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ORIGINAL ARTICLE

The presence of *Epichloë* sp. in *Bromus auleticus* (Trin.) seeds enhances micropropagation and growth of micropropagated plantlets from these seeds

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Abstract

Bromus auleticus (Trin.) is a grass native to the southern cone with important agronomical potential as fodder. Different breeding programs have been initiated with this grass, but plant tissue culture techniques could not be used because *B. auleticus* is recalcitrant. The aim of the present study was to develop a micropropagation protocol in the genus *Bromus* and to investigate if the association between *B. auleticus* and *Epichloë* endophytes affected in vitro culture and growth of micropropagated plantlets. In different micropropagation stages, better results were obtained with endophyte-infected (E+) seeds compared to endophyte-free (E−) seeds. The E+ seeds presented higher percentages of in vitro germination (82 ± 5 vs. 57 ± 6%), callus induction (72 ± 6 vs. 37 ± 6%), and plant regeneration from callus (89 ± 5 vs. 13 ± 5%). We also compared the biomass of shoot complexes and regenerated plantlets. After 4 weeks of culture, shoot complexes obtained from E+ seeds reached greater weight than the ones regenerated from E− seeds (173 ± 24 vs. 74 ± 9 mg). More than the 80% of the regenerated shoot complexes were rooted ex vitro and acclimated, regardless of their origin (E+ or E−). Finally, after 4 weeks of acclimatization, the plantlets regenerated from E+ seeds reached a greater weight than the ones from E− seeds, (461 ± 64 vs. 172 ± 25 mg). These results indicate that the use of endophyte-infected (E+) seeds enhances significantly *B. auleticus* micropropagation and promotes growth of the regenerated plantlets.

Keywords Callus culture · Ex vitro rooting · Fungal endophytes · Native pastures · Organogenesis

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Introduction

Bromus auleticus Trin ex Nees (*Pooideae*) is a winter perennial C3 grass, native to central Argentina, southern Brazil and Uruguay. The majority of the populations of this grass are natural ones, since their domestication is recent (Millot 2001). *Bromus auleticus* is considered one of the most valuables grasses of the Southern Cone (Millot 1999; Ayala et al. 2010), due to its high productivity, palatability, protein abundance, field persistence and drought resistance (Gasser et al. 2005). *Bromus auleticus* breeding has been the focus of different studies (Scheffer-Basso et al. 2009; Gutiérrez et al. 2015; Condón et al. 2017), and some varieties of this grass are commercialized in Uruguay (Ayala et al. 2010).

In the last decades, the development of diverse biotechniques has widened the range of possibilities classic breeding offers. To adapt these biotechnological techniques to a specific species, reliable micropropagation protocols are a pre-requisite (Song et al. 2012; Ran et al. 2014). However, *B. auleticus*, like other grasses, is recalcitrant to plant

tissue culture techniques (Giri and Praveena 2015). The only species of the genus *Bromus* for which a micropropagation protocol has been described is *B. inermis* Leyss (Wattanasiri and Walton 1993). The only reference to genetic transformation in the genus *Bromus* is also in *B. inermis* (Nakamura and Ishikawa 2006). Nakamura and Ishikawa transformed *B. inermis* calli but did not regenerate plantlets from them. Protocols for *B. inermis* callus culture were developed in the early 90's (Tanino et al. 1991; Ishikawa et al. 1990) and have been used in different studies but plants were never regenerated from these calli (Nakamura et al. 2013; Ishikawa et al. 2006).

A recent study, carried out by our research group, showed that the endophytic fungus *Epichloë occultans* influences micropropagation competence positively in *Lolium multiflorum* (Regalado et al. 2017), another grass considered recalcitrant to in vitro culture. *Epichloë* spp. are fungal endophytes associated with some cool-season grasses of the subfamily *Pooideae*. The asexual *Epichloë* are mainly vertically transmitted via the seeds of the host plant (Clay and Schardl 2002). These associations are in general considered mutualistic. Endophyte-infected plants may be subject to morphological and physiological modifications (Torres et al. 2012) that confer increased growth and tolerance to abiotic stresses (Clay 1987; Nagabhyru et al. 2013), and resistance to fungal pathogens (Gwinn and Gavin 1992; Xia et al. 2016). The associations with *Epichloë* endophytes have been used in the grasses breeding (Johnson et al. 2013). In the last years, in vitro culture techniques have been employed to study the effect of different microorganisms, such as endophytic fungi (Thomas et al. 2010; Verma et al. 2015; Wang et al. 2016). However, the effect of *Epichloë* endophytes has never been studied in in vitro cultivated grasses.

