Improved grain yield of cowpea (*Vigna unguiculata*) under water deficit after inoculation with *Bradyrhizobium elkanii* and *Rhizophagus irregularis*

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Abstract. Cowpea (*Vigna unguiculata* (L.) Walp.), a plant broadly cultivated for human consumption and animal feed, is among the most nutritious grain legumes. Most of the areas where cowpea is grown are drought-prone, and there is a need to address this issue, with water scarcity becoming a major concern in agriculture. Cowpea is known to form mutualistic associations with nitrogen-fixing (NF) bacteria and arbuscular mycorrhizal (AM) fungi. These beneficial soil microorganisms have the capacity to benefit plants by reducing the effects of environmental stresses, including drought. Our aim was to study the effect of inoculation with *Bradyrhizobium elkanii* and *Rhizophagus irregularis* on the growth and grain yield of cowpea under water-deficit conditions. Under moderate water deficit, grain yield was increased by 63%, 55% and 84% in plants inoculated with *B. elkanii*, *R. irregularis* and *B. elkanii* + *R. irregularis*, respectively. Under severe water deficit, inoculation with *B. elkanii* and *B. elkanii* + *R. irregularis* resulted in grain-yield enhancement of 45% and 42%, respectively. The use of cowpea inoculated with NF bacteria and AM fungi has great potential for sustainable agricultural production under drought conditions.

Additional keywords: plant-microbe interactions, pulses, rhizobia, sustainable agriculture, tripartite symbiosis, water stress.

Introduction

Cowpea (*Vigna unguiculata* (L.) Walp.) is highly versatile and one of the most important grain legume crops. It is widely cultivated, especially in the semi-arid tropical regions, where it provides a cheap source of rich vegetable protein for human consumption (Ehlers and Hall 1997; Timko and Singh 2008). Valued as food, forage and a green manure crop in many parts of the world, cowpea contributes to the sustainability of cropping systems and improves soil fertility through nitrogen (N) fixation. Cowpea can grow successfully with few economic inputs in low-fertility and marginal soils, and it can be used to increase the yields of cereal crops when grown in rotation (Fery 2002; Bell *et al*. 2017). In addition to being drought-tolerant, cowpea has high yield potential. Worldwide, the cultivated area of cowpea is estimated to have increased from ~2.4 Mha in 1961 to >12.5 Mha in 2014, with an economic value of US$1.5 billion (FAOSTAT 2017). Although exact statistics are not available, Singh *et al*. (2002) estimated an annual global production of

cowpea of 4.5 Mt. The region encompassing West and Central Africa contributes ~70% of world cowpea production. Other major production areas are Asia, Central and South America, and southern and south-eastern Europe (Singh *et al*. 2002). Farmers from Africa and Asia not only grow cowpea for dry seed, but also utilise the leaves and fruits for human consumption and animal feed. In high-income countries, cowpea is becoming regarded as an alternative to soybean as consumers seek more traditional foods with health benefits (Rangel *et al*. 2004; Timko and Singh 2008). Cowpea seeds are rich in proteins and carbohydrates and contain substantial amounts of lysine, tryptophan, minerals and vitamins (folic acid and vitamin B) (Nielsen *et al*. 1993; Timko and Singh 2008).

Most areas where cowpea is grown are semi-arid and often experience severe to moderate droughts (Hall 2012). Global water scarcity is increasing pressure to produce more food with less water; therefore, drought-tolerant crops such as cowpea are of great interest for sustainable agriculture. Its long taproot and resistance mechanisms such as closing the stomata when soil-water supply is insufficient and turning the leaves upwards to protect them from excessive temperatures give cowpea a unique ability to survive extreme droughts that would kill most other crop plants (Schakel and Hall 1979; Hall 2012; Halilou *et al*. 2015). For example, under irrigated and optimal conditions, production was up to 4000 kg ha^{-1} in Senegal (Hall 2012). The growth performance of cowpea can be significantly improved through associations with nitrogen-fixing (NF) rhizobia (Figueiredo *et al*. 1998, 1999). *Bradyrhizobium elkanii*, *B. yuanmingense* and *B. japonicum* are among the main rhizobial species associated with cowpea (Zhang *et al*. 2008). However, drought can reduce significantly the biological N_2 fixation by associated rhizobia and cause plants to rely more on available inorganic N (Elowad and Hall 1987).

