$ 0.5, KCl 0.5, $FeSO_4$ 0.01, bacteriological agar 18, pH 7.

## Molecular identification of endophytic yeast isolates

The 5.8S internal transcribed spacer (ITS) rDNAs of yeast isolates ARDMC1 and *S. cerevisiae* and *P. norvegensis* were amplified using the primers ITS-1 ($5^0$-TCCGTAGGTGAACCTGCGG-$3^0$) and ITS-4 ($5^0$-TCCTCCGCTTATTGATATGC-$3^0$) according to White et al., 1990. The D1/D2 domain of the 26S rDNA gene was amplified using the primer pair NL1 ($5^0$-GCATATCAATAAGCGGAGGAAAAG-$3^0$) and NL4 ($5^0$-GGTCCGTGTTTCAAGACGG-$3^0$). The polymerase chain reaction (PCR) amplification was performed in an Eppendorf Thermocycler according to a protocol[14] amplification parameters consisted of an initial denaturation step of 3 minutes at 95°C, followed by 30 cycles of 95°C for 30 seconds, primer annealing for 30 seconds at 58°C, elongation for 1 minute at 72°C, and final extension of 10 minutes at 72°C for one cycle. The amplified PCR product was purified and subjected to automated DNA sequencing using a 3130 Genetic Analyzer (Applied Biosystems, Rotkreuz, Switzerland).

## Inoculation of seeds of *P. vulgaris* with endophytic yeast and *X. autotrophicus*

The *P. vulgaris* seeds were disinfected with 5% NaClO (sodium hypochlorite) for 5 minutes, then washed 5 times with sterile water, disinfected in 70% alcohol for 5 minutes, washed 5 times with sterile water: for each 10 *P. vulgaris* seeds were inoculated, with 1 mL of *P. norvegensis* at a density of 6.4 x$10^6$ CFU/mL, *S. cerevisiae* at a density of 8.1 x$10^6$ CFU/ml, *X. autotrophicus* at a density of 4.3x$10^6$ CFU/mL, then were sown in soil, according to the Table 1, under an experimental design of random blocks: six treatments and six repetitions: *P. vulgaris* uninoculated irrigated with water, or absolute control (AC). *P. vulgaris* uninoculated, fed with 100% or relative control (RC); (T1) = *P. vulgaris* with *P. norvegensis* 70% $NH_4NO_3$; (T2) =*P. vulgaris* with *S. cervesiae* fed $NH_4NO_3$ at 70%; (T3) =*P. vulgaris* with *P. norvegensis* and *S. cervesiae* fed $NH_4NO_3$ at 70%; (T4) = *P. vulgaris* with *P. norvegensis* and *X. autotrophicus* fed $NH_4NO_3$ at 70%; (T5) = *P. vulgaris* with *S. cervesiae* and *X. autotrophicus* with $NH_4NO_3$ at 70%; (T6)= *P. vulgaris* with *X. autotrophicus* fed $NH_4NO_3$ at 70%. The plants were fed with mineral solution with the following chemical composition (g/L): $NH_4Cl$ or $NH_4NO_3$ 10.0, $K_2HPO_4$ 2.5, $KH_2PO_4$ 2.0, $MgSO_4$ 1.0, NaCl 0.1, $CaCl_2$ 0.1, $FeSO_4$ traces, trace element solution 10.0 mL, adjusted to a pH of 6.5-6.8; for the trial regard as a RC so that it was 100% $NH_4NO_3$ 10.0 g was applied and in the inoculated *P. vulgaris* to 7.0 g of $NH_4NO_3$ was added. The response variables were based on the days of emergence and percentage of germination; phenology: plant height (PH) and root length (RL); in biomass: aerial fresh weight (AFW) and radical fresh weight (RFW); for the aerial dry weight (ADW) and radical dry weight (RDW) of *P. vulgaris* seedling stage (Sanchez-Yañez, 2007). The experimental results obtained were analyzed by ANOVA and Tukey ($P \leq 0.05$), to establish the minimum significant difference Figure 1.[15]