Different endophytes of the genus *Epichloë* have been isolated from Argentine populations of *B. auleticus: E. pampeana* (Iannone et al. 2009), *E. tembladerae* (Iannone et al. 2009) and other *Epichloë* spp. not yet formally described (Mc Cargo 2015). The association with *Epichloë* endophytes is of interest in breeding programs because it provides different advantages to the infected plants of *B. auleticus*. For instance, *B. auleticus* plants infected with *Epichloë* endophytes (E+) produce not only more biomass and seeds (Iannone and Cabral 2006; Iannone et al. 2012), but also larger seedlings that increase survival rates (Novas et al. 2003). Furthermore, *Epichloë* endophytes interact with other microorganisms present in *B. auleticus* plants by inhibiting the growth of *Ustilago bullata*, which causes a disease known as the head smut of grasses (Vignale et al. 2013; Iannone et al. 2017), increasing the diversity of phosphorus-solubilizing rhizospheric fungi (Arrieta et al. 2015) and promoting arbuscular mycorrhizal fungi development (Novas et al. 2011; Vignale et al. 2016, 2017).

The aim of this research was to examine if the association between *B. auleticus* and *Epichloë* endophytes enhances micropropagation of this native grass and promotes growth of the micropropagated plantlets. The development of a micropropagation protocol adapted to *B. auleticus* would allow the use of other biotechnological tools in the breeding of this grass.

Materials and methods

Plant material and endophytic status

The seeds used in this work are original from a population located at El Palmar Natl. Park, whose plants are naturally associated with an *Epichloë* sp. not yet formally described, but different from the other *Epichloë* species described in association with *B. auleticus* (Mc Cargo 2015). In this population, all the plants are associated with *Epichloë* and the incidence of the endophytes in the seeds is 100%. In 2004, seeds from E+ plants of this population were collected and stored. In 2007, these seeds were sown in the field of the INTA-Concepción del Uruguay Agronomic Experimental Station, Entre Rios Province, Argentina. Endophyte-free (E−) plants were obtained from some seeds in which the endophyte had lost its viability during storage. Since then, nursery of E+ and E− plants were established and seeds are collected every year. In December 2015, seeds from E+ and E− plants were collected and bulked according to the endophytic status of the mother plant to establish E+ and E− seed lots used in this work. Notwithstanding, prior to the experiments, we used the protocol described in Vignale et al. (2017) to check the endophytic status of the seed lots. The endophytic status was examined in 50 seeds randomly chosen from each pool. The seeds were immersed in a 10% sodium hydroxide solution at room temperature (22 °C) for 5 h, then rinsed and stained with aniline blue. *Epichloë* presence in seeds was confirmed by conventional histological techniques (Clark et al. 1983). Endophytic mycelia were visualized by staining tissue using aniline blue (0.1% aqueous). A seed was considered as endophyte-infected (E+) if twisted hyphae were observed to be associated with the aleurone layer cells in seed preparations.

Seed disinfection

B. auleticus seeds were disinfected to avoid contamination during the in vitro culture. We used the protocol developed by our research group for the disinfection of *Lolium multiflorum* seeds (Regalado et al. 2017) and, to increase its effectiveness, the glumels were previously removed.

In vitro germination of E+ and E− seeds

The viability of E+ and E− seeds was examined through a germination test. 20 E+ and 20 E− seeds were used in the germination test and three independent repetitions were carried out, totaling 60 E+ and 60 E− seeds. The seeds were cultured in Petri dishes, five seeds per Petri dish, on 25 ml of MS (Murashige and Skoog 1962) culture medium supplemented with 30 g l^{-1} sucrose, solidified with 8 g l^{-1} of bacteriological agar and pH adjusted to 5.74 before autoclaving. All culture media employed in this research had the same pH and gelling agent. The Petri dishes were placed in an incubator model I-291PF (Ingelab) at 25 ± 2 °C under dark for 4 weeks. This incubator was used in all in vitro experiments. The germination percentage was calculated, as the number of seeds with visible radicle with respect to the number of total seeds, and compared between E+ and E− seeds.

Induction of calli in E+ and E− seeds

In order to obtain calli to regenerate *B. auleticus* plantlets, a total of 60 E+ and 60 E− seeds were cultured in Petri dishes, five seeds per Petri dish, on 25 ml of CIM culture medium (callus induction medium). The seeds were distributed in three independent repetitions, 20 E+ seeds and 20 E− seeds in each repetition. CIM consists of MS medium (Murashige and Skoog 1962) supplemented with 30 g l^{-1} sucrose, 2 mg l^{-1} 2,4-D and 0.1 mg l^{-1} BA. The Petri dishes were incubated at 25 ± 2 °C under dark for 4 weeks. The callus induction percentage in the E+ and E− seeds was calculated as the number of seeds in which callus is produced with respect to the total seeds. The seed germination percentage in CIM medium was also evaluated.

Callus proliferation

The calli obtained from E+ and E− seeds were subcultured every 4 weeks, in new Petri dishes with CIM culture medium, until they reached a pre-established size to begin regeneration of *B. auleticus* plantlets (15 mm). The Petri dishes were placed at 25 ± 2 °C in dark conditions.