Like most land plants, cowpea also associates with arbuscular mycorrhizal (AM) fungi. Work on the tripartite symbiosis of legume–rhizobia–AM fungi has showed that AM fungi can improve nodulation and biological N_2 fixation within the nodule (Wilson *et al*. 2012; Ossler *et al*. 2015). Enhanced performance of legumes by AM fungal symbiosis is the result of promotion of key processes benefiting plant vigour, biomass production and fitness (Chalk *et al*. 2006). AM fungi can benefit plants by reducing the effects of environmental stresses (Oliveira *et al*. 2005*a*, 2010). They have the capacity to improve uptake of nutrients (Oliveira *et al*. 2006, 2016*a*, 2016*b*) and to increase drought tolerance (Augé *et al*. 2015), which may contribute to improved crop yield under adverse environmental conditions.

We aimed at assessing the effects of single and dual inoculation with an NF bacterium and an AM fungus on the biomass, leaf chlorophyll concentration, crude protein content and grain yield of cowpea under water-deficit conditions.

Materials and methods

Biological material and soil

The bacterial isolate used in this work, *Bradyrhizobium elkanii* FF24-2, was isolated from a surface-sterilised nodule present in the roots of a cowpea plant in a field in Vila Real, Portugal. This bacterial strain was chosen for its fast-growing capacity and its ability to nodulate cowpea successfully (G. Marques, pers. comm.). The bacterium was isolated in yeast mannitol agar (YMA) medium containing (g L^{-1}): yeast extract, 1; mannitol, 10; K_2HPO_4, 0.5; $MgSO_4.7H_2O$, 0.2; NaCl, 0.1; agar, 15. First, it was streaked in YMA medium supplemented with 0.025 g L^{-1} of Congo red. A single colony was picked and streaked in the same medium supplemented with 0.1 g L^{-1} of bromothymol blue (BTB) (Somasegaran and Hoben 1985). The isolate was purified by repeated streaking in YMA medium supplemented with BTB, and then, for molecular identification, the DNA was extracted manually according to Sikora *et al*. (2002). The nearly full-length 16S rDNA gene (Weisburg *et al*. 1991) was amplified, and in order to identify the bacterium at the species level, this analysis was complemented with *nodC* (Laguerre *et al*. 2001) genes. Based on the alignment of the obtained sequences of 16S rDNA and partial *nodC* gene, a 100% pair-wise identity was observed with *B. elkanii*. The GenBank accession numbers of the publically available sequences showing the best match with the obtained sequences for 16S rDNA and partial *nodC* gene were KX396582 and FJ418725, respectively. The bacterial isolate *B. elkanii* FF24-2 was grown in yeast mannitol broth (YMB) (Sigma-Aldrich, St. Louis, MO, USA) for 3 days at 28°C and 0.5*g*. The culture was then centrifuged at 4930*g* for 10 min and the pellet was washed with saline solution (0.85% NaCl). The pellet was resuspended in saline solution and the colony forming unit adjusted to 10^9 mL^{-1}.

The AM fungal isolate *Rhizophagus irregularis* BEG140 was grown for 8 months in a multi-spore pot culture containing a 1 : 1 (v/v) mixture of zeolite and expanded clay with *Zea mays* L. as the host plant (Symbiom Ltd, Sázava, Czech Republic). This AM fungal isolate was chosen because of root-colonisation capacity shown in previous experiments (Oliveira *et al*. 2016*b*). The inoculum had 400 AM fungal propagules g^{-1}, estimated by the most probable number method (Porter 1979).