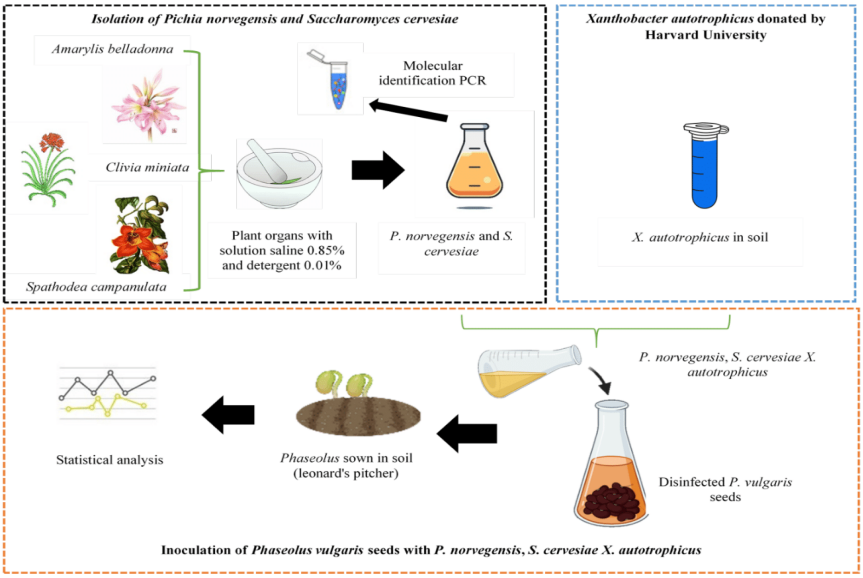


**Figure 1** Scheme of the experimental design to evaluate the response of *Phaseolus vulgaris* to *Pichia norvegensis*, *Saccharomyces cerevisiae* and *Xanthobacter autotrophicus* with 70% $NH_4NO_3$.

## Results

According to biochemical and molecular tests, endophytic plant yeasts were identified as *P. norvegensis* and *S. cervesiae* from *Amarylis belladonna*, *Clivia miniata* and *Spanthodea campanulata* respectively.

Table 1 demonstrated the positive effect of *S. cervesiae* and *X. autotrophicus,* on the germination of *P. vulgaris* seed with $NH_4NO_3$ at 70%, registed 97.7% germination, and the emergence was 3 days after sowing. Was evident that the seeds of *P. vulgaris* responded positively to all types of yeast, as well as did to *X. autotrophicus,* compared to seed of *P. vulgaris* used as a relative control, uninoculated fed with $NH_4NO_3$ at 100%, as well as with days to emergency.

Figure 2. Effect of *P. norvegensis, S. cervesiae* and *X. autotrophicus* on the germination, of *Phaseolus vulgaris* seeds with $NH_4NO_3$ at 70%, shows the positive effect of endophytic yeasts, and *X. autotrophicus* with 50% $NH_4NO_3$. There it was easy to observe, that both the stem and root primordium, showed an evident greater and better growth, compared to the seed of *P. vulgaris*, uninoculated with endophytic yeast and *X. autotrophicus,* fed with 100% $NH_4NO_3$ used as a relative control (RC), as well as the seed of *P. vulgaris* irrigated with water or absolute control (AC).

Table 2 shows the response of *P. vulgaris* in seedlings to *P. norvegensis* and *X. autotrophicus* with $NH_4NO_3$ that registered 37.48 cm of PH, 18 cm of RL, 1.96 g of AFW, 1.55 g of RFW, 0.24 g of ADW and 0.14 g of RDW, followed by the response of *P. vulgaris* to *S. cervesiae* and *X. autotrophicus* with $NH_4NO_3$ at 70%, that registered 36.83 cm of PH, 17.83 cm of RL, 1.93 g of AFW, 1.58 g of RFW, 0.22 g of ADW and 0.13 g of RDW. The results of the positive response of *P. vulgaris* in phenology and biomass to *P. norvegensis* and *X. autotrophicus* with $NH_4NO_3$ at 70% had a statistical difference with 28.8 cm of PH, 10.66 of RL, 0.82 g of AFW, 0.29 g of RFW, 0.12 g of ADW and 0.03 of RDW of *P. vulgaris* uninoculated by *P. norvegensis* or *X. autotrophicus* with 100% $NH_4NO_3$ or RC.

