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Impact of double inoculation with arbuscular mycorrhiza and native rhizobia on *Phaseolus vulgaris* L. and *Triticum durum*

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Abstract

The aim of this study was to analyse the effect of double inoculation with mycorrhizal and rhizobial symbioses on the growth of *Phaseolus vulgaris* L., var. el jadida and *Triticum durum* in monoculture and association. The experiment consists of sowing the plants on sterile soil: non-inoculated, inoculated with endomycorrhizal fungi, inoculated with rhizobia, inoculated with both symbionts. After 45 and 70 days, the morphometric parameters height, diameter, aerial and root biomass and number of leaves as well as the mycorrhizal and rhizobial colonisation were quantified. Inoculated bean and wheat showed highly significant growth differences. The total yield of the mycorrhizal bean plants was 1.76 times higher than that of the non-inoculated plants when the two species were combined. Double inoculation optimised endomycorrhizal colonisation of the roots of both species. The frequency of colonisation reached 86.67% and 93.33% and the arbuscular intensity reached 70.57% and 8.21% in bean and wheat, respectively, when double inoculated and when associated at the final stage. The double inoculation with rhizobia and mycorrhizae has a positive effect. It increases the growth and yield of beans and wheat, especially when they are grown together.

Key words: Endomycorrhiza, Rhizobia, inoculation, growth, bean, wheat.

Introduction

It is well known that the use of chemical fertilizers often leads to nutrient imbalance, reduction in the quality of agricultural products, soil degradation and thus

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destruction of the ecological environment (Ma et al., 2021). Plant Growth Promoting Bacteria (PGPB) and Arbuscular mycorrhizal (AMF) symbiosis are sustainable agronomic practices that promote plant growth and yield like chemical fertilizers without compromising product quality (Cruz et al., 2020). This symbiosis reduces the consumption of chemical nitrogen fertilizers, which are major challenges in agriculture today (Corre-Hellou et al., 2013).

Arbuscular mycorrhizal symbiosis is the most widespread and ancient terrestrial symbiosis (Fitter, 2005). It is a species-specific interaction (Emmanuel and Babalola, 2020; Nacoon et al., 2021). The efficiency of mycorrhizal root systems is mainly due to an extension of the absorbent surface and the volume of soil explored by fungal hyphae. Indeed, Plenchette et al. (1982) showed that the zone of mineral depletion by root hairs is limited to a few millimetres around the roots, while the zone of exploration by mycorrhizae can extend up to 10 cm. It is involved in the uptake of low-mobility nutrients (Gianinazzi-Pearson, 1996) with growth-promoting and sustainability effects. Furthermore, Wu et al. (2022) also recorded a bioprotective effect of mycorrhizal fungal colonization in sorghum-sudan and common bean. Bimmoy and Valdez (2024) have also confirmed the positive effect of inoculation of Betel nut plants which showed better growth despite the water stress to which they were subjected compared to non-inoculated plants.

The bacteria symbiosis is implicated on nitrogen fixation (Pawlowski and Bisseling, 1996), while Rhizobial bacteria live symbiotically within the root nodules of leguminous plants and fix atmospheric nitrogen in a form that can be used by plants (Brewin, 2004). In addition, legume plants can establish at the same time a symbiotic association with the AMF of the order *Glomales* (Duhoux *et al*., 2001).

Willey (1979) reported that crop associations such as cereals and legumes are a way to better use environmental resources compared to the corresponding "pure" or monospecific crops by reducing the use of chemical fertilizers. It would improve the protein content of cereal grains (Hauggaard-Nielsen et al., 2001), reduce the pressure of bio-aggressors (diseases, weeds and pests) compared to pure crops (Hauggaard-Nielsen et al., 2001). Indeed, Latati et al. (2013) confirmed that the facilitation of nutrient resources to cereals in association with legumes is ensured via the symbiotic fixation of atmospheric nitrogen by legumes.

The objective of this study was to compare the effects of single and co-inoculation with *Rhizobia* and AMF on the growth of *el jadida* variety of bean (*Phaseolus vulgaris* L.) and durum wheat (*Triticum durum*) grown alone and in combination culture.

Material and methods

The local bean and durum wheat were provided by the agricultural cooperative of Naciria (Boumerdes, Algeria). The experiment was carried out in 2022 in a plastic greenhouse with an average temperature of 25°C in the region of Naciria. The soil used came from a fallow land in the Naciria region. Samples were taken from a depth of 0-30 cm and air-dried, then sieved with a 2 mm diameter sieve before being placed in pots. Cultivation was carried out in plastic pots with a capacity of 5 liters, filled with 4kg of soil. They were irrigated at a rate of 250 ml to maintain the pots at their field capacity. The soil analyses were performed out for pH, electrical conductivity (EC), cation exchange capacity (CEC), particle size, total limestone, organic carbon, organic matter (OM), assimilable and total phosphorus (Joret-Hebert, 1955), assimilable and total Kjeldahl nitrogen (Bremner, 1960).