The seeds used in this study were from cowpea cv. Fradel obtained from the collection of the University of Trás-os-Montes e Alto Douro, Vila Real, Portugal. Soil was collected from the uppermost 10-cm layer of an organic farm in northern Portugal, sieved through a 4-mm mesh and autoclaved twice (121°C for 25 min) on consecutive days. This was a sandy soil with pH (1 : 2.5 w/v water) 6.5, electrical conductivity 0.1 dS m^{-1}, 1.2% organic matter, total N 3.8 g kg^{-1}, extractable (Egner-Riehm) phosphorus 48.8 mg kg^{-1}, potassium 4.3 g kg^{-1}, calcium 1.6 g kg^{-1}, magnesium 66 mg kg^{-1} and sodium 147 mg kg^{-1}.

Experimental treatments and setup

Experimental units (1-L pots filled with soil) were arranged in a fully randomised manner using a 2 × 2 × 3 factorial design. The first factor was bacterial inoculation (non-inoculated plants and plants inoculated with *B. elkanii*), the second factor was fungal inoculation (non-inoculated plants and plants inoculated with *R. irregularis*), and the third factor was water deficit (no water deficit, moderate water deficit and severe water deficit). Thus, for each water regime there were four treatments: non-inoculated plants, plants inoculated with *B. elkanii*, plants inoculated with *R. irregularis* and dually inoculated plants. Each treatment combination was replicated 10 times. Seeds of cowpea were surface-sterilised with 0.5% (v/v) sodium hypochlorite for 20 min, placed on moist paper towels and germinated at 20°C in the dark. After germination, seedlings of similar size were

transplanted, one plant into each pot. A nitrocellulose membrane filter (diameter 24 mm and pore size 0.4 µm) (Pragopore; Pragochema Ltd, Prague) was inserted vertically in each pot for future measurements of extraradical mycelium (ERM) length (Baláz and Vosátka 2001). At transplanting, each pot from the bacterial treatments received 4 mL bacterial suspension (described above). Every pot from the non-bacterial treatments received 4 mL autoclaved bacterial suspension. Each pot from the mycorrhizal treatments received 10 g inoculum consisting of colonised root fragments, hyphae and spores in the mixture of zeolite and expanded clay, placed 2 cm below the root system. Every pot from the non-mycorrhizal treatments received 10 g inoculum autoclaved twice (121°C for 25 min) on consecutive days. In order to eliminate differences in bacterial populations introduced with the AM fungal inoculum, a 5-mL filtrate of AM fungal inoculum was added to all pots from the non-mycorrhizal treatments (Koide and Li 1989). The filtrate was prepared as described in Oliveira *et al*. (2010). Field capacity of the soil in the pots was determined (Grewal *et al*. 1990), and during the first 4 weeks, soil moisture in all pots was kept at 75% of field capacity by weighing the pots every 2 days and watering accordingly with deionised water. Soil moisture was kept at 75%, 50% and 25% of field capacity by weighing the pots for the treatments with no water deficit, moderate water deficit and severe water deficit, respectively. Plants were grown in a greenhouse under natural light with an average photoperiod of 12 h. Temperature and relative humidity ranges were 12−42°C and 55–85%, respectively. Pots of different treatments were periodically rotated to different bench positions and re-randomised to minimise differences due to their location in the greenhouse.

Measurements and analyses

After a growth period of 3 months, grains were harvested and the number of grains per plant, fresh weight of grains per plant and fresh weight per grain were determined. Grain samples were dried at 80°C for 48 h and analysed for total Kjeldahl N following the methods of the Association of Official Analytical Chemists (2006). Crude protein was calculated as N × 6.25 (FAO 2003). A fresh subsample (0.02 g) was cut from the second mature leaf from the plant apex and the concentrations of chlorophyll a + b determined after extraction with N,N-dimethylformamide according to Wellburn (1994). Plants were removed from the pots, and the root system was separated from the shoot and gently washed to remove adhered soil. Number of root nodules was counted. A fresh subsample (0.2 g) of roots was collected to assess AM colonisation (described below). The remaining root system was weighed and dried at 80°C for 48 h together with the shoot. The dried root system and shoot were then re-weighed. The dry root mass of the subsample was calculated by multiplying its fresh mass by the dry : fresh mass ratio of the root system. The sum of the dry mass of the root subsample, the dry mass of the root system and the dry mass of the shoot gave the total dry weight per plant. The subsample of fresh roots was cut into 1-cm pieces and stained with trypan blue, using a modified (Phillips and Hayman 1970) protocol (Oliveira *et al*. 2005*b*). Percentage root-length colonised (RLC) by AM fungi was assessed for each plant species by using the grid-line intersect method (Giovannetti and Mosse 1980) under a stereomicroscope (Olympus SZ61; Olympus, Tokyo). The ERM length was determined by using the inserted membrane technique (Baláz and Vosátka 2001) followed by the grid-line intersect method under a compound microscope (Leica DM 750: Leica, Wetzlar, Germany), using an ocular grid at 200× magnification (Brundrett *et al*. 1994). Background lengths of mycelium found in non-mycorrhizal treatments were subtracted from the values obtained in the corresponding mycorrhizal treatments and the ERM length was expressed in cm hyphae per cm^2 inserted membrane filter.