**Table 1** Effect of *Pichia norvegensis, Saccharomyces cervesiae and Xanthobacter autotrophicus* on the germination of *Phaseolus vulgaris* seeds with $NH_4NO_3$ at 70%

| Tratamiento Phaseolus vulgaris** | Emergency days | Germination percent |
|---|---|---|
| Absolute Control (AC) | $5^{b**}$ | $50.6^{f}$ |
| Relative Control (RC) | $5^{b}$ | $70.7^{d}$ |
| *Pichia norvegensis* + $NH_4NO_3$ at 70% | $3^{a}$ | $66.6^{e}$ |
| *Saccharomyces cervesiae* + $NH_4NO_3$ at 70% | $3^{a}$ | $94.4^{a}$ |
| *P. norvegensis* + *S. cervesiae* + $NH_4NO_3$ at 70% | $3^{a}$ | $88.8^{c}$ |
| *P. norvegensis*+ *Xanthobacter autotrophicus* + $NH_4NO_3$ at 70% | $3^{a}$ | $92.2^{b}$ |
| *S. cervesiae* + *X. autotrophicus* + $NH_4NO_3$ at 70% | $3^{a}$ | $97.7^{a}$ |
| *X. autotrophicus* + $NH_4NO_3$ at 70% | $3^{a}$ | $93^{b}$ |

n=6, **Different letters indicate statistical difference by ANOVA Tukey P>0.05.

**Table 2** Response of *Phaseolus vulgaris* at seedlings stage to *Pichia norvegensis*, *Saccharomyces cervesiae* and *Xanthobacter autotrophicus* $NH_4NO_3$ at 70%

| Treatments * *P. vulgaris* | Plant height (PH) cm | Radical length (RL) Cm | Aerial fresh weight (AFW) g | Radical fresh weight (RFW) g | Aerial dry weight (ADW) g | Radical dry weight (RDW) g |
|---|---|---|---|---|---|---|
| Absolute control (AC) | $21.41^{d**}$ | $7.8^{d}$ | $0.66^{d}$ | $0.11^{d}$ | $0.04^{c}$ | $0.01^{c}$ |
| Relative control (RC) | $23.8^{d}$ | $10.66^{c}$ | $0.82^{c}$ | $0.29^{c}$ | $0.12^{b}$ | $0.03^{d}$ |
| *P. norvegensis* + $NH_4NO_3$ at 70% | $30.46^{b}$ | $11.46^{c}$ | $1.32^{b}$ | $0.37^{c}$ | $0.15^{b}$ | $0.03^{d}$ |
| *Saccharomyces cervesiae*. + $NH_4NO_3$ at 70% | $29.66^{b}$ | 15.88b | $1.80^{a}$ | 0.67c | $0.18^{b}$ | $0.09^{b}$ |
| *S. cervesiae* + *P. norvegensis* $NH_4NO_3$ at 70% | $34.5^{a}$ | $14.76^{b}$ | $1.37^{b}$ | 0.63c | $0.14^{b}$ | $0.07^{b}$ |
| *P. norvegensis* +**X. autotrophicus**+ $NH_4NO_3$ at 70% | $37.48^{a}$ | $18.0^{a}$ | $1.96^{a}$ | $1.55^{a}$ | $0.24^{a}$ | $0.14^{a}$ |
| *S. cervesiae* +*X. autotrophicus* + $NH_4NO_3$ at70% | $36.83^{a}$ | $17.83^{a}$ | $1.93^{a}$ | $1.58^{a}$ | 0.22a | $0.13^{a}$ |
| X. autotrophicus + $NH_4NO_3$ at 70% | $29.5^{b}$ | $15.16^{b}$ | $1.76^{a}$ | $1.17^{a}$ | 0.20a | $0.10^{a}$ |

*n=6 **Different letters indicate statistical difference by ANOVA Tukey P>0,05.

Figure 2 showed the positive response in the phenology of the *P. vulgaris* seedling stage to *P. norvegensis*, *S. cervesiae* and *X. autotrophicus* with $NH_4NO_3$ at 70 %, with an increase in the number of stems, size of leaves, intense green coloration and increase in the density of the root system, the foregoing supports the fact that *P. norvegensis*, *S. cervesiae* and *X. autotrophicus* improved the uptake and optimization of $NH_4NO_3$ at 70% compared to *P. vulgaris* uninoculated with *P. norvegensis*, *S. cervesiae* and *X. autotrophicus* with 100% $NH_4NO_3$ or RC that showed fewer stems and leaves together with a less dense root system, indicating that the recommended dose was not uptake and was optimized to the maximum, that cause greenhouse gasses for global warming[6] acute problem and lost soil fertility that agriculture has to solve.[1]

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## Discussion

Endophytic yeasts: *P. norvegensis* and *S. cervesiae* has been living with legume as *P. vulgaris* for long time ago, in that sense the positive interaction among them or mixing with *X. autotrophicus* is interesting tool for sustainable agriculture to adjust dose of nitrogen fertilizer in order to avoid greenhouse gases[6] and global warming[16–18] as it has showed in this research with $NH_4NO_3$ at 70% of the recommended dose for *P. vulgaris* where is cropping in Mexico.

