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Research Note

Germination performance of different forage grass species at different salinity (NaCl) concentrations

M Mangwane[1,2], IC Madakadze[1*], FV Nherera-Chokuda[2], S Dube[3] and M Mndela[1,2]

[1] *Department of Plant and Soil Sciences, University of Pretoria, Pretoria, South Africa*
[2] *Agricultural Research Council (ARC), Irene, South Africa*
[3] *International Livestock Research Institute (ILRI), Mount Pleasant Harare, Zimbabwe*
**Correspondence: casper.madakadze@up.ac.za*

Certain grasses show potential for the rehabilitation of coalmine spoils. Species selection and evaluation are used to guide the choice of the most appropriate grass species. This study evaluated the germination performance of seven forage grass species, with some represented by two varieties, under varying salinity conditions of 0 (distilled H_2O), 100, 200, 400, 600, 800 and 1 000 mS m^{-1} of NaCl. Cumulative germination, final germination percentage (FG%), and time taken to reach 50% of the final germination (T_{50}) were determined for each species–treatment combination. Species × salinity interaction was significant (*p < 0.01*) for cumulative germination, FG% and T_{50}. Cumulative germination increased gradually up to 17 days and thereafter declined. The highest FG% for all grass species was attained under distilled water (0 mS m^{-1}), ranging from 38% to 94%, and declined significantly (*p < 0.01*) with an increase in salinity. T_{50} increased with increasing salinity for all grass species. *Eragrostis curvula* var. Ermelo and *Lolium multiflorum* var. Archie were the quickest to germinate and attained significantly (*p < 0.01*) higher values of FG%, of 45% and 50%, respectively, at 600 mS m^{-1}, indicating higher salt tolerance than the other species. Overall, increasing salinity reduced the germination performance of all grass species tested; however, Archie and Ermelo showed higher potential for rehabilitation of coalmine spoils irrigated with saline water.

Keywords: *Eragrostis curvula*, *Lolium multiflorum*, mine rehabilitation, saline conditions, South Africa

Coal mining in South Africa is often associated with negative environmental impacts, hence rehabilitation by mean of revegetation is required post mining (Mhlongo and Amponsah-Dacosta 2016; Mentis 2020). During mining, acid mine drainage releases toxic substances that pose a serious threat to ecosystem services and functioning around the mined area (Kumar 2013). Low pH (2.5–3.2) and a high concentration of heavy metals do not allow acid mine drainage to be discharged into streams; therefore, acid mine drainage is treated with gypsum ($CaSO_4 \cdot 2H_2O$) to neutralise the pH and reduce toxicity (Annandale et al. 2001). However, the salinity of mine water may be as high as 557 mS m^{-1}, which might negatively affect plant germination and growth (Maree et al. 2004). When treated, mine water is used mainly for irrigation of forage grasses during active restoration of opencast coalmines (Annandale et al. 2006). However, gypsum tends to precipitate, thereby increasing soil pH above threshold levels (neutral state) to a saline state, as a result of the build-up of salt ions (Na^+ and Cl^-) (Li et al. 2014). This condition can restrict seed germination and plant establishment through the impairment of metabolic processes in the seeds (Li et al. 2014). Among other outcomes, the uptake of salt ions increases osmotic stress, by reducing the imbibition of water into the seeds (Kaymakanova 2009).

Germination is the first stage of a plant's lifecycle, and its degree of success indicates whether a plant will become fully established. Germination involves breaking of seed dormancy to support the developing embryo through metabolic processes, such as oxidation of stored lipids and carbohydrates required to nourish the embryo (Tsegay and Gebreslassie 2014). Aloui et al. (2013) reported that high levels of salinity affect radicle and plumule development. Variations in salinity tolerance were reported by Ma et al. (2014) in a germination study of 18 grass species. Zhang et al. (2011) described that germination and seedling growth significantly diminished with increasing salinity. Excess salt ions limit not only the absorption of water by germinating seeds, but also nutrient uptake, leading to poor germination performance (Ayaz et al. 2000; Easton and Kleindorfer 2009). Moreover, the reduction in water uptake may result in death of the germinating seeds, because salt ions inhibit enzymes involved in metabolic processes during germination and can ultimately destroy the developing embryo (Keshavarzi et al. 2011).