1-Inoculum

1-1- Rhizobium inoculum

The indigenous Rhizobium strain used was isolated from the nodules of *Phaseolus vulgaris* L. roots grown on soil collected in Naciria, then purified and grown in the laboratory. The Rhizobium strain was selected on Yeast Extract Mannitol (YEM) agar medium, and multiplied on liquid YEM medium using the method of Somasegaran andHoben, (1985). The liquid rhizobial inoculum is poured in the vicinity of the roots of each seedling at a rate of 5ml of bacteria containing 109 cells ml^{-1}. The isolated Rhizobia strain presented in colonies of isolates of 2 to 3 mm in diameter, circular, regular in outline, whitish in color with a viscous appearance, compact and opaque. The Bacteria had the shape of Gram-negative coco bacilli, with pink color and fast growing.

1-2-Endomycorrhizal inoculum

The production of endomycorrhizal inoculum was carried out on leeks (*Allium porrum* L.) grown in greenhouses in pots and inoculated with indigenous spore's witch were isolated in the same Naciria soil: 10 spores morphotypes of *Glomus*, 3 spores morphotypes of *Scutellospora* (3 species) and 2 spores morphotypes of *Acaulospora* genus. The mycorrhized roots were cut into 2 mm pieces used to inoculate bean and durum wheat seedlings. Each seedling received 10 g of endomycorrhizal root.

2-Experimental protocol

The experimental design was carried out in a randomized complete block design. Bean and wheat seeds were disinfected in a bleach solution (5%), rinsed and soaked in warm water overnight. Seeds floating in the liquid were removed. The healthy seeds were germinated in Petri dishes containing sterilized and moistened filter paper. The dishes were placed in the oven at 25°C. The young bean and wheat seedlings, having reached a height of about 3.5 cm, were taken and transplanted into pots at a rate of 6 bean and 12 wheat seedlings for the monoculture and 5 bean and 10 wheat seedlings for the combination.

The pots were placed in a plastic greenhouse, in 60 pots divided into 4 treatments (T) and 5 replications. Four blocks of pots were filled with sterile soil (twice for 1 hour at 120°C, with an interval of 48 hours between each sterilization) divided into: T1 corresponds to plants grown on sterile soil without inoculation (control): *P. vulgaris* grown alone (PT1); *T. durum* grown alone (TrT1); *P. vulgaris* and *T. durum* grown in association (PTrT1), T2 corresponds to plants raised on sterile soil and inoculated with AMF : *P. vulgaris* grown alone (PT2), *T. durum* grown alone (TrT2), *P. vulgaris* and *T. durum* grown in association (PTrT2); T3 corresponds to plants raised on sterile soil and inoculated with *Rhizobia sp*.: *P. vulgaris* grown alone (PT3), *T. durum* grown alone (TrT3); *P. vulgaris* and *T. durum* grown in

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association (PTrT3) and T4 corresponds to plants on sterile soil and inoculated with both *Rhizobia sp.* and AMF : *P. vulgaris* grown alone (PT4), *T. durum* grown alone (TrT4,*P. vulgaris* and *T. durum* grown in association (PTrT4).

3-Morphometric parameters

The plants were harvested in two periods of 45 and 70 days of cultivation on February and March, 2022. we measured the growth parameters height, diameter, fresh weight of stem and root and the weight of the dry matter of the aerial and root after their drying in the oven at 80°C for 48 hours**.**

4- Land Equivalent Ratio

According to Willey and Osiru (1972), the Land Equivalent Ratio (LER) was the area of land that would be required under pure crops to obtain the same yields as under combined crops. The LER was determined according to the formula used by Balde (2011).

$$LER = \frac{DW\ Bean\ IC}{DW\ Bean\ PC} + \frac{DWWheatIC}{DWWheatPC}$$

Where IC: intercrop; PC: pure crop and DW: dry weight; if: LER=1: there is no difference between the crops, LER<1: there is a decrease in productivity of the association, LER>1: a productive advantage of the association.