Statistical analyses

Normality and homogeneity of variances were confirmed and data analysed by using three-way analysis of variance (ANOVA) for each dependent variable (plant parameters) vs. independent variables (bacterial inoculation, fungal inoculation and water deficit). When a significant *F*-value was obtained ($P < 0.05$), treatment means were compared by Duncan's multiple range test. Microbial parameter data were analysed by two-way ANOVA without including the respective non-inoculated control treatments. All statistical analyses were performed with the SPSS 23.0.0.0 software package (IBM, Armonk, NY, USA).

Results

Cowpea growth

Inoculation with *R. irregularis* significantly increased root, shoot and total plant dry weight of cowpea without water deficit (Fig. 1*a–c*). Overall, under moderate and severe water deficit there was no effect of microbial inoculation (either singly or dually) on plant biomass. However, leaf chlorophyll a + b concentration was significantly increased under moderate water deficit with bacterial inoculation and under severe after deficit with dual inoculation (Fig. 1*d*). Water deficit significantly influenced plant biomass and leaf chlorophyll a + b concentration, whereas inoculation with *B. elkanii* had a significant effect only on leaf chlorophyll a + b concentration (Table 1).

Microbial inoculation did not cause any significant reduction in root, shoot and total plant dry weight or in leaf chlorophyll a + b concentration compared with the respective non-inoculated controls, regardless of the water regime (Fig. 1).

Cowpea grain yield

Bacterial inoculation and water deficit significantly influenced all grain-yield-related parameters (number of grains per plant, fresh weight of grains per plant, fresh weight per grain), whereas fungal inoculation had no significant effect (Table 2).

Without water deficit, inoculation with *B. elkanii* + *R. irregularis* significantly increased the number of cowpea grains produced per plant (Fig. 2*a*). Under moderate water deficit, inoculation with *B. elkanii* alone and with *B. elkanii* + *R. irregularis* resulted in a significantly higher number of grains per plant. However, when severe water deficit was imposed, there was no improvement in the number of grains per plant in any inoculation treatment.

Bacterial inoculation and dual inoculation led to a significant increase in the fresh weight of grains produced per plant under both moderate and severe water deficit, whereas fungal inoculation produced a significant improvement only under moderate water deficit (Fig. 2*b*). Without water deficit, dual