High soil salinity has the potential to prevent active restoration of opencast coalmines unless salt-tolerant grass species are used. Tolerance to salinity is species-specific and can also vary with the plant variety (Ma et al. 2014). Therefore, it is crucial to identify grass species and varieties that can successfully germinate within a short period under saline conditions (Kaouther et al. 2013) and to conduct follow-up assessments of seedling establishment. Active rehabilitation through phytoremediation therefore entails screening of the most salt-tolerant species (Kaouther et al. 2017). Once forage grasses are established, the revegetated area can be utilised for grazing cattle (Limpitlaw and Briel 2014). Therefore, proper selection of forage grass species for mine rehabilitation should consider not only the most salt-tolerant, but also highly productive species. Here, we selected both subtropical and temperate grass species, because the use of species with alternate growing seasons can ensure active grass cover throughout the year. The objective of the study was to evaluate the potential salt tolerance of the forage grass species by determining their germination performance under saline conditions.

A greenhouse experiment was conducted at the University of Pretoria Experimental Farm, Pretoria, South Africa. The glasshouse was air-conditioned with constant temperatures set to 25 °C during the day and 10 °C at night, with 12 hours of daylight. The grass species selected (with seeds sourced from Agricol) were: *Eragrostis curvula* L. (var. Ermelo and Agpal), *Chloris gayana* L. (var. Katambora), *Digitaria eriantha* L. (var. Irene), *Penisetum clandestinum* L. (var. Whittet), *Lolium multiflorum* L. (var. Archie and AgriBoost), *Lolium perenne* L. (var. Belay and Halo) and *Festuca arundinacea* L. (var. Dovey and Fuego). Here, we refer to each variety of each species as a species, accordingly we discuss 11 species. The minimum and maximum temperatures at the Kleinkopje Colliery opencast mine in Mpumalanga province, taken for consideration in this study, varied widely, at approximately 8 °C and 23 °C, respectively (Platt 2009). Therefore, the temperatures at which the seeds were germinated were set such that they suited both subtropical and temperate species. A study by Lin et al. (2018) showed that these *Lolium* species attained optimal germination at approximately 25 °C; moreover, the subtropical species selected in this study are promising for forage production in the context of mine rehabilitation (Mentis 2020), whereas temperate *Lolium* species are reported to be tolerant to some degree to saline conditions (Lin et al. 2018; Uslu and Gedik 2019). Seeds of these species were subjected to seed viability tests using tetrazolium chloride, before and at the end of the experiment. One hundred seeds of each grass species were placed in petri dishes lined with Whatman No. 2 filter paper. The seeds were allowed to germinate in distilled water (0 mS m^{-1}, controls) or in varying sodium chloride (NaCl) solutions of 100, 200, 400, 600, 800 and 1 000 mS m^{-1} . The 11 grass species × 7 treatment combinations were replicated three times in a randomised completely design on germination benches. The experiment was repeated once to eliminate measurement errors, while still observing variability among individual measurements.

The petri dishes were watered twice daily (in the morning and afternoon). Observations were done daily in the morning and afternoon. Seeds were considered germinated after the appearance of a 2-mm radicle (Madsen et al. 2018). All germinated seeds were counted and removed from each petri dish daily, until there was no sign of further germination for at least three consecutive days. Germinated seeds were removed from the petri dishes using a toothpick. Germination performance was assessed through final germination percentage (FG%) and time (in days) taken to 50% final germination (T_{50}) fitted to seed germination curves (cumulative germination vs time).

The FG% and T_{50} data were first tested for normality and heteroscedasticity using the Kolmogorov–Smirnov test and the White test, and were normally distributed. Thereafter, a two-way analysis of variance (ANOVA) was used to test the fixed effects of the salinity treatments and grass species and their interaction on FG% and T_{50}, using the GLM procedure of SAS (SAS Institute 2003). Mean separation on FG% and T_{50} was determined using Fisher's protected least significant difference (LSD) test, with significant differences between means reported at the 95% confidence level.