Regeneration of shoots

For plantlet regeneration, 45 callus pieces (approximately 15 mm of diameter), in three independent repetitions, each with 15 callus pieces, derived from E+ and E− seeds were transferred to Petri dishes containing 25 ml of regeneration medium (RM medium), which consisted of MS medium (Murashige and Skoog 1962) supplemented with 30 g l^{-1} sucrose and 0.2 mg l^{-1} kinetin. Five calli were cultured on each Petri dish, three Petri dishes with E+ seeds and three with E− seeds in each independent repetition, and incubated for 4 weeks at 25 ± 2 °C under 16:8 h (L:D) photoperiod with a light intensity level of 40 µmol photon $m^{-2}s^{-1}$. After 4 weeks, the percentage of shoots regenerated from calli was determined and compared between the calli obtained from E+ and E− seeds. The percentage was calculated as the number of callus with regenerated shoots versus the total number of cultured callus.

Elongation and multiplication of shoots

For elongation, the shoots regenerated were transferred to individual test tubes with 10 ml of RM medium and incubated under the same conditions of light and temperature used for shoot regeneration. During this elongation, new shoots were formed together with the regenerated ones, but none of these shoot complexes formed roots. To multiply the shoot complexes, they were subcultured every 4 weeks in test tubes with 10 ml of RM medium. The multiplication consisted in the mechanical division of each shoot complex.

Effect of *Epichloë* sp. on the growth of in vitro shoot complexes

Once the shoot complexes were ready to be subcultured, we measured the biomass of thirty shoot complexes regenerated from E+ seeds and thirty from E− seeds. The shoot complexes were distributed in three independent experiments. After 4 weeks of culture, the biomass of these explants was measured by removing the explants from the agar and weighing them. The biomass of shoot complexes regenerated from E+ seeds and E− seeds was then compared. These same shoot complexes were used in ex vitro rooting and acclimatization.

Ex vitro rooting and acclimatization

To induce the ex vitro formation of roots, thirty shoot complexes regenerated from E+ seeds and thirty shoot complexes from E− seeds, both without roots, were acclimatized using the grasses acclimatization protocol developed by our research group (Regalado et al. 2017). The shoots complexes were distributed in three independent experiments, ten shoot complexes regenerated from E+ seeds and ten from E− seeds in each experiment. The shoots complexes were thoroughly washed with tap water, transplanted to 5 × 5 cm polyethylene alveolus trays containing a mixture of tyndallized sand:peat:perlite (1:1:1), and incubated in a culture chamber at 22 °C, 60% relative humidity and 14:10 h (L:D) photoperiod with a light intensity of 30 µmol photon m^{-2} s^{-1}. The tray with the plantlets was wrapped with plastic film for 2 weeks to maintain high humidity. During the next 2 weeks, holes were made in the plastic film to reduce the humidity down to 60%, and finally the plastic wrap was

removed. After 4 weeks, the acclimatization percentage was calculated and the plantlets were removed from the pots to check the ex vitro rooting.

Effect of *Epichloë* sp. on the growth of acclimatized plantlets

Once the substrate residues were removed, we measured the biomass (fresh weight) of the acclimatized plantlets and compared it between the plantlets regenerated from E+ and E− seeds.

Statistical analysis

All data were analyzed using SPSS software package (version 19.0; SPPS INC., Chicago, IL, USA). The percentage of germination, callus induction, plantlet regeneration and acclimatization obtained for E+ and E− seeds were analyzed by Generalized Linear Models using Logit as the link function and Binomial as the probability distribution. Pairwise comparisons among groups were performed by Bonferroni test. The biomass of the shoots ready to be subcultured, the biomass of shoots after 4 weeks of in vitro culture and the biomass of the acclimatized plantlets were analyzed by one-way ANOVA, using a HSD-Tukey test in the post-hoc analysis for comparisons between E+ and E− seeds.

Results

In vitro seed germination

The percentage of germination in MS medium of *B. auleticus* E+ and E− seeds are presented in Table 1. The germination percentage of E+ seeds was 82 ± 5%, while in E− seeds it was 57 ± 6%, and this difference was statistically significant. In both classes of seeds, germination percentages in CIM medium, which is used for callus induction, were lower than in MS medium (Table 1): 55 ± 6% in E+ and 43 ± 6% in E−. This difference in the germination percentage was statistically significant for E+ seeds but not for E− seeds.

Callus induction

Regardless of the endophytic status of the seeds, callus formation was not induced in any seed (0%) cultured in MS medium without growth regulators (data obtained from In vitro germination assay). However, in seeds cultured in CIM medium, the callus induction percentage of E+ seeds (72 ± 6%) was significantly higher than in E− seeds (37 ± 6%) (Table 1).

Table 1 Germination percentage, induction callus percentage and plant regeneration percentage of *Bromus auleticus* E+ and E− seeds

Culture medium*	Type of seeds	Germination percentage	Callus induction percentage	Plant regeneration percentage
MS	E+	82 ± 5^{a}	0 ± 0^{c}	
	E−	57 ± 6^{b}	0 ± 0^{c}	
CIM	E+	55 ± 6^{b}	72 ± 6^{a}	
	E−	43 ± 6^{b}	37 ± 6^{b}	
RM	E+			89 ± 5^{a}
	E−			13 ± 5^{b}

Different letters in each percentage indicate groups that were significantly different by LSD at $\alpha = 0.05$

*Composition of the different culture media is shown in "Materials and methods"

Regeneration, elongation and multiplication of shoots

After 4 weeks of culture in RM medium, shoots regenerated from 89 ± 5% of the calli from E+ seeds (Table 1), obtaining a total of 40 shoots. However, only 6 shoots were regenerated from E− calli, with a regeneration success percentage of 13 ± 5%. All the shoots transferred to individual test tubes with RM medium elongated correctly and new shoots were formed together with the regenerated shoots, constituting shoot complexes. However, the shoot complexes did not root in the RM medium. During the multiplication of the shoot complexes, depending on the initial size of the shoot complex, 2–4 new shoot complexes were obtained in each division.