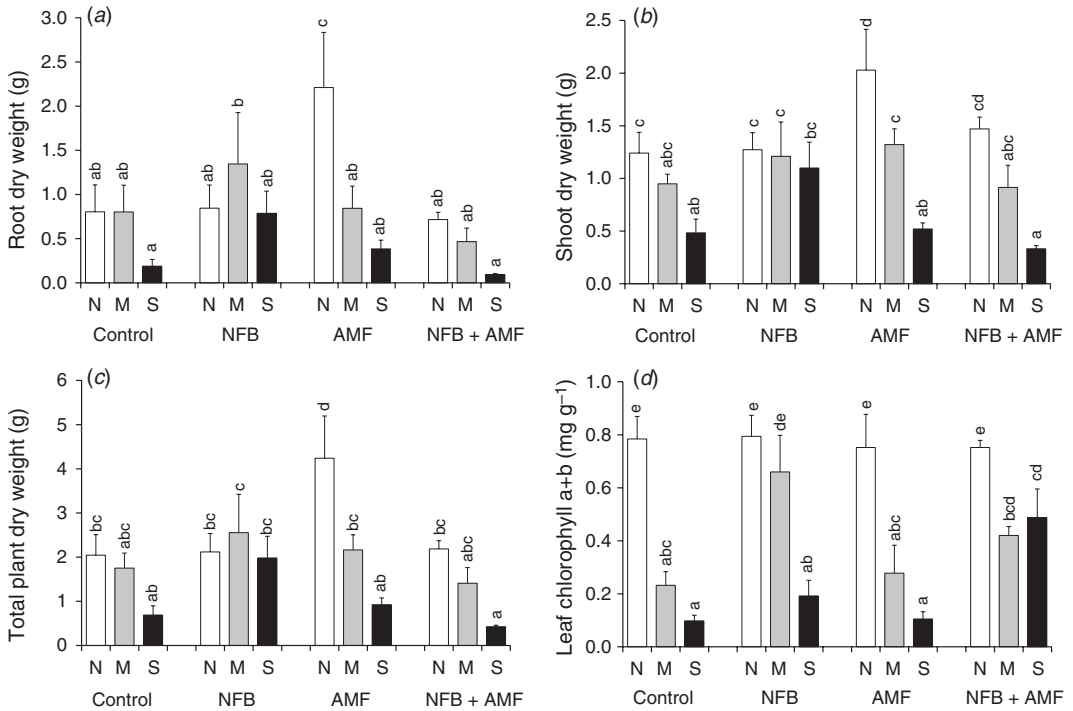

Fig. 1. (*a*) Root dry weight, (*b*) shoot dry weight, (*c*) total plant dry weight, and (*d*) leaf chlorophyll a + b concentration of cowpea grown under different water regimes and inoculated with *Bradyrhizobium elkanii* and *Rhizophagus irregularis*. Values are means ± 1 s.e. Means with the same letter are not significantly different according to Duncan's multiple range test at $P = 0.05$. NFB, Nitrogen-fixing bacterium; AMF, arbuscular mycorrhizal fungus; N, no water deficit; M, moderate water deficit; S, severe water deficit.

Table 1. Three-way ANOVA *F*-values and significances of cowpea biomass and leaf chlorophyll a + b concentration according to bacterial inoculation, fungal inoculation and water-deficit factors

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; n.s., not significant ($P > 0.05$)

	Root dry weight	Shoot dry weight	Total plant dry weight	Leaf chlorophyll a + b concentration
Bacterial inoculation (B)	0.8n.s.	0.1n.s.	0.5n.s.	13.4***
Fungal inoculation (F)	0.003n.s.	0.2n.s.	0.01n.s.	0.01n.s.
Water deficit (W)	6.4**	19.9***	11.5***	45.4***
B × F	9.8**	8.8**	10.8**	0.001n.s.
B × W	2.5n.s.	1.4n.s.	2.2n.s.	3.3*
F × W	3.4*	4.6*	4.0	2.4n.s.
B × F × W	0.4n.s.	0.1n.s.	0.1n.s.	3.0n.s.

Table 2. Three-way ANOVA *F*-values and significance of number, weight and crude protein content of cowpea grains according to bacterial inoculation, fungal inoculation and water-deficit factors

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; n.s., not significant ($P > 0.05$)

	No. of grains per plant	Fresh weight of grains per plant	Fresh weight per grain	Crude protein of grains
Bacterial inoculation (B)	14.3***	50.0***	21.6***	14.9***
Fungal inoculation (F)	2.3n.s.	1.7n.s.	0.1n.s.	0.07n.s.
Water deficit (W)	170.0***	242.3***	4.8**	2.7n.s.
B × F	0.4n.s.	5.2*	0.7n.s.	0.4n.s.
B × W	0.1n.s.	3.7*	0.8n.s.	0.1n.s.
F × W	0.3n.s.	1.7n.s.	1.9n.s.	1.2n.s.
B × F × W	5.4**	12.7***	2.7n.s.	0.5n.s.