The interaction between grass species and treatment was significant ($p < 0.05$) for FG%. Relative to the controls (0 mS m^{-1}), the FG% of all grass species was less when watered with a salt solution (Figure 1). The species responded differently under varying salinity levels. The response of *L. multiflorum* var. Archie, *E. curvula* var. Ermelo, *L. multiflorum* var. AgriBoost, and *P. clandestinum* var. Whittet was a linear decline in FG% ($r^2 > 0.8$ for all) with increased salinity. *Lolium perenne* var. Belay, *D. eriantha* var. Irene, *C. gayana* var. Katambora, *L. perenne* var. Halo, *F. arundinacea* var. Fuego, and *F. arundinacea* var. Dovey showed exponential decline in FG% with increasing salinity. *Lolium multiflorum* var. Archie, *E. curvula* var. Ermelo, and *E. curvula* var. Agpal attained a generally higher FG% (≥80%) at 0 mS m^{-1} and at 100 mS m^{-1}, compared with the other germinated grass species (Figure 1).

The seed germination curves of all grass species differed significantly ($p < 0.01$) in terms of cumulative germination and germination rate (Figure 2). In general, germination was quicker under low-salinity conditions (100 and 200 mS m^{-1}), but took ≥7 days to commence at salinities beyond 400 mS m^{-1}. *Lolium multiflorum* var. Archie and *E. curvula* var. Ermelo were the first grass species to germinate, within 2–3 days, and stopped germinating after 7–9 days. At salinities of ≥400 mS m^{-1}, only *L. multiflorum* var. Archie displayed high cumulative germination values, ranging from 13 ± 3.6% to 57 ± 3.6%, up to day 13. Under all salinity treatments, *D. eriantha* var. Irene and *C. gayana* var. Katambora showed relatively slow germination and low cumulative germination of <50%. Even though *L. multiflorum* var. AgriBoost and *E. curvula* var. Agpal showed a somewhat slower onset of germination, at 3–4 days, they attained high values of FG% at 0 and 100 mS m^{-1}. *Pennisetum clandestinum* var. Whittet, *F. arundinacea* var. Dovey, and *F. arundinacea* var. Fuego were slowest to germinate, at 7–8 days, but thereafter the germination rate abruptly increased up to day 16. *Lolium perenne* var. Belay and *L. perenne* var. Halo were slowest to germinate, at 7–8 days, under most salinity treatments.

The T_{50} was significantly affected ($p < 0.001$) by the interaction of grass species and salinity treatment. Across all species, the T_{50} increased with increasing salinity (Table 1), but the values of T_{50} varied among species. Significant differences ($p < 0.01$) in T_{50} appeared among the grasses that germinated at salinities of ≥400 mS m^{-1} . *Eragrostis curvula* var. Ermelo, *C. gayana* var. Katambora, and *L. multiflorum* var. Archie attained T_{50} earlier than the other species under all salinity conditions. The earliest T_{50} was recorded in the distilled water treatments and did not differ ($p > 0.05$) from that of 100 and 200 mS m^{-1} for *E. curvula* var. Ermelo; *L. multiflorum* var. Archie attained

