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SEED PRIMING WITH ASFAC-BCO-4 IMPROVES FESTUCA ARUNDINACEA SCHREB. SEED GERMINATION AND SEEDLINGS GROWTH UNDER MOISTURE STRESS

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Abstract

Festuca arundinacea Schreb. is an important cool-season perennial grass, used in parks, home lawns, athletic fields, golf courses and soil conservation sites. Recent researches are directed towards a deeper understanding of its behaviour under different stress conditions and increasing the ability to counteract the effects of global climate change. This study was carried out with a view to evaluate the effects of saline and drought on some standard germination and early seedlings growth indicators, in laboratory conditions. Both types of stress had significant negative effects on physiological indicators, but the influence of saline stress have proven to be more prominent, especially as regard as mean germination time. The effect of seeds priming was also evaluated. Priming with ASFAC-BCO-4 (a Romanian agricultural biostimulator) stimulated the germination process, i.e. it hastened it, compared with the untreated control, and the effects were more noticeable at the beginning of germination. Also, it positively affected early seedling growth, as well as chlorophyll synthesis, even if the saline stress was present.

Key words*:Festuca arundinacea, osmotic stress, seed germination, priming.*

INTRODUCTION

Festuca arundinacea Schreb., commonly known as tall fescue is one member of Festuca genus, which comprises more than 360 species, that are very different as appearance. It is native to large parts of Europe, Asia and North Africa (USDA-NRCS, 2016). It is an important cool-season perennial grass (Lou et al., 2015), prevalent in temperate climates worldwide, with a vigorous growth, especially during the spring and autumn seasons. Tall fescue is widely used in parks, home lawns, athletic fields, golf courses and soil conservation sites (Wang and Xie, 2007; Yuan et al., 2014), and more than that, it has begun to be used for biogas production (Kanapeckas et al., 2011). Due to its deep and extensive root system, the specie adapted to a wide variability of conditions, as regard as soil and climatic ones (Wiecko, 2006), exhibiting a remarkable potential under water deficit, also a notable capacity to recover on re-watering (Turner et al., 2012).
Moreover, fescue has been shown to facilitate the petroleum phytoremediation (Ebadi et al., 2018), thanks to its increased degradation capacity in the case of contaminated soil, due to root exudates (such as palmitic acid), which also favours the growth of functional bacteria into the rhizosphere and stimulates *F. arundinacea* plant growth promoting bacteria (PGPB) (Liu et al., 2015). In addition, recent studies conducted by Liu et al. (2014) proved that fescue resists on saline alkaline contaminated soils, especially when it was inoculated with PGPB.
Having all the strengths of this species, recent research focus on improving them: for the plant to successfully cope with climate change, including those relating to water availability in the soil and its accessibility respectively, and the action of other stressors. In this context, improve plant resistance to the action of multiple biotic and abiotic stress factors during the summer, called briefly as summer stress tolerance (SST) is a major objective in tall fescue breeding programs (Yuan et al., 2014). Generally, drought stress is one of the most important abiotic limiting factors regarding growth and development of plants (Shahidi et al., 2017). So, plant pronounced ability to

recover after the action of such a factor is a basic condition for survival during stress and rehydration (Yu et al., 2015).

Accordingly, given that seed germination is a key step of the plant life (Taiz and Tiger, 2010), being the first physiological process that must face action of stressors (Rouhi et al., 2011; Lou et al., 2016) and taking into account that the increased in salinity is one of the major concerns of farmers worldwide (FAO, 2002), improving the germination characteristics is particularly needed in the areas with irregular rainfall and drought conditions (Tilaki et al., 2012). Along the time, studies on this topic are numerous and address different ways to improve germination, based on physiological tools (Rouhi et al., 2011; Gao et al., 2015; Rekik et al., 2017), as well as using genetically approaches (Martin et al., 2012).