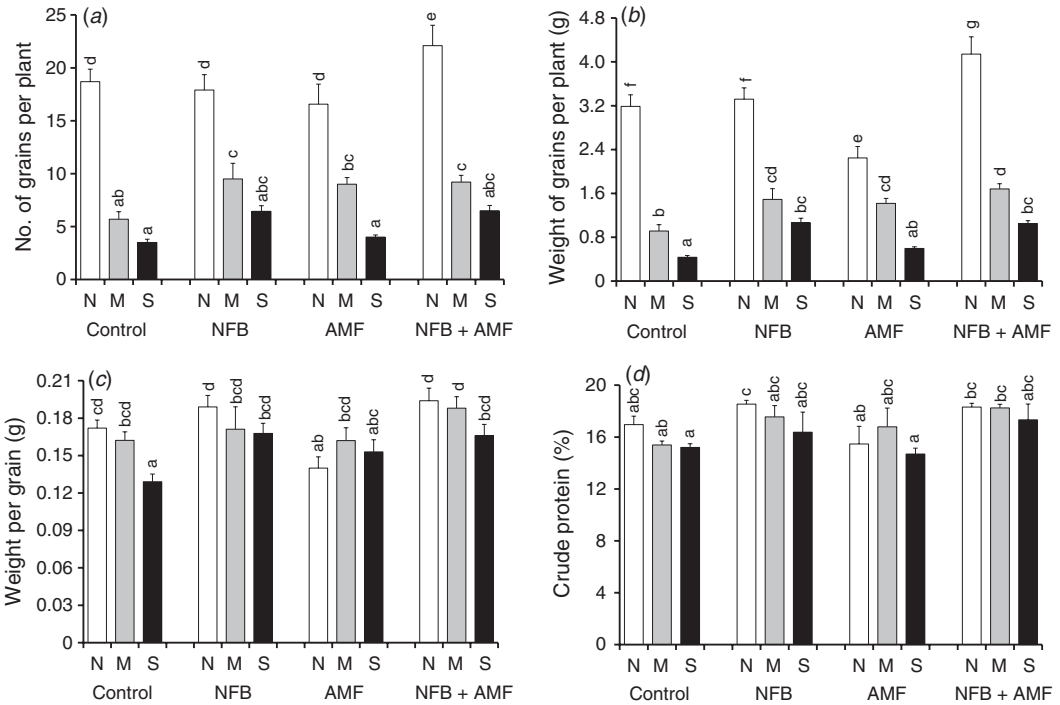

Fig. 2. (*a*) Number of grains per plant, (*b*) fresh weight of grains per plant, (*c*) fresh weight per grain, and (*d*) crude protein percentage of grains of cowpea grown under different water regimes and inoculated with *Bradyrhizobium elkanii* and *Rhizophagus irregularis*. Values are means ± 1 s.e. Means with the same letter are not significantly different according to Duncan's multiple range test at $P = 0.05$. NFB, Nitrogen-fixing bacterium; AMF, arbuscular mycorrhizal fungus; N, no water deficit; M, moderate water deficit; S, severe water deficit.

inoculation significantly increased the weight of grains per cowpea plant.

There was no enhancement of the weight per grain produced by cowpea without water deficit or under moderate water deficit in any microbial inoculation. However, under severe water deficit, plants inoculated with *B. elkanii* or with *B. elkanii* + *R. irregularis* produced heavier grains than the respective non-inoculated control (Fig. 2*c*).

There were no significant differences in the crude protein content of cowpea grains between non-inoculated controls and inoculated plants, irrespective of inoculation treatment and water regime (Fig. 2*d*).

Microbial performance

There were no nodules of *B. elkanii* and no mycorrhizal colonisation in the roots of non-inoculated cowpea (Table 3). Plants under severe water deficit had a significantly reduced number of root nodules compared with those without water deficit and those under moderate water deficit in both inoculation treatments (*B. elkanii* and *B. elkanii* + *R. irregularis*).