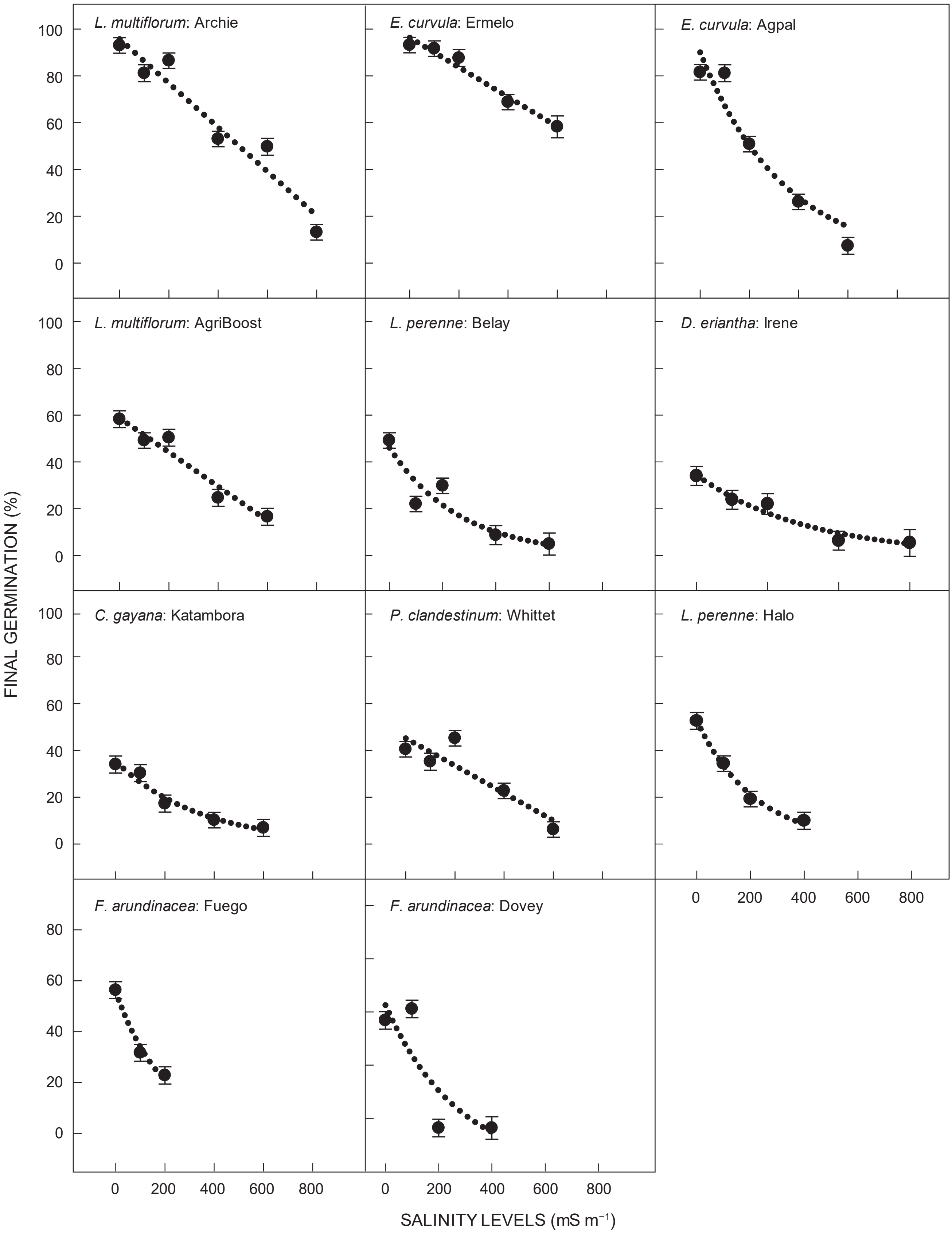

Figure 1: Final germination percentage of forage grass species under different salinity conditions in petri dish experiments. Bars extending beyond each point denote the standard error of mean (SEM). See Table 1 for full species names