5- Relative mycorrhizal dependence index

Mycorrhizal dependence is the degree to which a plant is required by its mycorrhizal state to produce its maximum dry weight under given fertility conditions (Gerdemann, 1975). It was estimated here by the relative mycorrhizal dependence index (R.M.D. I) proposed by Plenchette *et al.* (1983) which we also adopted for plants inoculated with *Rhizobia* and AMF (R.M.Rz.D.I):

$$RMDI = 100\frac{(DWIn - DWNIn)}{DWIn}$$

With: DWIn = dry weight of inoculated plants and DW.NIn= dry weight of non-inoculated plants. The index varies between 0 and 100% ; with 100%: if the plant cannot grow without mycorrhizae and 0% when mycorrhization does not influence growth.

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6- Root staining

The roots of the plants were cleaned of soil particles by rinsing gently and abundantly with running water. The roots are thinned with 10% potash and heated in a water bath for 30 minutes (this time can be optimized, sometimes 10-15 minutes are sufficient if the roots are fragile). The KOH has the role of emptying the cellular contents. The roots were then rinsed thoroughly with tap water and neutralized with lactic acid for 5 minutes. After rinsing with water, the roots were stained with Trypan blue with heating on a water bath for 15 min. The roots have been rinsed and preserved in lactoglycerol (Phillips and Hayman, 1970). The roots were observed under a Zeiss light microscope at magnifications of 100 and 400.

6-1- Evaluation of rhizobial colonization parameters

The rhizobial infection rate of *Phaseolus vulgaris* L. seedling roots was estimated by direct counting of the number of nodules on a 30 cm root length of each plant.

6-2- Assessment of endomycorrhizal colonization

A length of 30 cm of roots from each plant was taken at random to estimate the endomycorrhizal colonization. We adopted the scoring system of the Trouvelot *et al.*, (1986) method calculates five infection parameters:

Colonization frequency (F), % of the number of endomycorrhized root fragments.

$$F\% = \left(\frac{Number\ of\ Mycorrhized\ fragements}{total\ Number\ of\ fragments}\right) * 100$$

Intensity of mycorrhization of the cortex. It reflects the importance of the colonization of the root system proportion of the colonized cortex estimated and expressed in %: $M\% = (95n5 + 70n4 + 30n3 + 5n2 + n1)$

With n5 =number of fragments rated 5, n4=number of fragments rated 4, etc.;

Intensity of colonization of the endomycorrhizal part of the root system (proportion of colonized cortex, expressed in %):

$$m\% = M\%\left(\frac{total\ number\ of\ fragments}{number\ Mycorrhized\ of\ Fragments}\right)$$

Arbuscular content in the mycorrhized parts of the root fragments (a %):

$$a\% = (100mA3 + 50mA2 + 10mA1)/100$$

With: $A(\%) = \left(\frac{95n5An+70n4An+30n3An+5n2An+N1An}{NbrMycorrhizedfragments}\right) * \frac{100}{m\%}$

Arbuscular content of the infection related to the whole root system (proportion of root cortex containing arbuscules, expressed in %) :

$$A\% = a\ \left(\ \frac{M\%}{100}\ \right)$$

7- Statistical analysis.

Growth measurement data were subjected to one-way analysis of variance (ANOVA) using R 3.6.1 software.

Results

1-Characterisation of the soil.

The analysis of the soil used revealed a fine clay-silt texture. The exchangeable bases Na+, Ca++ and K+ ($Cmol^{+}kg^{-1}$soil) inducing an average Cation Exchange Capacity of 20.53 $Cmol^{+}kg^{-1}$ suggesting an average storage capacity. The soil is non-calcareous and has a low alkaline pH. The concentration of organic matter and the C/N ratio 59,24 > 25 indicate a good capacity of mineralization, productivity and a More type humus. According to LCA (2008), the soil is classified as moderately rich and rich in total and assimilable phosphorus respectively. The total and assimilable nitrogen concentration indicates a poor soil in nitrogen according to Calvet and Villemin (1986).

Table 1: Results of chemical analysis of Naciria soil.

Parameters	TLi (%)	CES (cm^{-1})	C (%)	MO (%)	P_{ass} (ppm)	P_T (ppm)	NT (%)	N_{ass} (ppm)	C/N
Value	0.52	0.31	3.14	5.4	10.5	23.5	0.053	7.84	59.24

T Li.: Total Limestone; P_{ass}: Assimilable Phosphorus; $P_{T:}$ Total phosphorus, NT: Total Nitrogen; N_{ass}: Assimilable Nitrogen.

2- Effect of symbiosis on biomass production.