Bacterial inoculation significantly increased mycorrhizal colonisation in moderate and non-deficit water regimes. There was no influence of bacterial inoculation or water deficit on the length of the ERM of *R. irregularis*.

Discussion

Cowpea is cropped mainly for its grain, but the leaves are also used for human consumption and the dried stalks are valuable as animal feed (Iqbal 2015). Our results showed that inoculation with an AM fungus significantly increased below- and aboveground biomass of cowpea without water deficit. This is in accordance with the work of Omirou *et al*. (2016) and indicates that AM fungi have the potential to improve the sustainable production of cowpea. However, when water deficit (moderate or severe) was imposed in our study, no benefit of microbial inoculation was observed. No benefit in plant biomass of cowpea inoculated with AM fungi under water stress was also reported by Diallo *et al*. (2001). Those authors showed that under water-stressed conditions (drought), the root and shoot dry matter of plants inoculated with *Glomus mosseae* or *G. versiforme* did not differ significantly from non-inoculated controls. They related this lack of effect to the lack of significant differences in leaf osmotic potential, stomatal conductance and leaf transpiration in mycorrhizal cowpea and suggested that AM fungal inoculation did not affect stomatal closure.

Oliveira *et al*. (2005*a*) showed that plants inoculated with an NF bacterium alone or in combination with an AM fungus exhibited significantly higher leaf chlorophyll a + b concentration. Similarly, in the present study, the leaf chlorophyll a + b concentration was significantly increased under moderate water deficit with bacterial inoculation and under severe water deficit with dual inoculation, indicating that cowpea inoculated with *B. elkanii* or with *B. elkanii* + *R. irregularis* had higher photosynthetic potential. Although this potential did not translate into increased plant biomass, it may have contributed to improve grain yield.

Table 3. Number of root nodules, arbuscular mycorrhizal fungal (AMF) colonisation and length of extraradical mycelium (ERM) of cowpea inoculated with *Bradyrhizobium elkanii* and *Rhizophagus irregularis* under different water regimes

RLC, Root length colonised. Within columns, means (± 1 s.e.) followed by the same letters are not significantly different according to Duncan's multiple range test at $P = 0.05$. $^{**}P < 0.01$; $^{***}P < 0.001$; n.s., not significant ($P > 0.05$)

Inoculation	Water deficit	No. of nodules	AMF colonisation (%RLC)	ERM length (cm cm^{-2})
Bradyrhizobium elkanii	None	61 ± 3b	0	0
	Moderate	79 ± 15b	0	0
	Severe	20 ± 2a	0	0
Rhizophagus irregularis	None	0	34 ± 3a	8 ± 2
	Moderate	0	28 ± 3a	13 ± 3
	Severe	0	30 ± 4a	14 ± 3
B. elkanii + *R. irregularis*	None	56 ± 6b	46 ± 3b	19 ± 12
	Moderate	63 ± 11	45 ± 6b	21 ± 8
	Severe	14 ± 4a	29 ± 4a	12 ± 5
Two-way ANOVA *F*-values and significances				
Bacterial inoculation (B)			8.9**	1.0n.s.
Fungal inoculation (F)		1.1n.s.		
Water deficit (W)		12.4***	3.3n.s.	0.2n.s.
B × W			2.9n.s.	0.4n.s.
F × W		0.2n.s.		