Effect of *Epichloë* sp. in the in vitro growth of shoot complexes

The biomass of shoot complexes ready to be subcultured was 82 ± 10 mg in shoot complexes regenerated from E+ seeds (Fig. 1a) and 42 ± 6 mg in shoot complexes from E− seeds (Fig. 1b) (Fig. 2). After 4 weeks of culture on RM medium, shoot complexes regenerated from E+ seeds (Fig. 1c) reached an average weight of 173 ± 24 mg, while the ones regenerated from E− seeds (Fig. 1d) reached an average weight of 74 ± 9 mg (Fig. 2). In both cases, the differences in biomass between the shoot complexes regenerated from E+ and E− seeds were statistically significant.

Ex vitro rooting, acclimatization and effect of the endophyte *Epichloë* sp. in the growth of acclimatized plantlets

Four weeks after acclimatization, completely functional roots grew in 83 ± 7% of the shoot complexes regenerated

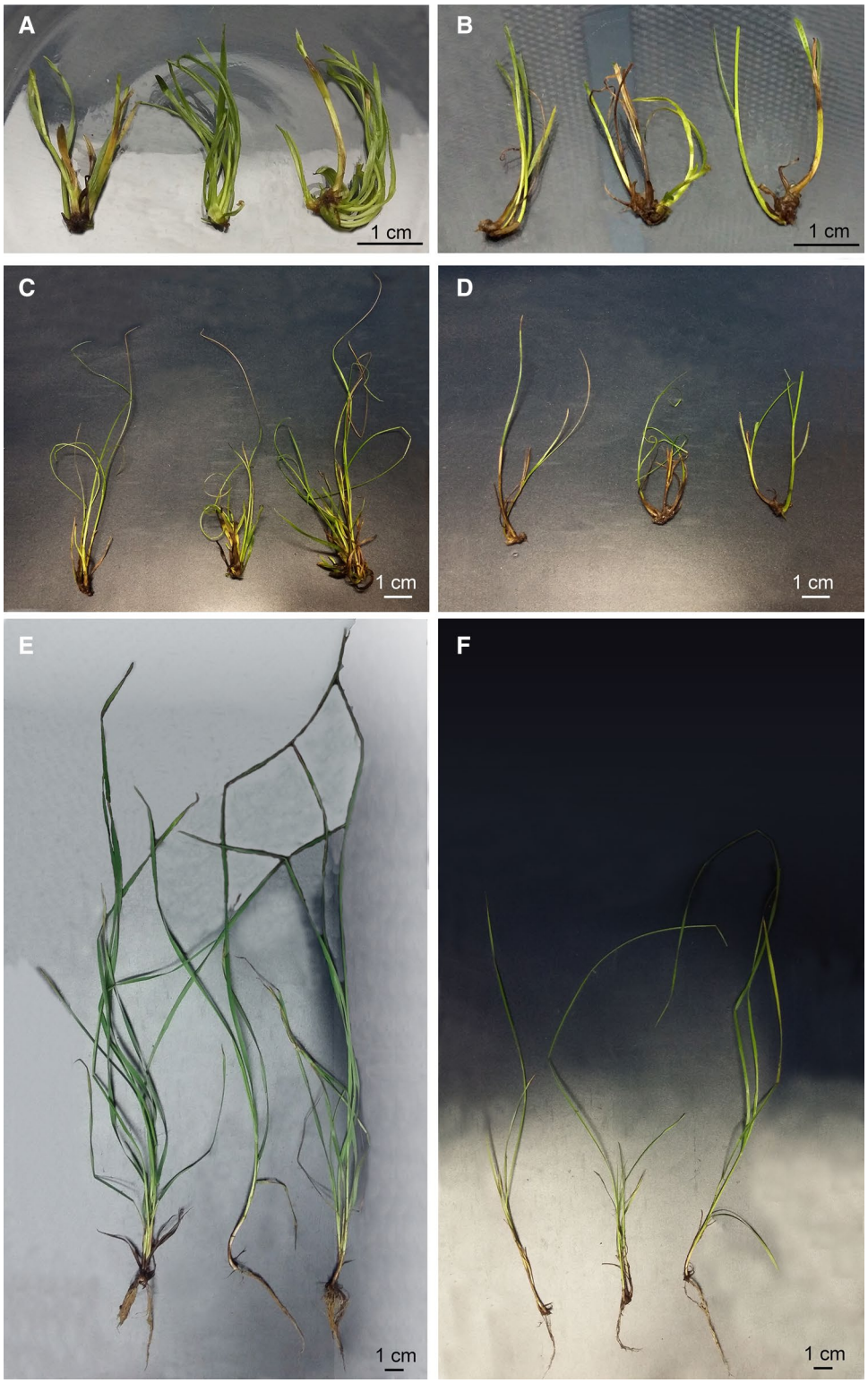
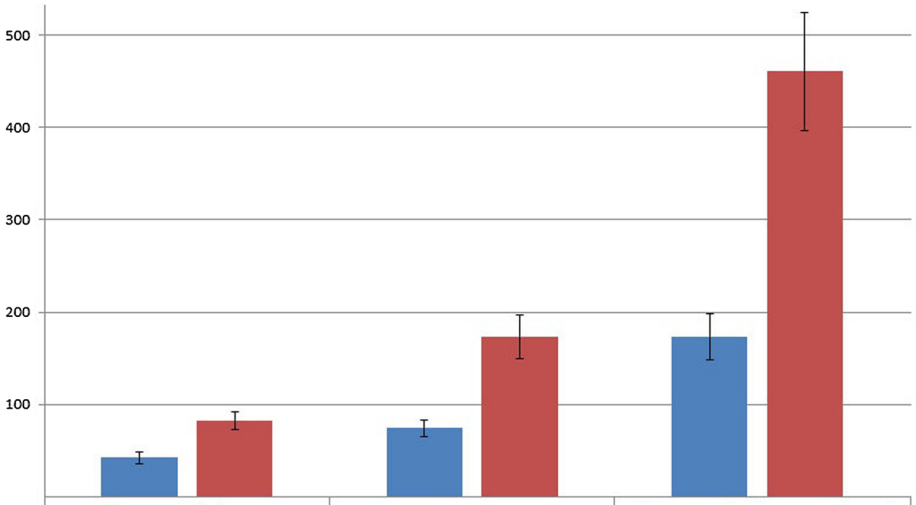