2-1-Biomass production of *Phaseolus vulgaris* L

The results obtained from the experiment and the analysis of variance showed that the *el jadida* bean variety had maximum growth and development for all the variables measured on natural soil and cultivated in association with wheat, with the exception of plants produced on sterile soil, suggesting a competition effect between the two species in the absence of symbiotic partners. Moreover, the behavior of bean biomass production recorded in plants grown on soil inoculated with rhizobium and AMF revealed the highest values compared to those produced respectively on soil inoculated with rhizobium followed by those of the soil inoculated treatment with AMF and finally on sterile soil. In the case of plants grown on sterile soil, we found the lowest growth in plants grown in monoculture with an average ratio of 1.52 and 2.66 times less for the diameter and height variables than those grown on natural soil. It is noted that according to the treatments, the bean plants raised in association with wheat recorded better production results than those raised in monoculture (Table 2). At the end of the 70 days' culture, the results obtained according to the treatments showed a similar pattern to that of the plants produced after 45 days of development. In fact, the highest production figures were recorded for plants produced on natural soil, followed respectively by those inoculated with rhizobium, those inoculated with AMF and those on sterile soil. We recorded a 56.6% increase in aboveground biomass growth in T4 plants compared to T1 controls plants (Table 3).

Table 2: Growth of *Phaseolus vulgaris* (P) after 45 days according to the treatments

Treatments	H (cm)	D (mm)	L Nbr	Nd Nbr	ABM (g)	RBM (g)	ADM (g)	RDM (g)
PTrT1	4.68 ±0.3a	3 ±0.2a	5 ±0.6a	//	1 ±0.01a	1 ±0.03ab	0.09 ±0.01 a	0.09 ±0.01 a
PT1	4.6 ±0.3a	3.6 ±0.2b	5± 0.9a	//	1.22 ±0.04ab	1 ±0.05b	0.1a	0.09 ±0.01a
PTrT2	7.2 ±0.5c	4.3 ±0.1cd	6.8 ±0.8b	//	1.7 ±0.4cd	1.2 ±0.2bc	0.3 ±0.04e	0.2 ±0.01c
PT2	6.4 ±0.3b	4 ±0.1bc	6.72 ±0.7b	//	1.4 ±0.1bc	1.12 ±0.2 ab	0.15 b	0.12 ±0.01b
PTrT3	7.41 ±0.1cd	4.5 ±0.2 de	6 ±0.9b	29 ±3.2b	1.87 ±0.2d	1.48 ±0.2c	0.2 ±0.02c	0.2 ±0.01c
PT3	6.75 ±0.2bc	4.1 ±0.2cd	7 ±1bc	22 ±1a	1.35 ±0.2b	1.3 ±0.2ac	0.17 ±0.04bc	0.2 ±0.03c
PTrT4	9.9 ±0.7e	5 ±0.5f	7.8 ±0.9cd	35 ±2.2c	2.5 ±0.1f	2.2 ±0.2e	0.27 ±0.02 de	0.28 ±0.03de
PT4	8 ±0.78d	4.5 ±0.2de	7 ±0.7bc	30 ±4.2b	2 ±0.1de	1.5 ±0.1c	0.26 ±0.04d	0.26 ±0.01d

P: Phaseolus vulgaris, Tr: *Triticum durum*, T1: sterile soil, T2: inoculated with AMF, T3: inoculated with Rhizobium, T4: double inoculation soil, H: height, D: diameter; Nd Nbr: nodule number, ABM: areal biomass; RBM: root Biomass; ADM: areal dry matter; RDM: root dry matter

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Table 3: Growth of *Phaseolus vulgaris* (P) after 70 days according to the treatments

Treatments	H(cm)	D(mm)	L Nbr	NdNbr	ABM (g)	RBM (g)	ADM (g)	RDM (g)
PTrT1	5.20 ±0.48a	0.36 ±0.02ab	5.6 ±0.5a	//	1.5 ±0.2 a	1.50 ±0.19a	0.18 ±0.01a	0.09 ±0.01a
PT1	6 ±0.76a	0.39 ±0.01b	6 ±0.7a	//	1.5 ±0.2a	2 ±0.34b	0.19 ±0.01a	0.14 ±0.01b
PTrT2	9.69 ±0.27bc	0.40 ±0.02b	9 ±0.8 bc	//	2.83 ±0.1b	2.67 ±0.03c	0.46 ±0.01d	0.35 ±0.01f
PT2	9.2 ±0.33b	0.34 ±0.01a	8.75 ±0.62b	//	3 ± 0.1b	2 ± 0.04b	0.26 ±0.01b	0.25 ±0.01c
PTrT3	10.67 ±1.2cd	0.47 ±0.03cd	10 ±1.05c	46 ±4.05b	4.58 ±0.2d	3.4 ±0.03d	0.67 ±0.03f	0.40 ±0.03g
PT3	10 ±0.94bd	0.44 ±0.04c	9 ± 0.85bc	40 ±2.5a	4 ± 0.3c	2.5 ±0.04c	0.42 ±0.03c	0.28 ±0.03cd
PTrT4	12 ± 0.41ef	0.54 ±0.02f	12 ±0.94de	65 ±4.96d	7.4 f	4.66 ±0.06f	0.75 ±0.02g	0.46 ±0.02h
PT4	11 ±0.44de	0.5 ±0.02de	11.5 ±0.67d	50 ±2.79b	4.58 ±0.1c	4.25 ±0.03e	0.60 ±0.02e	0.3 ±0.03de