Under moderate water deficit, grain yield (in terms of both number of seeds produced and total weight of seeds per plant) was significantly higher with microbial inoculation. There was an increase in the number of seeds of 67% and 61% in cowpea inoculated with *B. elkanii* and *B. elkanii* + *R. irregularis*, respectively; and an increase of 63%, 55% and 84% in the total weight of seeds per plant inoculated with *B. elkanii*, *R. irregularis* and *B. elkanii* + *R. irregularis*, respectively. Under severe water deficit, inoculation with *B. elkanii* and *B. elkanii* + *R. irregularis* resulted in an enhancement of total weight of seeds per plant of 45% and 42%, respectively. These results show that NF bacteria and AM fungi can increase grain yield of cowpea under both moderate and severe water-deficit conditions; however, the benefit of adding NF bacteria and the combination of both microorganisms varies with the level of water deficit. Overall, dual inoculations (NF bacterium + AM fungus) were the treatments in which superior grain yield was achieved. Furthermore, dually inoculated cowpea plants produced heavier seeds under severe water stress compared with the respective non-inoculated control. Ngakou *et al*. (2008) also reported improved grain yield of cowpea inoculated with NF bacterium + AM fungus. Microbial inoculation brought no increase in crude protein content of cowpea grains under our experimental conditions. In a meta-analysis with 12 legume species, Kaschuk *et al*. (2010) found increases in grain protein of 7% and 14% in plants inoculated with rhizobia in the field and with AM fungi in pot experiments, respectively. However, cowpea was not included in their study.

Nodulation was not affected by moderate water deficit; nonetheless, there was a significant reduction of nodulation in the severe water-stress treatments. Inoculation with *R. irregularis* was unable to alleviate this reduction. Similarly, Omirou *et al*. (2016) found no effect of AM fungal inoculation on cowpea nodulation. Water-deficit stress has been shown to reduce nodulation and N_2 fixation of different *Bradyrhizobium* strains in symbiosis with cowpea (Figueiredo *et al*. 1998, 1999). However, it was demonstrated that the susceptibility to drought is strain-specific.

Mycorrhizal colonisation (%RLC) of cowpea inoculated with *R. irregularis* varied between 29% and 46%, which is in accordance with previous studies on interactions between cowpea and AM fungi (Bagayoko *et al*. 2000; Augé *et al*. 2001; Omirou *et al*. 2016). Inoculation with *B. elkanii* increased root colonisation of *R. irregularis* under all water regimes except severe water deficit. Significantly higher mycorrhizal root colonisation in cowpea inoculated with NF bacteria was previously found in experiments without water stress (Ames *et al*. 1991; Taiwo *et al*. 2001). Severe water deficit was detrimental to nodulation of *B. elkanii* and might have reduced its ability to fix N_2 (Figueiredo *et al*. 1998, 1999). The reduced cowpea fitness under severe water deficit and the higher drain on plant photosynthates during the tripartite symbiosis might have contributed to the observed decrease in mycorrhizal colonisation.

Inoculation with *B. elkanii* + *R. irregularis* improved grain yield of cowpea under water deficit. Drought is one of the main challenges in agriculture. Hence, the use of cowpea inoculated with NF bacteria + AM fungi has great potential to tackle problems arising from water scarcity. These beneficial soil microorganisms can be regarded as biotechnological tools for sustainable agriculture in drought scenarios. The study also indicated that the NF bacterial inoculant is potentially resistant to moderate water stress. The AM fungal inoculant shows considerable resistance to moderate and even severe drought stress, and that applies to both the intra- and extraradical phase of the symbiosis. There is the prospect that, in the field, the inoculants might persist over drought periods and maintain mutualistic capacity with the host. Nevertheless, this would have to be tested under field conditions, using different isolates of AM fungi and NF bacteria.

Acknowledgements

RS Oliveira, Y Ma and I Rocha acknowledge the support of Fundação para a Ciência e a Tecnologia (FCT) through the research grants SFRH/BPD/85008/2012, SFRH/BPD/76028/2011 and SFRH/BD/100484/2014, Fundo Social Europeu (FSE) and Programa Operacional do Capital Humano (POCH). This work was financed by Portuguese national funds through Programa Operacional Competitividade e Internacionalização (POCI), Project 3599—Promover a Produção Científica e Desenvolvimento Tecnológico e a Constituição de Redes Temáticas (3599-PPCDT) and Fundo Europeu de Desenvolvimento Regional (FEDER) under Project POCI-01-0145-FEDER-016801 and by FCT under Project PTDC/AGR-TEC/1140/2014. G Marques acknowledges the European Union's Seventh Framework Program for research, technological development and demonstration under grant agreement no. 613781. M Vosátka was supported by the project NPUI LO1417 of the Ministry of Education, Youth and Sports of the Czech Republic.

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