Fig. 1 Different micropropagation stages of *Bromus auleticus*. **a** Shoot complexes regenerated from E+ seeds ready to be subcultured. **b** Shoot complexes regenerated from E− seeds ready to be subcultured. **c** Shoot complexes regenerated from E+ seeds cultured on RM medium for 4 weeks. **d** Shoot complexes regenerated from E− seeds cultured on RM medium for 4 weeks. **e** Micropropagated plantlets regenerated from E+ callus and rooted ex vitro, 4 weeks after acclimatization. **f** Micropropagated plantlets regenerated from callus E− and rooted ex vitro, 4 weeks after acclimatization

from E+ seeds (Fig. 1e) and in 86 ± 8% of the shoot complexes regenerated from E− seed growth seeds (Fig. 1f). All plantlets that were rooted ex vitro also acclimatized correctly, thus, the acclimatization percentage was 83 ± 7 and 86 ± 8% in the plantlets regenerated from E+ and E− seeds respectively.

Finally, 4 weeks after acclimatization, the average weight of the plantlets regenerated from E+ seeds was significantly



Biomass of shoot complexes ready to be subcultured		Biomass of shoot complexes cultured on RM for four weeks		Biomass of micropropagated plantlets four weeks after the acclimatization.	
From E- seeds	From E+ seeds	From E- seeds	From E+ seeds	From E- seeds	From E+ seeds
42 ± 6 mg ^{b}	82 ± 10 mg ^{a}	74 ± 9 mg ^{b}	173 ± 24 mg ^{a}	173 ± 25 mg ^{b}	461 ± 64 mg ^{a}

Fig. 2 Biomass of shoot complexes regenerated from E+ and E− *B. auleticus* seeds in different stages of the micropropagation protocol

greater than the average weight of the plantlets regenerated from E− seeds, 461 ± 64 and 173 ± 25 mg respectively (Fig. 2).

Discussion

As in the most grasses, species of the genus *Bromus* are recalcitrant to micropropagation (Giri and Praveena 2015). In this work, we examined if the symbiosis with endophytic fungus of the genus *Epichloë* could enhance the micropropagation of *B. auleticus*. Once we obtained in vitro *B. auleticus* plants, we analyzed how this association affected plant growth.

First, we studied the effect of an *Epichloë* endophyte in the germination of *B. auleticus* seeds. Our results showed that the presence of the endophyte increased in vitro germination (Table 1). This is in agreement with the results obtained by our research group for the ex vitro germination of *B. auleticus* (Iannone and Cabral 2006) and *B. setifolius* seeds (Novas et al. 2003) infected with *Epichloë* endophytes, and for the in vitro germination of *Lolium multiflorum* (Regalado et al. 2017).