P: Phaseolus vulgaris, Tr: Triticum durum, T1: sterile soil, T2: inoculated with AMF, T3: inoculated with Rhizobium, T4: double inoculation soil, H: height, D: diameter; Nd Nbr: nodule number, ABM: areal biomass; RBM: root Biomass; ADM: areal dry matter; RDM: root dry matter

2-3- Biomass production of *Triticum durum*

In greenhouse trials conducted after 45 and 70 days, inoculated wheat plants showed a better development and biomass production for the plants with symbiosis. The different growth parameters were strongly impacted by the presence of symbiotic partners in the soil. Indeed, from the first stage, better plant growth results were obtained at T4, T3 and T2 respectively in the case of associated crops and at T4, T2 and T3 in the case of wheat crops alone in the presence of symbionts (Table 4).

After 70 days, the inoculated wheat plants also showed higher growth compared to the non-inoculated. In fact, the plants with dual inoculation and in combination with bean recorded average values of AB = 7.40 g and RB = 4.66 g, D= 0.85 cm and L Nbr = 12.5, much higher than those recorded in the non-inoculated plants which were respectively AB = 0.97 ± 0.07g, RB = 2.49 ± 0.04 g, D= 0.30 ± 0.01 cm, L Nbr = 1.52 ± 0.04 (Table 5)

Table 4: Growth of *Triticum durum* (Tr) growth after 45 days according to the treatments

Treatments	H (cm)	D (mm)	L Nbr	AFB(g)	RFB(g)	ADM(g)	RDM(g)
PTrT1	8 ±0.56b	2.5 ±0.1a	5 ±0.69a	1 ±0.044a	1 ±0.04b	0.11 ±0.01b	0.11 ±0.01a
TrT1	7 ±0.75 a	2.8 ±0.1b	6 ±0.86b	1 ±0.04a	0.85 ±0.2a	0.08 ±0.02a	0.15 ±0.02b
PTrT2	9.8 ±0.47d	0.5 ±0.3e	8.7 ±0.59cd	2.84 ±0.35c	1.5 ±0.24de	0.25 ±0.02d	0.4 ±0.0d
TrT2	8 ±0.39b	4.2 ±0.2c	9 ± 0.47d	2.84 ±0.35c	1.4 ±0.19d	0.16 ±0.03c	0.17 ±0.02b
PTrT3	8.5 ±0.48c	4.5 ±0.2d	8.36 ±0.49 c	2.5 ±0.51b	1.2 ±0.14c	0.17 ±0.01c	0.17 ±0.01b
PTrT4	12.6 ±0,7e	5.8 ±0.2f	9.7 ±1.29e	3.2 ±0.55d	1.7 ±0.15f	0.25 ±0.02d	0.33 ±0.02c

P: Phaseolus vulgaris, T1: sterile soil, T2: inoculated with AMF, T3: inoculated with Rhizobium, T4: double inoculation soil; H: height, D: diameter; L Nbr: leafnumber; ABM: areal biomass; RBM: root Biomass; ADM: areal dry matter; RDM: root dry matter

Table 5: Growth of wheat (*Triticum durum*) after 70 days of growth according to treatments

Treatments	H (cm)	D (mm)	L Nbr	AFB(g)	RFB(g)	ADM(g)	RDM(g)
PTrT1	30 ± 1.58b	2.9 ±0.1a	6.8 ± 0.41a	1.01 ± 0.07a	1.19 ± 0.04b	0.5 ±0.05a	0.2 ±0.06a
TrT1	27.29 ±1.36a	3 ±0.1a	6.25 ±0.44a	1.52 ± 0.04a	0.97 ± 0.07a	0.62 ±0.02b	0.23 ±0.01a
PTrT2	46.45 ±3.38d	5.4 ±0.4c	11.45 ±1.9d	5.14 ± 0.97b	2.43 ± 0.04e	1.37 ±0.02e	0.5 ±0.04d
TrT2	40 ±1.99c	6.5 ±0.2d	10 ± 0.78c	5.10 ± 0.32b	1.47 ± 0.02c	1.18 ±0.02c	0.4 ±0.01c
PTrT3	47.55 ±1.44d	5 ±0.2 b	9 ± 0.64b	5 ± 0.35b	1.93 ± 0.03d	1.23 ±0.06d	0.30 ±0.04b
PTrT4	54.72 ±2.78f	8.5 ± 0.3f	12.5 ±1.35e	7.40 ±0.84e	4.66 ± 0.28h	1.50 ±0.02f	0.53 ±0.04d

