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REPORT ON SCREENING OF PLANT SPECIES FOR SUITABILITY FOR REHABILITATION OF DUMPS AND TAILINGS OF PALABORWA COPPER MINING COMPANY, 2015

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INTRODUCTION

A short-term rehabilitation screening study was initiated by SAEON in 2014, in collaboration with the University of Stellenbosch. An MSc student was appointed to test for levels of a particular plant hormone (strigolactone) in plant species that are or might be used for rehabilitation work on PMC. The overall aim of this research is to determine whether strigolactone levels are a reliable indicator a species suitability for rehabilitation, thus providing a tool for screening a far greater range of species than those currently used.

The plant hormone strigolactone was selected as it is known to induce mycorrhizal symbiosis and modulate responsiveness to environmental stresses. These include salinity, pH, low or excessive nutrient concentrations, moisture limitations and heavy metal accumulation.

PROGRESS TO DATE

Germination trials

Commercial seed was obtained from Diverse Ecological Solutions (Pty. Ltd.) These include the 5 species presently used by Palabora Copper (*Anthephora pubescens, Cenchrus ciliaris, Chloris gayana, Cynodon dactylon and Panicum maximum*) and 6 alternative species. The latter were selected according to the following criteria: commercial availability, indigenous, mycorrhizal, perennial and so-called pioneer species. The species selected were *Enneapogon cenchroides, Eragrostis teff, Melinis repens, Paspalum notatum, Stripagrostis uniplumus and Tragus berteronianus*.

Germination trials were carried out using commercial vermiculite and raw capping material collected from PC, and under ambient and greenhouse conditions. Trials were repeated in Jan-Mar, Apr-May, Jun-Jul and Aug-Sept 2015. Germinants (seedlings) from the germination trials were used in subsequent treatments accessing the effect of plant-growth promoting substances (including the synthetic strigolactone analogue GR24) on seedling vigour (as an indicator of likely survivorship). Of the 11 species tested, only 7 species germinated; being the 5 species presently used by PC in seeding the mine dumps, as well as *E. teff* and *M. repens*. The remaining species (*Tragus, Paspalum, Stipagrostis uniplumis* and *Enneapogon*) did not germinate, despite several attempts and modifications to the protocols.

Unfortunately the results from the germination trials varied largley between repeats. However, *Eragrsotis teff* and *Anthephora pubescens* consistently showed the most rapid, synchronous and highest % germination. Poor germination was recorded for *Panicum maximum*; with intermediate responses seen for *Cenchrus, Cynodon, Chloris* and *Melinis*.

High levels of mortality were recorded following the transplant of seedlings to pots containing raw capping material for treatment with growth-promoters; especially for *Panicum maximum, Eragrsotis teff, Melinis repens* and *Cynodon dactylon.*

A second germination trial was conducted to assess indigenous grass species from the area. Student M. Jacob Rossouw and project supervisor Dr PN Hill visited PC in late May 2015 and collected seed from 5 naturally-occurring, dominant grass species viz. *Stripagrostis hirtigluma, Cenchrus ciliaris, Pennisetum setaceum, Aristida adscensionis* and *Enneapogon cenchroides*. In addition, rhizosphere soil samples were collected from around the roots of *Enneapogon, Cenchrus, Pennisetum, Stripagrostis hirtigluma* and *Tephrosia polystachya* – a dominant forb species, growing on recently (2 yr) and previously (+10 yr) capped rock dump slopes. Seed was subjected to the same germination trials as outlined above. Poor germination was recorded for *Stripagrostis hirtigluma* and *Aristida adscensionis*, whilst the seed of the remaining 3 species germinated within the range seen for the commercially available seed of the same species

Growth promoters

The role of various commercially available plant growth promoting substances (PGPS) as treatment applications were investigated to determine their effect on growth for the grass species. Lumichrome, strigolactones (GR24), flavonoids (CropbioLife™), smoke-water (karrikins) and arbuscular mycorrhizal fungi (Mycoroot™) were tested as possible growth-promoting additives.

The positive or negative effect of the PGPS, relative to control seedlings treated with distilled water, varied markedly between species, with no single PGPS being consistently superior. For example, GR24 increased rooting in *Chloris*, but Lumichrome increased the number of leave sand fresh mass in the same species. Lumichrome increased root length and fresh mass in *Panicum maximum*, but decreased fresh mass for *Melinis repens*. Both GR24 and Lumichrome were necessary for the survival of *Eragrsotis teff* seedlings. Lumichrome increased overall seedling vigour for *Anthephora*, whereas Cropbiolife simply increased leaf number. Treatment with smoke-water decreased rooting in this species. Lumichrome also increased vigour in *Enneapogon*. Mycoroot increased seedling vigour in *Chloris*, but had no effect for *Cenchrus* or *Anthephora*.

For the 5 indigenous grass species, the effect of PGPS on the seedlings was also highly variable.

Rhizosphere characterization

Microbial DNA was extracted from the rhizospheres of the 5 dominant species present on the rehabilitation sites. Between 2-9 bacterial strains were found in association with their roots. For the most part, the bacterial strains belong to the genera *Baccillus, Brevibacterium* and *Cupriavidus* – all with reported plant benefits (promoting N-fixation and root nodulation, enhancing heavy metal tolerance, increased elemental availability) that would promote survival under stress-conditions. Fungal associates were typically in the *Glomeromycota*, a group of AMF known to assist in the acquisition of nutrients in nutrient poor environments and to improve plant tolerance to heavy metal stress in polluted soils.

For the indigenous grass species, arbuscular mycorrhizal associations were seen in *Cenchrus, Chloris, Pennisetum, Cynodon* and *Enneapogon*, in the PGPS trials involving Mycoroot. *Anthephora* was not mychorrizal. However, only *Chloris* seedlings showed increased growth metrics as a result of the association.

Given the unconvincing response of commercial and indigenous, locally dominant grass species to exogenous strigolactone (GR24) application, gene expression in affected plants and the development of a protocol for sensitivity screening were not pursued.

Reports

Several drafts of a complete MSc thesis, including management/industry recommendations, have been prepared by the MSc student (Mr Jacob Roussouw). Following final inputs from project supervisors, the thesis will be submitted for examination by 31 December 2015. A final report to PMC will be submitted in February 2016.

RECOMMENDATIONS FOR MANAGEMENT

While results have not yet been finalized, there are a number of clear conclusions that can already be used to improve rehabilitation efforts at PMC:

- The composition of grass species used to initiate revegetation should be changed, to include *Eragrsotis teff* and *Melinis repens* and exclude *Panicum maximum*. This is based on the high and synchronous germination, wide tolerance for germination substrates and environmental conditions, and response to PGPS application, shown by the former two species.
- Smoke-water and CropbioLife can be excluded from the list of potential growth promoters, whereas application of Lumichrome should be tested in the field, as it shows positive effects even at very low concentrations.
- Given the theoretical benefits of AMFs (here only seen in *Chloris gayana*) and the rich rhizosphere of species growing in the revegetated areas, the use of Mycoroot is also supported.
- Strigolactone application, although having little direct effect on seedling survivorship, could be considered further given the proven role of strigolactones in supporting mycorrhizal associations.