The first stage of micropropagation consisted in inducing callus from seeds. The callus induction percentage of the control seeds (E−) was 37 ± 6% (Table 1). Wattanasiri and Walton (1993) obtained a callus induction percentage of 88.9% using as explants 10–15 cm long young unemerged inflorescences of *B. inermis*. Since this result was obtained in another species of the genus *Bromus* and with a different initial explant, it is less comparable to our results. The presence of *Epichloë* endophytes in *B. auleticus* seeds (E+ seeds) increased callus induction up to 72 ± 6%. Similar effects were produced by *E. occultans* on the callus induction of *Lolium multiflorum* seeds (Regalado et al. 2017). The production of AIA (indole acetic acid) was detected in culture extracts of *Epichloë coenophialum*, endophytic fungus associated to *Festuca arundinaceu* (De Battista et al. 1990). This production of AIA could be associated with an increment in callus induction in *B. auleticus* and *L. multiflorum* seeds infected with *Epichloë* sp. It is possible that this positive influence on callus induction could also be produced by other species of the genus *Epichloë*, but additional assays are necessary to confirm this.

Regarding the regeneration of shoots from the callus of *B. auleticus*, the regeneration percentage in callus obtained from E− seeds (control seeds) was 13 ± 5% (Table 1). This percentage is low compared with the regeneration percentages obtained for calli of *B. inermis* (Wattanasiri and Walton 1993), *Lolium multiflorum* (Lee et al. 2009; Regalado et al. 2017) and *Lolium perenne* (Liu et al. 2006), which ranged from 20 to 50% depending on the genotype. The high regeneration percentage obtained with E+ seeds (89 ± 5%) indicates that the influence of *Epichloë* sp. increased noticeably the regeneration percentage. This increase in the regeneration percentage has also been described for *E. occultans* in shoot regeneration from *L. multiflorum* callus (Regalado et al. 2017), but additional studies are necessary to establish if this effect is also observed in other species of the genus

Epichloë and to elucidate the mechanism by which these endophytes enhance the micropropagation of their hosts.

Once regenerated, we studied the influence of the *Epichloë* endophyte in the in vitro growth of the shoot complexes. The shoot complexes, regenerated from callus E+ reached a higher biomass after 4 weeks of culture in RM in comparison to the ones regenerated from callus E− (Fig. 2). Thus, the presence of *Epichloë* endophytes in the *B. auleticus* seeds resulted in greater growth in the shoot complexes regenerated from these seeds. This work is the first study pertaining to the effect of *Epichloë* endophytes in the in vitro growth of their hosts. The results are in agreement with those obtained by our research group in ex vitro plants of *B. auleticus* (Iannone and Cabral 2006) and *B. setifolius* (Novas et al. 2003) infected with *Epichloë* endophytes.

To attain high acclimatization percentages, the plantlets must have a well-developed in vitro root system. However, since the strategies tested to root the shoot complexes in vitro were unsuccessful (data not shown), we decided to carry out ex vitro rooting. In the literature, there are some examples where the authors combined ex vitro rooting with the acclimatization phase (Agarwal et al. 2015; Aygun and Dumanoglu 2015; Ahmed et al. 2017). Usually, the micropropagated shoots were dipped in different rooting solutions to induce root formation. In many cases, without these rooting solutions ex vitro rooting failed (Agarwal et al. 2015; Aygun and Dumanoglu 2015; Ahmed et al. 2017). In *B. auleticus* the use of rooting solution was not necessary, since the stress produced during the acclimatization was sufficient to induce ex vitro rooting in more than 80% of the shoots complexes. All ex vitro rooted shoot complexes survived acclimatization and resulted in viable plants, regardless of their origin (E+ or E− seeds). However, the origin of the shoot complexes influenced the biomass of the plantlets 4 weeks after acclimatization, and as a result, the plantlets regenerated from E+ seeds were larger (Fig. 2). This plant growth enhancement was previously described in the in vitro growth of shoot complexes in this work and also in previous works in potted plants obtained by the germination of *B. auleticus* and *B. setifolius* seeds infected with different *Epichloë* spp. (Novas et al. 2003; Iannone and Cabral 2006), but never in micropropagated pastures. The production of AIA by *Epichloë* sp. has been detected (De Battista et al. 1990), but the studies concerning the relation between this production and the promotion of plant growth in the endophyte-infected plants were inconclusive (De Battista et al. 1990). Therefore, additional studies will be necessary to explain the mechanism by which the presence of different *Epichloë* spp. promotes the growth of their hosts.

In conclusion, the use of seeds infected with *Epichloë* endophytes is essential for *B. auleticus* micropropagation. The interaction with *Epichloë* endophytes enhances the success percentage in key phases of micropropagation, such as callus induction or shoots regeneration. Furthermore, our results indicate that the presence of *Epichloë* endophytes in *B. auleticus* seeds promotes in vitro and ex vitro plant growth.