P: *Phaseolus vulgaris*, T1: sterile soil, T2: inoculated with AMF, T3: inoculated with Rhizobium, T4: double inoculation; H: height, D: diameter; L Nbr : leaf number ; Nd Nbr: nodule number, ABM: areal biomass; RBM: root Biomass; ADM: areal dry matter; RDM: root dry matter.

2-4-Association effect on biomass production.

In order to highlight the contribution of symbiosis on the biomass production of wheat in association with bean, we calculated the Land Equivalent Ratio (LER). This allowed us to quantify the advantage of the association crop and the contribution of rhizobial symbiosis compared to the monoculture without *Rhizobia* (TrT2). The mixed crops showed a productivity higher than 1 and at least twice that

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of the pure crop for T2 affirming the productive advantage of the bean-wheat association (Table 6).

Table 6: Land Equivalent Ratio (LER) of bean-wheat combinations

Period	45 Days	70 Days
LER T1	1.95	1.46
LER T2	3.62	2.58

T1: sterile soil, T2: inoculated with AMF

3-RootSymbiosis Colonization.

3-1- Endomycorrhizal colonization.

For the parameter mycorrhizal intensity (M%), the rates varied from 15.77% to 0.43% for the double inoculation at 45 days and from 31.03% to 0.4% for the mycorrhizal inoculation alone. Mycorrhizal intensity was higher in both the root system (M%) and in the root fragments (m%). The abundance of arbuscules was very high, both in the root system (A%) and in the mycorrhized parts of the root fragments (a%). They were higher in the plants produced on soil with double inoculation for both stages studied. Indeed, the colonization frequency (F%) for beans varies from 26.67% to 70% for seedlings inoculated with AMF, from 76.67 to 30% for those inoculated with both *AMF* and *Rhizobia* after 70 days of cultivation (Table7).

Table 7: Frequency, mycorrhization intensity and arbuscular content of bean (P) and Wheat (Tr) plants at 45 and 70 days according to treatments

Period	45 Days					70 Days				
Treatment	F (%)	M%	m%	a%	A%	F (%)	M%	m%	a%	A%
PTrT2	43.33	0.7	1.62	24.69	0.17	80	5.95	7.44	0.63	0.04
PT2	26.67	0.4	0.44	37.88	0.15	53.33	4.03	7.56	78.62	3.17
PTrT4	43.33	2.1	4.85	24.27	0.51	86.67	8.9	10.27	35.69	9.18
PT4	30	0.43	1.43	40.62	0.18	56.66	4.2	7.41	12.7	0.53
Tr/PTrT2	70	20.17	28.81	79.55	16.05	90	14.11	15.67	26.23	3.7
TrT2	53.33	37.03	69.43	69.39	25.7	83.33	1.77	2.12	32.85	0.58
Tr/PTrT4	76.67	15.77	20.57	86.1	13.58	93.33	15.27	16.36	58.21	8.89

PTr :*Phaseolus* and *Triticum* association, T2: inoculated with AMF, T4: dual inoculation

The associated crops recorded a higher endomycorrhizal colonization than the monocultures in all treatments and for both species. It is also interesting to note that the highest mycorrhization factors M%, m%, A% and a% were quantified in the plants produced on natural soil.

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3-2- Relative symbiosis dependency index.

In order to express the symbiotic association contribution, we calculated the relative dependence mycorrhizal index (RMDI) and VAM and rhizobium with relative dependence mycorrhizal VAMs and rhizobium index (R.M.Rz.D.I) in stimulating bean plant growth (formula adapted), mycorrhizal and symbiotic dependency index. The RMDI varied between 29.62% and 35.29% depending on the treatment. The highest R.M.Rz.D.I of the bean plants was recorded in the plants in the double symbiosis treatment (T4) evoking a dependence of more than 60% on the double symbiosis and relatively higher than the mycorrhizal dependence (Table 8).