Thereby, the aim of the present study was: (1) to determine the influence of salt and drought stress on fescue seeds physiological quality; (2) to evaluate the effect of stress factors on fescue seedlings growth; 3) to overcome stress impact by seeds priming.

MATERIALS AND METHODS

The experiment was carried out at the Plant Physiology Laboratory, of the Faculty of Horticulture, Bucharest.

Biological material

Seeds of *Festuca arundinacea* Schreb. cv. Tomahawk were obtained from Barenburg Export and were surfaced disinfected with ethanol 70 %, during 5 min. Then, the seeds were washed three times with sterile distilled water and air-dried before use in the germination experiments.

Germination test and related parameters

For germination indicators twenty seeds (in two replicates and two repeats) were placed on sterile Whatman No. 1 filter paper in Petri dishes (diameter of 9 cm), moistened initially with 5 ml distillate water (control – 0 MPa) or different concentration of NaCl/ mannitol, with a view to obtain different water potential for the solutions, corresponding to -0.3 MPa; -0.6 MPa; -1.2 MPa; and -2.4 MPa, respectively (Braccini et al., 1996). The dishes were covered with lids, also paper being used, and they were moistened, as well as it was mentioned above. To ensure a good seeds germination, the mentioned solutions have been applied at the need.

Seeds priming was performed by hydro-priming and priming with ASFAC-BCO-4 (0.1%, 0.2% and 0.4%), for 6 hours. After return of the seeds to the pre-treatment weight, these were subjected to germination tests on filter paper moistened with 5 ml distillate water or different saline solutions, as follows: 1) control-non primed / distilled water (DW); 2) hydro-primed (HP)/(DW); 3) primed with ASFAC-BCO-4 (A) (a biostimulator - based on 4-clor-2 potasium amidosulfonil-fenoxiacetat + microelements and additives; produced in our country by Romchim Protect, Bacău - invention of Corneliu Oniscu) 0.1%/DW; 4) primed with A at 0.2%/DW; 5) primed with A at 0.4%/DW; 6) primed with A at 0.2%/-0.3 MPa; 7) primed with A at 0.2%/-0.6 MPa; 8) primed with A at 0.2% /-1.2 MPa; 9)primed with A at 0.4% /-0.3 MPa; 10) primed with A at 0.4% /-0.6 MPa; 11) primed with A at 0.4% /-1.2 MPa.

The environmental conditions were those specific to the laboratory: 23±2 °C, 16 h light/8 h dark.

In order to determine the kinetics and percentage of germination, daily, for a period of 10 days, at a fixed hour, there was count the number of the germinated seeds, in fact those with 2 mm protrusion of radicle, until the germination of seeds became constant, over 2 consecutive days. By daily counting of the germinated seeds, the obtained data were used to calculate the specific indicators based on the formulas: germination percentage (GP) (%) and final germination percentage (FGP) (%) (Czabator,1962; ISTA (1999); mean germination time (MGT) (days) (Patil et al., 2012); mean daily germination (MDG) (number) (Gairola et al., 2011).

Seedlings growth

At the end of the germination test, for each variant and repetition seedlings growth indicators such as the radicle and plumule was measured manually with a ruler (ten repetitions) obtaining radicle length (RL) (cm) and plumule length (PL) (cm). Also, fresh weight of these seedlings parts was assessed (RFW, PFW) (g).

The total chlorophyll content of plumule was determined by using the chlorophyll meter CCM 300 and expressed as mg m^{-2}.

Statistical analysis

The experimental design was completely randomized (CRD) with two replications. The obtained data were statistically analysed by two-way analysis of variance (ANOVA) and T test's method was used for pairwise comparison at a 5% level of significance ($P \leq 0.05$).

RESULTS AND DISCUSSIONS

Germination tests results

Dynamics of germination percentage (GP) (%)

Cumulative germination percentage of *F. arundinacea* seeds in different conditions are shown in Figure 1. Germination was noticed on the third day, at the control variants, also for the conditions when the water potential assured had a higher value (-0.3 MPa). During the tested period GP increases, and the maximum value was noticed at the eighth day for the control (85%). In the case of the stress incidence GP had lower value, even at the ninth day (25% in the case of -1.2 MPa NaCl and 40% at -1.2. MPa mannitol).

