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

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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 H2O), 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 (T₅₀) were determined for each species–treatment combination. Species × salinity interaction was significant (p < 0.01) for cumulative germination, FG% and T₅₀. 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. T₅₀ increased with increasing salinity for all grass species. Eragrostis curvula var. Archie and Lolium multiform 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.

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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. Delay 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 (T50) fitted to seed germination curves (cumulative germination vs time).

The FG% and T50 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 T50, using the GLM procedure of SAS (SAS Institute 2003). Mean separation on FG% and T50 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% (r2 > 0.8 for all) with increased salinity. *L. perenne* var. Delay, *D. eriantha* var. Irene, *C. gayana* var. Katambora, *L. perenne* var. Halo, *F. arundinacea* var. Fuego, and *P. clandestinum* var. Dovey showed exponential decline in FG% with increasing salinity. *L. 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. *L. 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. *P. 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. *L. perenne* var. Delay and *L. perenne* var. Halo were slowest to germinate, at 7–8 days, under most salinity treatments.

The T50 was significantly affected (p < 0.001) by the interaction of grass species and salinity treatment. Across all species, the T50 increased with increasing salinity (Table 1), but the values of T50 varied among species. Significant differences (p < 0.01) in T50 appeared among the grasses that germinated at salinities ≥2400 mS m−1. *E. curvula* var. Ermelo, *C. gayana* var. Katambora, and *L. multiflorum* var. Archie attained T50 earlier than the other species under all salinity conditions. The earliest T50 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.
T50 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 T50 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 T50, at approximately 10 to 12 days (Table 1).

The significant interaction between treatments and species on FG% and T50 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).

*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 T50 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

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**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.
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 T₅₀ between the species might be ascribed to interspecific differences in seed dormancy, hardness and thickness of the seed coat. Longer T₅₀ 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 T₅₀ (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 T₅₀ varied greatly, because of differences among grass species, and that T₅₀ 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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