In Table 1 showed these facts support when exuding the seed of *P. vulgaris,* released sugars such as glucose and maltose, organic acids and amino acids as sources of carbon and energy, that *S. cervesiae* plus *X. autotrophicus* transformed, into phytohormones that interrupted the latency period of the seeds, to the emergence of root and stem primordium. This corroborates the research by Tawfiq et al., 2018, whom reported that *S. cervesiae* was capable of synthesizing phythormones. Lencinas et al.,[19] analyzed *S. cerevisiae* and *S. pombe* in *Lactuca sativa* seeds, reported an increase in germination per centage. The results of the positive effect of *S. cervesiae* and *X. autotrophicus* in the *P. vulgaris* seed, fed $NH_4NO_3$ at 70% had a statistical difference, compared to 70.7% germination and that emerged 5 days after the sowing of *P. vulgaris,* non-inoculated fed with 100% $NH_4NO_3$ or relative control (RC).

On Figure 2 there an increase in root primordia was observed, this supports that *P. norvegensis, S. cervesiae* and *X. autotrophicus,* accelerated plant growth by increasing root density, that improved exploration in the soil to uptake and maximize $NH_4NO_3$ at 70%. Compared to the seed of *P. vulgaris* uninoculated, with *P. norvegensis, S. cervesiae* and *X. autotrophicus* fed 100 % $NH_4NO_3$ or RC, there it was shown with a smaller root and stem primordium of *P. vulgaris,* these facts support that *P. norvegensis. S. cervesiae* and *X. autotrophicus* endophytically, in the root and stem primordium of *P. vulgaris,* transformed vegetal intermediate metabolites into phytohormones, inducing karyokinesis;[20] increasing the number of root hairs that improved the uptake and optimization of $NH_4NO_3$ at 70%, and thus achieved healthy plant growth. In few analyzes the promoting effect of yeast plant growth has been reported, by Amprayn et al.,[21] that showed the positive response of *Oryza sativa,* to *Candida tropicalis* which improved uptake of nitrogen fertilizer by *O. sativa* root system was improved, with to increase in the dry weight of the plant by 35%, due to synthesis of phytohormones; in that sense some investigations, reported the mechanisms of promotion of plant growth of the genus *X. autotrophicus:* Santos et al.,[22] explain that solubilization of phosphates by *X. autotrophicus,* that was capable to solubilize phosphates precipitated in soil, for to uptake by *P. vulgaris*.[23,24] When phosphates as well as unregulated $NH_4NO_3$ dose could not be uptake, by the root system of *P. vulgaris* efficiently, is possible to solve by applying natural microbial consortium, as we did at the present research work.[22,7,2,11]