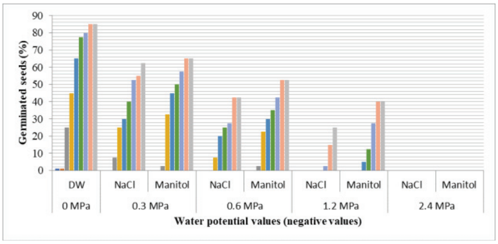

Figure 1. Cumulative germination percentage of *F. arundinacea* seeds for 9 days exposed to different water deficit inducedbydifferentconcentrationsofNaClandmannitol

The negative impact of NaCl may be due not only by its osmotic effects(Keisham et al., 2018), impairment of water uptake to assure seeds imbibition (Woodstock, 1988), or by the influence of Na ions absorbed. But, as recently it was largely explained, Cl- toxicity in plants is an actual issue, so, a reduction of its accumulation in the shoot, would be a plausible way to reduce Cl toxicity (Li et al., 2017). Moreover, no germination was noticed in the case of -2.4 MPa. Our results are consistent with those obtained by Peng et al. (2013). They found that in the case of *Festuca sinensis*, there was no germination under sever water stress (-1.2 MPa), if hydro-priming treatment had not been applied.

Final germination percentage (FGP)

Instead, as expected, the final germination percentage shows lower values compared to the control, for both stress conditions (saline and drought conditions) (Figure 2), but especially in the saline stress situation, where from -0.6 MPa, differences from the control were significantly lower from statistically view point, at P≤ 0.5 level of significance.

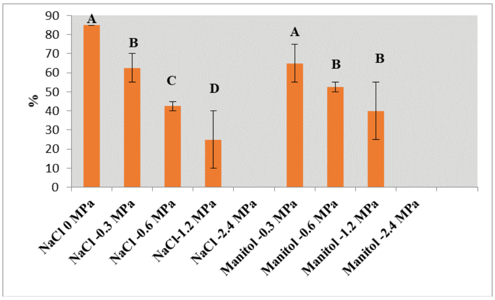

Figure 2. Final germination percentage of *F. arundinacea* seeds as influenced by different water potential induced by NaCl and mannitol solutions.

Values are means ±SE of two independent replications (n=2). Means followed by the same letter(s) are not significantly different, at P≤0.05. The graph shows the results of the T test, when the stressed variants are compared with the control.

As the ANOVA results showed FGP was statistically significantly affected by the stress severity ($F= 33.42$, $P<0.001$). Moreover, germination has been blocked in the case of the lowest water potential (-2.4 MPa).

In this context, it is necessary to mention that the soil water potential for plants ranges from -0.03 to -1.5 MPa (O'Geen, 2012) and mild to severe salt stress in the field ranges from 40 to 160 mM NaCl (Abrol et al., 1988).In the case of drought stress induced by mannitol, although the values are different, there were no statistically significant differences as compared with the control, at -0.3 MPa.

It is known that the percentage of germination is a common indicator which quantifies the effect of different factors on physiological seed quality. However, this indicator does not only offer the possibility of explaining the delay germination. Consequently, all other indicators are very useful for interpreting positive or negative impact from the physiological point of view.

Mean germination time (MGT)
As regard as mean germination time, as we can see in Figure 3, the highest value (8.19 days) was registered in the case of seeds germination on saline conditions that assured a water potential of -1.2 MPa. Also, a higher value (6.74 days) was noticed in the case of the drought stress assured by mannitol, at -1.2 MPa, but the differences between these two variants are significantly from the statistical view point ($P<0.05$).