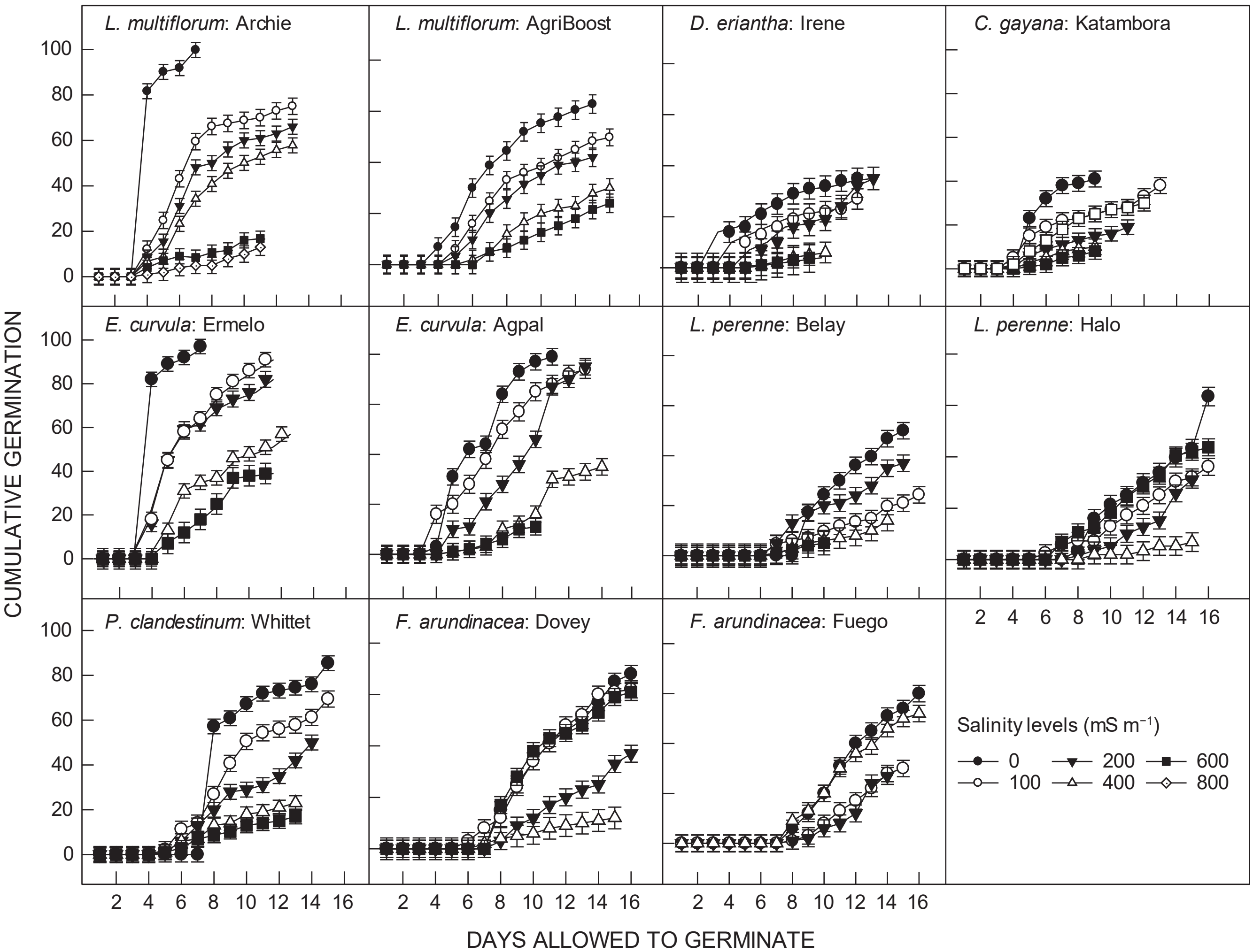

Figure 2: Seed germination curves of different forage grass species under varying salinity conditions (NaCl solutions) in petri dish experiments. Bars extending beyond each point denote standard error of the mean (SEM). See Table 1 for full species names

T_{50} within 8 days at 800 mS m^{-1} and was the only species that germinated at this salinity level. For *D. eriantha* var. Irene, *P. clandestinum* var. Whittet, and *L. multiflorum* var. AgriBoost, the T_{50} ranged from 7 to 9 days at salinity levels of 0–600 mS m^{-1} . Across all treatments, *L. perenne* var. Belay, *L. perenne* var. Halo, *F. arundinacea* var. Dovey, and *F. arundinacea* var. Fuego took longest to reach T_{50}, at approximately 10 to 12 days (Table 1).

The significant interaction between treatments and species on FG% and T_{50} indicated interspecific differences in their tolerance to salinity, probably owing to different seed characteristics among these grasses. The response patterns of all species in terms of FG% declined with increasing salinity (Figure 1), but the rates and extent of decline in germination were different. This suggests that some species that are able to germinate at higher salinity levels (e.g. *L. multiflorum* var. Archie and *E. curvula* var. Ermelo at 800 mS m^{-1}) are possibly more salt-tolerant, because of high proline levels in the seeds. High proline levels in seeds protect the seed enzymes against salt-ion toxicity (Wu et al. 2015). *Lolium multiflorum* var. Archie and *E. curvula* var. Ermelo not only showed better germination performance at higher salinities, but also germinated earlier (on day 3) than the other species (Figure 2). The better performance of *L. multiflorum* var. Archie relative to the other varieties of the same species suggests that, apart from interspecific differences, the variation in germination performance might also be explained by genetic differences.

The early commencement of germination and faster time to reach T_{50} for *L. multiflorum* var. Archie and *E. curvula* var. Ermelo (Figure 2; Table 1) suggests that the seeds of these species are easily scarified by saline conditions. This further indicates that seedling establishment may occur much earlier than with other species, thereby hastening the rehabilitation of opencast coalmines. Other studies (e.g. Uslu and Gedik 2019) have reported response patterns for *L. multiflorum* similar to this study, whereby germination performance declined with increasing salinity. However, Lin et al. (2018) found optimal germination of *Lolium* seeds at 400 mS m^{-1} at temperatures of 20 °C to 30 °C, and concluded that the *Lolium* species may be more salt-tolerant at these temperatures. A low FG% and delayed germination