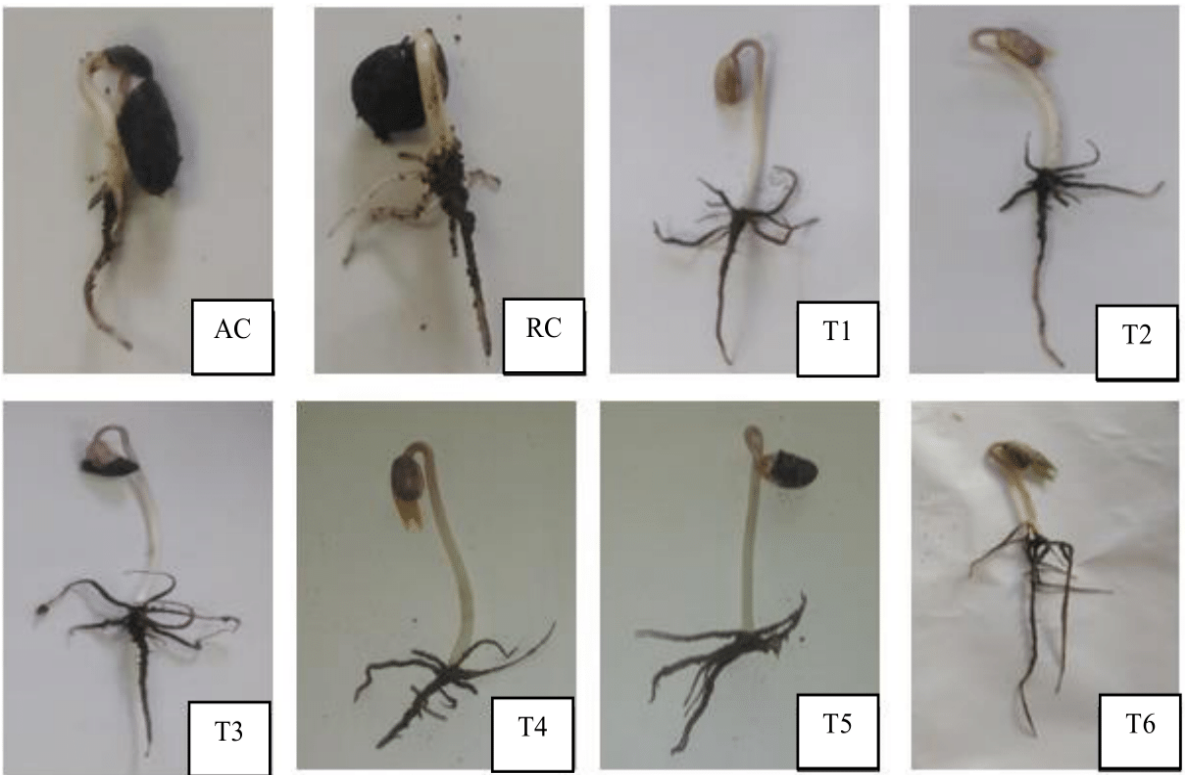


**Figure 2** Effect of *P. norvegensis, S. cervesiae* individually or in combination with *X. autotrophicus* in *P. vulgaris* fed $NH_4NO_3$ at 70%.

AC= *P. vulgaris* uninoculated irrigated with water; RC= *P. vulgaris* uninoculated fed with $NH_4NO_3$ at 100%;

T1= *P. vulgaris* + *P. norvegensis* + $NH_4NO_3$ at 70%;T2= *P. vulgaris* + *S. cervesiae*+ $NH_4NO_3$ at 70%;

T3= *P. vulgaris* + *P. norvegensis*, + *S. cervesiae* + $NH_4NO_3$ at 70%;

T4= *P. vulgaris* + *P. norvegensis* + *X. autotrophicus* + $NH_4NO_3$ at 70%;

T5= *P. vulgaris* + *S. cervesiae* + *X. autotrophicus* + $NH_4NO_3$ at 70% T6= *P. vulgaris* + *X. autotrophicus* + $NH_4NO_3$ at 70%

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Table 2 shows the response of *P. vulgaris* with 70% NH4NO3 and *P. norvegensis, S. cervesiae* plus *X. autotrophicus*. In general, regardless of which genus and species of yeast, individually or in mixture with *X. autotrophicus*, were able to transform organic compounds from the root metabolism of *P. vulgaris* into phytohormones, as an increase in root system density and the highest efficient uptake activity of $NH_4NO_3$ at 70% was observed (Agmy et al., 2013);[21,10] especially in plant height (PH) and root length (RL). This showed that the endophytic yeasts and *X. autotrophicus* invaded the root system of *P. vulgaris*, so in the phenology, numerical values were recorded, statistically similar or different from the numerical values in plant height (PH) in *P. vulgaris* not inoculated with 100% $NH_4NO_3$.

While in *P. vulgaris* biomass in fresh and dry weight of aerial part (FAW/DAW) and root system with *P. norvegensis,* or *S. cerevisiae*, individually or in consortium and *X. autotrophicus,* a synergistic action was recorded, to transform organic compounds from *P. vulgaris* metabolism, into phytohormones that optimized maximum $NH_4NO_3$ uptake to 70% (Lencinos et al., 2020).[4,11] Both endophytic yeasts and X. autotrophicus, share biochemical mechanisms of recognition of compounds from *P. vulgaris* metabolism (Tawfiq et al., 2018);[15,12] dependent on $NH_4NO_3$ concentration at 70%, that facilitated a positive interaction between endophytic yeasts and *X. autotrophicus* with the *P. vulgaris* root system0.[8,18] According to the numerical biomass values of *P. vulgaris,* endophytic yeasts in consortium with *X. autotrophicus* reached statistically different numerical values compared to the same parameters of non-inoculated *P. vulgaris* and 100% $NH_4NO_3$.