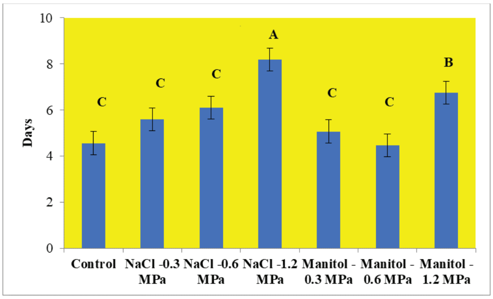

Figure 3. Mean germination time of *F. arundinacea* seeds (mean±SE)(n=2). Means followed by the same letter(s) are not significantly different, at $P\leq0.05$. The graph shows the results of the T test, when the stressed variants are compared with the control.

Of course, MGT was significantly reduced when seeds were not stressed during germination and even if in the case of the slightly stress this indicator had higher values, there were not significantly differences as against the control, at $P<0.05$.

Mean daily germination (MDG)
As noted in Figure 4, regardless of the stress factor and its severity, there are significant differences against the control, at which the maximum value was 10.62.
Instead, it should be obvious that the lowest values were recorded in saline stress conditions, and they decreased statistically significantly as compared with the control, as seen in the graph for sodium chloride, which provided a water potential of -0.3 MPa, and respectively -0.6 MPa ($P <0.001$).
Environmental conditions, as well as different water sources, have a different influence on herbaceous plants and as a result their degree of tolerance is variable. From this point of view, the behaviour at the fescue is an intermediate one (Cameron and Hitchmough, 2016). The authors mention, however, that the salinity impact is a major one during the germination process and during the early growth of the plants.

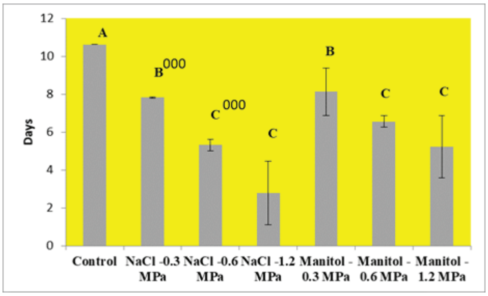

Figure 4. Mean daily germination of *F. arundinacea* seeds as influenced by different water potential induced by NaCl and mannitol solutions (mean ± SE) (n=2). Bars labelled with the same letter(s) are not significantly different, as compared with the control at $P\leq0.05$.

Therefore, salt and drought stress negatively affected the germination process, both in terms of total germination percentage, but also about speed germination.

Seedlings growth
Seedlings growth data are presented in Table 1. Like germination, salt stress has significant effect on the seedling growth. It is noted that salt stress highly significantly affected the plumule length, the maximum being recorded in control solution and the lower one on the solution of -1.2. MPa. The longest plumule was observed at the control, while the values have decreased as the severity of stress has increased.
Similar results were obtained by Manuchehri and Salehi (2015) who studied the interaction between irrigation and salinity.
As Kramer (1983) explained, the first physiological indicator that can be measured to be affected in the case of water deficit is the growth process, which is reduced because of the decline of the first step of the cell enlargement, which has been shown to be more sensitive to water scarcity, compared to the cell division stage.

Table 1. Seedlings and biomass attributes of *F. arundinacea* under saline stress (mean ± SE) (n=10). Means followed by the same letter(s) are not significantly different, at P≤0.05, when the stressed variants are compared with the control.

Variant	RL (cm)	PL (cm)	SL (cm)	SVI	RFW (g) (10 seedlings)	PFW (g) (10 seedlings)
Control	3.69±0.25A	5.13±0.21A	8.82±0.39A	749.70	0.028±0.01A	0.0383±0.001A
-0.3 MPa NaCl	2.38±0.28B	3.44±0.25B	5.82±0.49B	392.85	0.0221±0.003A	0.0344±0.002A
-0.6 MPa NaCl	1.44±0.24C	1.61±0.26C	3.05±0.48C	129.62	0.0035±0.0003B	0.0213±0.0002B
-1.2 MPa NaCl	0.56±0.06D	0.87±0.08D	1.43±0.12D	28.60	0.0019±0.0001C	0.0103±0.0016C

RL=roots length; PL=plumule length; SL= seedling length; SVI= seedlings vigor index; RFW=roots fresh weight; PFW= plumule fresh weight

Seed priming results

As we can see in Table 2, application of seeds priming treatments produce different changes as regard as some germination parameters, as well as concerning the seedlings growth.