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Author contribution RJJ and PASI designed the micropropagation experiments. RJJ and BV executed the micropropagation experiments. VMV checked endophytic status of plant material. NMV and ILJ provided the plant material. RJJ wrote the manuscript. VMV, NMV and ILJ reviewed the manuscript. PASI reviewed the English of the manuscript. ILJ supervised the work.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

Agarwal T, Gupta AK, Patel AK, Shekhawat NS (2015) Micropropagation and validation of genetic homogeneity of *Alhagimaurorum* using SCoT, ISSR and RAPD markers. Plant Cell Tiss Organ Cult 120:313–323

Ahmed MdR, Anis M, Alatar AA, Faisal M (2017) In vitro clonal propagation and evaluation of genetic fidelity using RAPD and ISSR marker in micropropagated plants of *Cassia alata* L.: a potential medicinal plant. Agroforest Syst 91:637–647

Arrieta AM, Iannone LJ, Scervino JM, Vignale MV, Novas MV (2015) A foliar endophyte increases the diversity of phosphorus-solubilizing rhizospheric fungi and mycorrhizal colonization in the wild grass *Bromus auleticus*. Fungal Ecol 17:146–154

Ayala W, Bemhaja M, Cotro B, Do Canto J, García J, Olmos F, Real D, Rebuffo M, Reyno R, Rossi C (2010) Forrajeras; catálogo de cultivares 2010. INIA, Montevideo

Aygun A, Dumanoglu H (2015) In vitro shoot proliferation and in vitro and ex vitro root formation of *Pyruselaea grifolia* Pallas. Front Plant Sci 6:225

Clark E, White J, Patterson R (1983) Improved histochemical techniques for the detection of *Acremonium coenophialum* in tall fescue and methods of in vitro culture of the fungus. J Microbiol Methods 1:149–155

Clay K (1987) Effects of fungal endophytes on the seed and seedling biology of *Lolium perenne* and *Festuca arundinacea*. Oecologia 73:358–362

Clay K, Schardl C (2002) Evolutionary origins and ecological consequences of endophyte symbiosis with grasses. Am Nat 160:99–127

Condón F, Jaurena M, Reyno R, Otaño C, Lattanzi FA (2017) Spatial analysis of genetic diversity in a comprehensive collection of the native grass *Bromus auleticus* Trinius (ex Nees) in Uruguay. Grass Forage Sci 72:723–733

De Battista JP, Bacon CW, Severson R, Plattner RD, Bouton JH (1990) Indole acetic acid production by the fungal endophyte of tall fescue. Agron J 82:878–880

Gasser M, Ramos J, Vegetti A, Tivano JC (2005) Digestión de láminas foliares de *Bromus auleticus* Trin. ex Nees sometidas a diferentes tiempos de incubación ruminal. Agricultura Técnica 65:48–54

Giri CC, Praveena M (2015) In vitro regeneration, somatic hybridization and genetic transformation studies: an appraisal on biotechnological interventions in grasses. Plant Cell Tiss Org Cult 120:843–860

Gutiérrez HF, Pensiero JF, Zabala JM (2015) Effect of population combinations on the reproductive success and germination of seeds of *Bromus auleticus* (Poaceae). Grass Forage Sci 70:176–184

Gwinn K, Gavin A (1992) Relationship between endophyte infestation level of tall fescue seed lots and *Rhizoctonia zeae* seedling disease. Plant Dis 76:911–914

Iannone LJ, Cabral D (2006) Effects of the *Neotyphodium* endophyte status on plant performance of *Bromus auleticus*, a wild native grass from South America. Symbiosis 41:61–69

Iannone LJ, Cabral D, Schardl CL, Rossi MS (2009) Phylogenetic divergence, morphological and physiological differences distinguish a new *Neotyphodium* endophyte species in the grass Bromus auleticus from South America. Mycologia 101:340–351

Iannone LJ, Pinget AD, Nagabhyru P, Schardl CL, De Battista JP (2012) Beneficial effects of *Neotyphodium* tembladerae and *Neotyphodium pampeanum* on a wild forage grass. Grass Forage Sci 67:382–390

Iannone LJ, Vignale MV, Pinget AD, Re A, Mc Cargo PD, Novas MV (2017) Seed-transmitted *Epichloë* sp. endophyte alleviates the negative effects of head smut of grasses (*Ustilago bullata*) on *Bromus auleticus*. Fungal Ecol 29:45–51

Ishikawa M, Robertson AJ, Gusta LV (1990) Effect of temperature, light, nutrients and dehardening on abscisic acid induced cold hardiness in *Bromus inermis* Leyss suspension cultured cells. Plant Cell Physiol 31:51–59

Ishikawa M, Suzuki M, Nakamura T, Kishimoto T, Robertson AJ, Gusta LV (2006) Effect of growth phase on survival of bromegrass suspension cells following cryopreservation and abiotic stresses. Ann Bot 97:453–459

Johnson L, de Bonth ACM, Briggs LR et al (2013) The exploitation of *Epichloë* endophytes for agricultural benefit. Fungal Divers 60:171–188

Lee KW, Choi GJ, Kim KY, Ji HC, Park HS, Yoon SH, Lee SH (2009) High frequency plant regeneration from mature seed derived callus of Italian ryegrass (*Lolium multiflorum*) cultivars. Afr J Biotechnol 8:6828–6833

Liu P, Zhang ZX, Yuan JG, Xi JB, Du XL, Yang ZY (2006) Callus induction and plant regeneration in eleven perennial ryegrass cultivars. Biotechnol Equip 20:30–37

Mc Cargo PD (2015) Evolución y diversidad de endófitos *Epichloë* de la forrajera nativa Bromus auleticus. Tesis Doctoral, p 136