Figure 3 shows the phenology of *P. vulgaris* with endophytic yeasts individually or in consortium with *X. autotrophicus* and 70% $NH_4NO_3$ compared to non-inoculated *P. vulgaris* fed with 100% $NH_4NO_3$. There it was evident that in all cases the endophytic yeasts invaded the root system of *P. vulgaris* with or without *X. autotrophicus* utilized organic compounds from root metabolism to convert them into various phytohormones[16,17] according to the aerial part of *P. vulgaris* with higher number of leaves and evident chlorophyll induction[25,21,19] that was higher than in non-inoculated *P. vulgaris* fed with 100% $NH_4NO_3$ according to 70% $NH_4NO_3$ concentration.[20,4]


**Figure 3** Response of *Phaseolus vulgaris* at seedlings to *P. norvegensis*, *S. cervesiae* and *X. autotrophicus* fed $NH_4NO_3$ at 70%.

AC= *Phaseolus vulgaris* uninoculated irrigated with water; RC= *P. vulgaris* uninoculated fed with $NH_4NO_3$ at 100%;

T1= *P. vulgaris* + *P. norvegensis*+ $NH_4NO_3$ at 70%;T2= *P. vulgaris* + *S. cervesiae*+ $NH_4NO_3$ at 70%;

T3= *P. vulgaris* + *P. norvegensis* + *S. cervesiae* + $NH_4NO_3$ at 70%;

T4= *P. vulgaris* + *P. norvegensis* + *X. autotrophicus* + $NH_4NO_3$ at 70%;

T5= *P. vulgaris* + *S. cervesiae* + *X. autotrophicus* + $NH_4NO_3$ at 70%

T6= *P. vulgaris* + *X. autotrophicus* + $NH_4NO_3$ at 70%

A similar positive response was observed in the dense and abundant root system of *P. vulgaris* treated with endophytic yeasts and *X. autotrophicus* due to the conversion of root metabolites into several phytohormones, so that despite reducing $NH_4NO_3$ to 70% its effective uptake without compromising the healthy growth of *P. vulgaris* to avoid the generation of greenhouse gases causing global warming, compared to non-inoculated *P. vulgaris* fed with 100% $NH_4NO_3$, that obviously the legume does not take advantage of *P. vulgaris,* to avoid the generation of greenhouse gases causing global warming, compared to non-inoculated *P. vulgaris* fed with 100% $NH_4NO_3$,

that obviously the legume does not take advantage of, according to what was observed in aerial and root growth, consequently, this remnant contributes to the release of greenhouse gases.[6] The growth of *P. vulgaris* with this natural consortium of two types of endophytic microorganisms supports the importance of the interaction between these three different types of biological groups that over time have adapted to environmental changes in the soil,[26,22,15] especially the response of P. *vulgaris*[3] dependent on the concentration of inorganic nitrogen[7] as $NH_4NO_3$ available to the root system of *P. vulgaris* that induces some specific ecological interaction with endophytic yeasts and *X. autotrophicus* in harmonic association.[9,4,2,27–29]

## Conclusion

Based on the above, it was demonstrated that an ancient positive interaction of endophytic yeasts from wild plants such as *P. norvegensis and S. cervesiae* in combination with *X. autotrophicus* are an alternative for the rapid and effective induction of seed germination and healthy growth of *P. vulgaris* at reduced doses of $NH_4NO_3$ to 70%, which facilitates the maximum uptake of nitrogen fertilizer, in turn allows a decrease in the generation of greenhouse gases, avoids the loss of soil productivity, being a useful tool for sustainable agriculture, that facilitates the maximum uptake of $NH_4NO_3$, in turn allows a decrease in the generation of greenhouse gases, avoids the loss of soil productivity, being a useful tool for sustainable agriculture. When $NH_4NO_3$ is reduced to 70%, which facilitates the maximum uptake of nitrogen fertilizer, in turn allows a decrease in the generation of greenhouse gases, avoids the loss of soil productivity, being a useful tool for sustainable agriculture.

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## Conflicts of interest

The authors declare no conflicts of interest.

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