As for instance, final germination percentage (87.5 %) (P3) was significantly increased as compared with the control (85%) (P1), due to seeds priming with ASFAC 0.1% for 6 hours, then germinated on distillate water. Higher ASFAC concentrations determined non-significant differences when normal conditions were assured. In the case of saline stress, the value of this indicator decreased significantly or highly significantly along with the stress severity increasing (14.4% at -1.2 MPa, even if seeds were primed with 0.4% ASFAC).

Mean germination time was highly significantly reduced to 3.47 days (P3), as compared with the control (4.56 days). At the opposite pole there is P7, when priming with ASFAC 0.2% followed by germination on -0.6 MPa, highly significantly increased the MGT. In other cases, values were not different from the statistical point of view.

Mean daily germination was not significantly influenced by seeds priming, when the germination was carried out on distillatewater, but, in line with the saline stress severity increasing, there were registered lower values of MDG, with the lowest one (1.81) at P11, when from statistically view point, difference against the control was strongly.

Early seedling growth was differentially affected. Instead, even normal germination conditions were assured, there were registered non-significant differences between non-priming and hydro-priming variants, considering total seedlings length, roots length and shoot length. Then, priming with a low dose of ASFC (P3) induced a significantly longer roots (6.23 cm), compared with the control (3.6 cm).

We mention that, for the length of the roots, all the radicles formed were measured and it was found that the formation of new radicles was favoured by P1; P4 and P5, respectively.

Higher ASFAC concentrations associated with saline severity increase determined a shoot reduction increase. So, ASFAC priming promoted the rates of radicle extension, concretized and favouring the branching of the root system. Blunk et al. (2019) also points out that early plant development in the case of primed seeds may be due to a better development of the root system than untreated control, which allows an increase in soil volume as the source of nutrient absorption.

The present results agree with those obtained by Peng et al. (2013) which revealed that seed hydro-priming, combined with the presence of the *Neotyphodium* endophyte improved seed germination and plant growth of *F. sinensis*.

Taking into consideration our results, as well as those obtained by Rouhi et al. (2011) and Tilaki et al. (2010), knowing the positive effects of seeds priming on germination and early seedlings growth, it is important to note that further field researches are needed, so that the results of the laboratory studies can be confirmed.

Table 2. Influence of *F. arundinacea* seed priming treatments, on germination, seedlings growth and shoot chlorophyll content under saline stress conditions (mean ± SE) (n=10).