The calculation of the mycorrhizal dependence showed a difference between treatments and periods (Table 8). Indeed, the Wheat tested showed the lowest dependence on arbuscular mycorrhization recorded for plants produced on inoculated soil for both periods from 30% to 46%. However, the R.M.Rz.D.I. of non-sterile Wheat soil showed the highest values with 75,32% after 45days and 67%s after 70 days suggesting a higher efficiency of symbiosis in the presence of the full symbiotic flora of the soil.

Table 8: Relative mycorrhizal dependency index (R.M.D.I) and mycorrhizal and Rhizobial dependency index (R.M.Rz.D.I) of Bean and Wheat

Treatment	45 Days	70 Days
R.M.D.I PT2 (%)	29.62	35.29
R.M.Rz.D.I PT4 (%)	63.46	63.33
R.M.D.I TrT2 (%)	30.30	46.20

Tr : Triticum durum, P : Phaseolus vulgaris,
T2: inoculated with AMF, T4: dual inoculation

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Discussion

1- Effect of symbiosis on bean growth

The results obtained in this study on positive effect of the dual *rhizobium* and *AMF* inoculation on growth of bean corroborate those reported respectively by Wang *et al*., (2011) in *Vicia faba* and *Glycine max* L. Indeed, the work Wang et al., (2011) specified that that co-inoculation with *Rhizobia* and AMF on soil poor in phosphorus and/or nitrogen significantly improved aboveground biomass growth in soybeans. Mycorrhizal inoculation alone and rhizobium alone also increased plant height by 1.5-fold and biomass production by 2-fold, compared to the sterile soil treatment. The quantification of the nodulation on the root system made it possible to note that the intensification of the nodulation was a function of the treatment and of the association. Indeed, bean plants subjected to the same treatment and grown in combination recorded more nodules than those produced alone.

We also noted that for the endomycorrhizal inoculation treatment, the plants recorded an improvement in the total biomass production of bean and wheat with a rate of 70%. Similar results were reported in cowpea by Haro et al. (2012) and Diop et al. (2013) in inoculated bean. Recent studies (Emmanuel and Babalola, 2020; Nacoon et al., 2021; Ishola et al., 2022) showed that inoculation of AMF fungi significantly increased the biomass of Sunchoke and cowpea.

Nodulation of common bean roots observed a similar pattern to the measured growth variables. It is, however, interesting to note that mycorrhizal inoculation of *Phaseolus vulgaris* plants resulted in a significant increase in nodulation compared to plants without mycorrhizal inoculation. The experiments of Tajini et al. (2012) also demonstrated that the combination of AMF and Rhizobium induced a significant increase in nodulation in common bean (*Phaseolus vulgaris* L.) and *Cicer arietinum* L., respectively. Our results concur with those reported by Iroume et al. (2008) who indicated that when bean plants are inoculated or fertilized, this species produces more dry matter. Endomycorrhizal inoculation increases the dry matter at mid-cycle (52 days after sowing) by 8 to 41% (Iroume et al., 2008). Haro

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(2020) reported an improvement in above-ground biomass production by 56.79%, root biomass by 70% and total biomass by 61.16% of Mucuna inoculated with AMF compared to uninoculated plants.

Makaure et al. (2022) reported that *Vignia. unguiculata* establishes symbiotic interactions with soil bacteria and naturally occurring endomycorrhizal fungi and has the ability to change its association preferences according to its needs, which gives it the ability to adapt to nutrient-poor ecosystems. The AMF and *Rhizobia* strains promote growth and provide the plant with the ability to alter biomass production and distribution during vegetative growth of alfalfa (Baslam et al., 2014). This appears to be the case for the bean.

The LER calculation showed that the association system significantly increases the total yield of both plants. The bean-wheat association achieved higher productivity than monocultures. The Land Equivalent Ratio averaged 2.53 and showed a 153% increase in productivity in the combination compared to separate production of the two species. Similar results have been reported by several authors (Ram and Meena, 2015; Trail et al., 2016) regarding the contribution of millet in association with cowpea in Senegal with an LER ranging from 1.68 and 1.77 in millet in association with mung bean. More recently, Elie et al. (2021) found that in associated maize, higher productivity was recorded compared to maize grown alone. Hussain et al. (2018) also reported that maize associated with the variety inoculated with Rhizobium bacteria had a significantly higher LER =1.68 than the other modalities.

2- Effect of symbiosis on Wheat growth

The absence of soil microflora induced a decrease in production of at least 25% for Bean and Wheat. Mycorrhizal and rhizobium inoculation alone resulted in a 1.5 fold increase in plant height and a 2-fold increase in biomass production. Monitoring of growth and development parameters showed a significant difference in favour of plants inoculated with either AMF or Rhizobium as well as with the combination of both symbionts. Similarly, the yield parameters showed a significant increase in all growth parameters. Our results agree with those of Pérez et al., (2016) who showed that mycorrhizal inoculation has a positive impact on yield in Wheat. In addition, Hemissi et al., (2019) also reported the effectiveness of mycorrhizal and rhizobial biofertilizers in improving yield and productivity in durum wheat.