Millot JC (1999) *Bromus auleticus* Trinius. Otra gramínea forrajera perenne invernal. Revista Oficial del Instituto Nacional de Semillas 2:25

Millot JC (2001) Bromus auleticus: una nueva especie domesticada. Documento de recursos fitogenéticos PROCISUR, Diálogo LVI, Montevideo, 3–6

Murashige T, Skoog F (1962) A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiol Plant 15(3):473–497

Nagabhyru P, Dinkins RD, Wood CL, Bacon CW, Schardl CL (2013) Tall fescue endophyte effects on tolerance to water-deficit stress. BMC Plant Biol 13:1

Nakamura T, Ishikawa M (2006) Transformation of suspension cultures of bromegrass (*Bromus inermis* Leyss) by *Agrobacterium tumefaciens*. Plant Cell Tiss Org Cult 84:293–299

Nakamura T, Yazaki J, Kishimoto N, Kikuchi S, Robertson AJ, Gusta LV, Ishikawa M (2013) Comparison of long-term up-regulated genes during induction of freezing tolerance by cold and ABA in bromegrass cell cultures revealed by microarray analyses. Plant Growth Regul 71:113–136

Novas MV, Gentile A, Cabral D (2003) Comparative study of growth parameters on diaspores and seedlings between populations of *Bromus setifolius* from Patagonia, differing in *Neotyphodium* endophyte infection. Flora 198:421–426

Novas MV, Iannone LJ, Godeas AM, Scervino JM (2011) Evidence for leaf endophytes regulation on root symbionts: effect of Neotyphodium endophytes on the pre-infective state of mycorrhizal fungi. Symbiosis 51(1):19–28

Ran Y, Patron N, Yu Q, Georges S, Mason J, Spangenberg G (2014) *Agrobacterium*-mediated transformation of *Lolium rigidum* Gaud. Plant Cell Tiss Organ Cult 118:67–75

Regalado JJ, Vignale MV, Novas MV, Pitta-Alavarez SI, Iannone L (2017) *Epichloë occultans* improve the *Lolium multiflorum* micropropagation. Plant Cell Tiss Organ Cult 130:37–46

Scheffer-Basso SM, Fávero F, Jouris C, Dall'Agnol M (2009) Selection of *Bromus auleticus* populations: a winter perennial grass. R Bras Zootec 38:249–255

Song G, Walworth A, Hancock JF (2012) Factors influencing *Agrobacterium*-mediated transformation of switchgrass cultivars. Plant Cell Tiss Organ Cult 108:445–453

Tanino KR, Chen THH, Fuchigami LH, Weiser CJ (1991) Abscisic acid-induced cellular alterations during the induction of freezing tolerance in bromegrass cells. J Plant Physiol 137:619–624

Thomas J, Ajay D, Kumar RR, Mandal AKA (2010) Influence of beneficial microorganisms during in vivo acclimatization of in vitro-derived tea (*Camellia sinensis*) plants. Plant Cell Tiss Organ Cult 101:365–370

Torres MS, White JF Jr, Zhang X, Hinton DM, Bacon CW (2012) Endophyte mediated adjustments in host morphology and physiology and effects on host fitness traits in grasses. Fungal Ecol 5:322–330

Verma P, Khan SA, Mathur AK, Shanker K, Kalra A (2015) Fungal endophytes enhanced the growth and production kinetics of *Vinca minor* hairy roots and cell suspensions grown in bioreactor. Plant Cell Tiss Organ Cult 118:257–268

Vignale MV, Astiz-Gassó MM, Novas MV, Iannone LJ (2013) Epichloid endophytes confer resistance to the smut *Ustilago bullata* in the wild grass *Bromus auleticus* (Trin.). Biol Control 67:1–7

Vignale MV, Iannone LJ, Pinget AD, De Battista JP, Novas MV (2016) Effect of epichloid endophytes and soil fertilization on arbuscular mycorrhizal colonization of a wild grass. Plant Soil 405:279–287

Vignale MV, Iannone LJ, Scervino JM, Novas MV (2017) *Epichloë* exudates promote in vitro and in vivo arbuscular mycorrhizal fungi development and plant growth. Plant Soil https://doi.org/10.1007/s11104-017-3173-5

Wang X, Yam TW, Meng Q, Zhu J, Zhang P, Wu H, Wang J, Zhao Y, Song X (2016) The dual inoculation of endophytic fungi and bacteria promotes seedlings growth in *Dendrobium catenatum* (Orchidaceae) under in vitro culture conditions. Plant Cell Tiss Organ Cult 126:523–531

Wattanasiri C, Walton PD (1993) Effects of growth regulators on callus cell growth, plant regeneration, and somaclonal variation of smooth bromegrass (*Bromus inermis* Leyss). Euphytica 69:77–82

Xia C, Zhang X, Christensen MJ, Nan Z, Li C (2016) Epichloë endophyte affects the ability of powdery mildew (*Blumeria graminis*) to colonise drunken horse grass (*Achnatherum inebrians*). Fungal Ecol 16:26–34