Priming treatment	FGP (%) (n=2)	MGT (days) (n=2)	MDG (days) (n=2)	Seedling Lenght (cm) (n=3)	Roots lenght (cm) (n=3)	Shoot lenght (cm) (n=3)	Total chlorophyll (n=5)
P1-Control	85.00± 0	4.56± 0.32	10.62± 0	8.8± 0.60	3.6± 0.38	5.2± 0.42	164.6± 4.58
P2	80.50± 4.94^{ns}	4.24± 0.14^{ns}	10.06± 0.44^{ns}	10.6± 1.73^{ns}	5.67± 1.69^{ns}	4.93± 0.07^{ns}	171.2± 2.35^{ns}
P3	87.50± 0.71^{*}	3.47± 0.26^{00}	10.93± 0.06^{ns}	11.47± 1.41^{ns}	6.23± 0.99^{*}	5.23± 0.43^{ns}	173± 4.27^{*}
P4	84.50± 0.71^{ns}	4.15± 0.03^{ns}	10.56± 0.06^{ns}	11.37± 0.93^{ns}	5.7± 0.87^{*}	5.67± 0.17^{ns}	180± 3.30^{*}
P5	86.50± 0.71^{ns}	4.29± 0.01^{ns}	10.8±1 0.06^{ns}	10.43± 1.62^{ns}	5.67± 1.20^{ns}	4.77± 0.43	185± 2.47^{**}
P6	74.00± 1.41^{0}	6.37± 0.83^{ns}	9.25± 0.12^{0}	6.07± 0.23^{00}	2.83± 0.17^{ns}	3.23± 0.14^{ns}	184.6± 1.75^{***}
P7	27.50± 0.71^{00}	6.20± 0.23^{**}	3.44± 0.06^{00}	2.13± 1.91^{00}	1.5± 0.76^{0}	0.63± 0.35^{000}	undetected
P8	0	0	0	0	0	0	undetected
P9	77.50± 0.71^{0}	3.99± 0.05^{ns}	9.69± 0.06^{0}	9.33± 0.96^{ns}	4.66± 0.60^{ns}	4.67± 0.55^{ns}	183.4± 2.91^{**}
P10	72.50± 2.12^{0}	4.67± 0.13 ^{ns}	9.06± 0.19^{0}	5.8± 0.94^{0}	3.07± 0.58^{ns}	2.73± 0.37^{0}	181.8± 3.26^{*}
P11	14.50± 2.12^{00}	5.95± 0.11^{0}	1.81± 0.19^{000}	0	0	0	undetected

*0,**00 and ***000 mean significant at P≤0.05; P≤0.01 and P≤0.001 level, respectively in T-test, when the variants are compared with the control. ns=not significant

Plant responses to the action of a stress factor is closely correlated with the dose of action of the stressor and involves a chain mechanism to quantify the stress factor action and to control the triggering of a control mechanism at the molecular level (Claeys et al., 2014).

Besides testing for germination, meaningful results can be obtained in connection with early seedlings growth potential. As it can be seen from the data given in Table 2, salt stress determined a significant reduction in plumule and radicle growth for all conditions tested. It should be emphasized that salt stress had an obvious inhibitory effect, but a stronger one was noticed for plumule. Similar results were obtained with the testing action of phytotoxic substances (Kusvuran et al., 2014), and the phenomenon was explained by the fact that the radicle is the first tissue that experiencing substance.

As is otherwise known and as mentioned recently by Gao et al. (2015a), the decrease of the water potential at the level of the soil solution leads to the impossibility of endosmosis, as the main process of water absorption by the plant cell. Moreover, at the cellular level there is a process of accumulation of reactive oxygen species (ROS), whose effect on the physiological and biochemical processes is negative. More than that, ROS activity leads to a reduction in the growth process.

For example, an interesting study was carried out by Gao et al. (2015b) who have demonstrated that He-Ne laser pre-illumination determined an increased *Festuca*seedlings tolerance to salt stress due to exceeding the damage caused by oxidative stress, through free radicals and the induction of some genes with a role in the functioning of the plant antioxidant system. The authors noticed that the induction of phytochrome B transcriptional level by He-Ne laser was probably correlated with these processes. Besides, as Martin et al. (2012) noticed, understanding the mechanisms that happen at the cellular level during a stressor action finds successful application to commercial crops worldwide.

According to Feki et al. (2015), for a good understanding of the key pathways that control plant tolerance to salinity, in the future, it is necessary to integrate the information provided by research undertaken in genomics, transcription, proteomics, and not utterly, of metabolomics.

CONCLUSIONS

In short, it can be concluded that the effects of salt and drought stress on tall fescue seed germination and seedlings growth depend on the characteristics and severity of stress.

By giving suitable seeds priming some common problems of germination can be overcome.

Seed priming with ASFAC-BCO-4 prior to sowing stimulates the physiological activities of seeds which resulted in rapid and uniform seed germination. This priming method also improvesupgrades early seedlings growth, stimulates the chlorophyll synthesis and vigorous seedlings are obtained.

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