Leaf number in Wheat inoculated with AMFincreased by 8-10 leaves between 45 and 70 days. Indeed, Khirani and Ould El Hadj-Khelil (2019), reported that a leaf number that varied between 10 and 12 leaves between 45 and 60 days, respectively. Similar results were reported by Ndonda (2018) who showed that VAM also have a positive effect on leaf quantity and quality especially during austere periods of low rainfall in cassava. Shola et al. (2022) reported that AMF improves cowpea production by increasing nutrient uptake and had a positive effect on cowpea growth, including height, number of leaves, fresh and dry weight. The absence of soil microflora induced a decrease in production of at least 25% for Bean and Wheat. Mycorrhizal and rhizobium inoculation alone resulted in a 1.5 fold increase in plant height and a 2-fold increase in biomass production

Taibi et al. (2016) reported, also, the presence of similar mycorrhizal structures in Durum Wheat and Barley, with total mycorrhization rates of 80.5% and 90.5% respectively while the rate of presence of arbuscules and vesicles is 36% and 45% in Wheat and 36% and 55% in Barley respectively in favor of vesicular fungal reserves.

3- Effect of symbiosis on bean and Wheat growth

Our study showed that the introduction of beans in association with durum wheat resulted in improved growth and development of the latter through a significant increase in root and areal biomass. Our results are in agreement with those of Latati et al, (2013) who reported significant biomass stimulation of barley when associated with cowpea. Shahbazi et al, (2012) who observed an increase in maize stem diameter when associated with cowpea and faba bean. The same incidence was also observed by the associated crop as compared to the monoculture of soybean especially under water shortage and poor soil conditions (Igiehon et al. 2020 and Igiehon and Babalola, 2021). Liu et al, (2020) explained that the mechanism of microbial inoculation to promote plant growth is an important way to promote nutrient uptake (N, P) in plants. Xavier andGermida (2003) report that in pea, double

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inoculation with Rhizobia species and compatible AMF intensely improve growth and nutrient uptake. They explained this by the universal consensus that mycorrhizal fungi enhance phosphorus nutrient uptake in legumes, which ultimately improves plant development and nitrogen fixation (Igiehon et al. 2020).

The estimation of endomycorrhizal colonization showed a variation in mycorrhization intensity between monoculture and associated crop. The estimation of endomycorrhizal colonization showed a variation in mycorrhization intensity between monoculture and associated crop. This was significantly higher in the plants grown in association and on natural soil followed by the double inoculated plants after 70 days. The highest frequency of 90% was observed at 70 days in seedlings inoculated with AMF grown in association and reached 93.33% for those inoculated with the double inoculation in association.

The abundance of arbuscules also reflected very high rates, both in the root system (A%) and in the mycorrhized parts of the root fragments (a%) for the same treatments. This was significantly higher in the plants grown in association and on natural soil followed by the double inoculated plants after 70 days. Our results are similar to those of Taibi et al. (2016) who also reported the presence of similar mycorrhizal structures in durum wheat and barley, with total mycorrhization rates of 80.5% and 90.5% respectively, while the rate of arbuscular presence is 36% in wheat and 36% in barley. They also reported the abundance of vesicular fungal reserves in both species as observed in our study.

However, the R.M.Rz.D.I. of non-sterile Wheat soil showed the highest values with 67% after 70 days suggesting a higher efficiency of symbiosis in the presence of the full symbiotic flora of the soil. Indeed, Ishola et al. (2022) reported that in the unsterilized soils a positive relationship has been established between the growth performance of cowpea with the mycorrhizal promotion of plant growth and enables plants to actively regulate the rhizosphere microbial communities.

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Conclusion

This study has highlighted the effect of mycorrhizal and rhizobial inoculation, both alone and in association, on the productivity of durum wheat and faba bean. A significant improvement was observed in the growth and production variables of both species, especially in the cropping systems in which they were associated. A higher productivity was also observed in the bean-wheat association than in the monocultures.

The symbionts and their colonisation seem to have evolved simultaneously and in a synergistic manner in both species. The dependence of the bean and the wheat on arbuscular mycorrhizae and rhizobia has been demonstrated. The association of cereals and legumes with inoculation is an alternative to improve the agricultural productivity of degraded soils. However, the best asset for tomorrow's agriculture is still a soil with its natural microflora intact.

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