Table 1: Effect of varying salinity levels on the number of days it took for 50% of germinating seeds to germinate (T_{50}) for different forage grass species. Means in the same column with different lowercase letters indicate significant ($p < 0.05$) differences between species; means within the same row with different uppercase letters indicate significant ($p < 0.05$) differences between treatments, using the least significant difference ($LSD_{(0.05)}$) test. Dashes indicate no germination

Grass species	Salinity level (mS m^{-1})						
	0	100	200	400	600	800	$LSD_{(0.05)}$
Eragrostis curvula var. Ermelo.	5.7^{aA}	5.8^{aA}	5.7^{aA}	6.0^{aA}	7.2^{aB}	–	0.68
Eragrostis curvula var. Agpal	6.5^{acdA}	7.0^{bAB}	7.2^{bAB}	7.7^{aB}	7.3^{aB}	–	0.79
Chloris gayana var. Katambora	6.0^{acA}	6.1^{aAB}	6.2^{abAB}	6.2^{aAB}	6.8^{aB}	–	0.72
Digitaria eriantha var. Irene	6.8^{cdA}	7.6^{bA}	7.8^{bA}	10.2^{bB}	7.5^{aA}	–	1.54
Pennisetum clandestinum var. Whittet	7.3^{dA}	7.7^{bA}	9.5^{cB}	9.8^{bB}	9.0^{bB}	–	1.18
Lolium multiflorum var. Archie	5.0^{aA}	6.0^{aB}	6.5^{abB}	6.5^{aB}	7.2^{aC}	7.8^{D}	0.59
Lolium multiflorum var. AgriBoost	7.2^{dA}	7.8^{bA}	9.0^{cB}	9.3^{bB}	9.2^{bB}	–	1.13
Lolium perenne var. Belay	10.2^{eAB}	10.3^{cAB}	9.5^{cA}	11.5^{cB}	–	–	1.92
Lolium perenne var. Halo	10.2^{eA}	11.0^{cAB}	11.3^{dAB}	12.7^{cB}	–	–	1.92
Festuca arundinacea var. Dovey	9.83^{eA}	10.0^{cA}	9.7^{cA}	12.2^{cB}	–	–	1.36
Festuca arundinacea var. Fuego	10.66^{eA}	11.5^{cA}	11.6^{dA}	–	–	–	1.02
$LSD_{(0.05)}$	0.95	1.14	1.30	1.82	1.12	–	

at higher salinity levels for the other species studied here could be ascribed to continuous uptake of Na^+ and Cl^- ions at the high salinity levels and an inability to regulate their internal osmotic potential (Zhang et al. 2015). Accumulation of these ions reduces enzymatic and hormonal activities, including mobilisation and synthesis of the embryonic sugars and proteins of the seeds (Adda et al. 2014). Among these enzymes is α-amylase, which assists with degrading the starch reserves during imbibition (Hopkins and Huner 2004).

Differences in FG% and T_{50} between the species might be ascribed to interspecific differences in seed dormancy, hardness and thickness of the seed coat. Longer T_{50} under high-salinity conditions, as observed for most of the grass species here, might be caused by reduced water uptake, because of a high accumulation of salt ions (Kaouther et al. 2013). The quicker the time to reach T_{50} (Table 1), the higher the likelihood that the species will escape the effects of salts that will build up later. Ma et al. (2014) reported that T_{50} varied greatly, because of differences among grass species, and that T_{50} increased gradually as the salt solutions increased for many species. For those grass species that took longer to reach 50% of final germination, toxic effects within the germinating seed might have resulted in a reduction of energy in the endosperm to nourish the developing embryo (Zhu 2003).

The results showed that high salinity levels of ≥600 mS m^{-1} had detrimental effects on seed germination of all the forage grass species tested in petri dish experiments. The grass species responded differently to salinity levels of 0−1 000 mS m^{-1}, with some germinating relatively quickly (within 2–3 days), whereas others were slower and displayed a relatively low percentage of final germination. *Lolium multiflorum* var. Archie and *Eragrostis curvula* var. Ermelo were the earliest to germinate and attained a higher percentage of final germination at higher salinity levels compared with the other species, consequently demonstrating better salt tolerance and overall germination performance.

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ORCIDs

M Mangwane: https://orcid.org/0000-0003-1980-540X
IC Madakadze: https://orcid.org/0000-0001-8594-8260

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