IMPACT OF DOMESTICATION ON AUSTRALIAN GRASS *MICROLAENA STIPOIDES* ROOT ARCHITECTURE AND TOUGHNESS

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Introduction

About 10,000 years ago (Hillman et al., 2001), humans started to settle in communities, rapidly making hunter-gatherer societies obsolete (first evidences of agriculture may be as old as 32,000 years (Mariotti Lippi et al., 2015)). To subsist, humans had to select, breed and crop wild cereals and legumes. Since then, plant domestication has artificially selected plants to increase their suitability to human requirements: taste, yield, and cultivation practices (Evans, 1993; Smartt and Simmonds, 1995). In general, crop domestication typically causes losses or deep modification in genetic diversity through genetic drift and bottlenecks (Chen et al., 2015 and references therein). There are several examples where selective breeding has profoundly changed plant traits such as morphology or resistance to herbivory. For instance, insect damaged maize emits volatiles to attract natural enemies of the herbivore. Most of the North American maize cultivars do not emit the volatile (*E*)-β-caryophyllene, a sesquiterpene involved in the attraction of insect killing nematodes, whereas European breeds and teosinte, the progenitor of domesticated maize, emit this volatile organic compound after induction by insect pest (Köllner et al., 2008). It is supposed that this trait has been bred out to manage certain pests (Robert et al., 2012a; Robert et al., 2012b). Such changes in plants' secondary metabolite profiles can have cascading effects on upper trophic levels and impair plant defenses (Hiltpold et al., 2010; de Lange et al., 2016). Crop domestication has frequently selected for a simplification of plant architecture, with reduced branching and tillering (Chen et al., 2015 and references therein), as these changes in morphology result in higher yields. The greater tillering ability of wild ancestors is often related to defense traits to tolerate herbivory, giving plants more opportunities and nodal points to compensate for the herbivore damage (Rosenthal and Welter, 1995; Chen and Romena, 2008). Such simplification also results in a lower habitat complexity and can hinder natural enemies (Chen and Bernal, 2011).

Not only crop plants are domesticated but also grasses have been bred for pastures and lawns. The impact of grass domestication on its morphology and defence traits remains overlooked. Therefore, we tested the impact of domestication on the root architecture and physical traits of *Microlaena stipoides* (Labill.) in a glasshouse set-up, hypothesizing that domestication would simplify grass root traits.

Methods

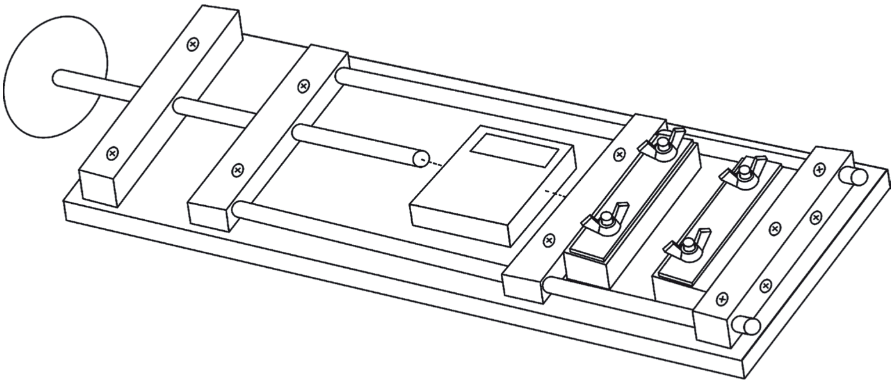
Plants

Yarramundi Loam (Hawkesbury Campus, Western Sydney University, NSW, Australia) was sieved to <2 mm and placed in rhizotubes (4.5 × 4.5 × 50 mm), up to approximately 2 cm from the surface. Rhizotubes were constructed of square plastic cable trunk cut to the appropriate length. One end of each section was sealed up with weed control mat (Coolaroo®, Gale Pacific Inc., VIC, Australia). To ensure that water was not a limiting factor during germination, the rhizotubes were saturated with water for two days prior to planting.

Seeds of both the wild type (WT, established in 1993, harvested 2006) and domesticated (DOM, bred until 2015) cultivars of *M. stipoides* were obtained from Native Seeds PTY Ltd (Cheltenham, VIC, Australia). 150 seeds per grass cultivar were individually sown in the rhizotubes (five per tube). Pots were then covered with thin wrap for one week to ensure enough moisture for germination and establishment of the seedlings.

Plants were grown in a glasshouse (25°C, 60% RH) with natural source of light for 12 weeks and watered three times per week. Then, the rhizotubes were disassembled and the soil was gently removed from the root systems. The roots were then washed from the remaining soil over 200 mm and 20 mm stacked sieves. Because of the low germination rate and the slow growth of the plants, four wild type (n = 4) and 21 domesticated plants (n = 23) have been harvested to record the data presented in this report.

Figure 1.
Device used to measure the tensile force required to tear the roots of the domesticated and wild *Microlaena stipoides.*



Root architectural properties

Immediately after washing, roots were immersed in water with 5% ethanol in a transparent tray and scanned (Expression 11000XL, Epson, NSW, Australia). Total root length, length per volume, root volume, root diameter and total root surface were measured using WinRHIZO Pro 2015 (Regent Instruments Inc., QC, Canada).

Root physical traits

Two physical traits of the roots of the wild and domesticated roots were measured immediately after scanning. First the physical strength of the root was evaluated using a tearing apparatus adapted from Pérez-Harguindeguy et al. (2013) (Figure 1). For each root system, the top 8 cm of the main root were cut with a razor blade and individually scanned and analyzed in WinRHIZO Pro 2015b. The average diameter of the root was recorded. Both ends of the section of root were wrapped in Parafilm (Bemis Inc., WI, USA), to prevent compression damage on the root, while ensuring a good hold with the clamps of the tearing apparatus, placed ca. 3 cm apart. Roots were torn apart. The force to fracture the root was recorded using a digital force gauge (FPG-5, Test-Equip, VIC, Australia). Tensile strength F_t was calculated as

$$F_t = \frac{\textit{Force to fracture}}{\textit{Diameter of the tested root}}$$

and expressed in N mm^{-1}.

In addition to the tensile strength, the exact distance between the root tearer clamps was measured prior (D_p, when the root was just under tension) and after (D_a) the fracture of the root. Root elongation R_e was calculated as

$$R_e = \frac{\textit{Da} - \textit{Dp}}{\textit{Diameter of the tested root}}$$

and expressed in mm mm^{-1}.

Statistical analyses

Root architectural properties

A Principal Component analysis (PCA) was performed to model the multivariate response of root architecture to domestication. The obtained model was tested with a Permutation test (999 permutations). Following Borcard et al. (2011), the function *prcomp( )* from the package *stats* (Oksanen et al., 2013) was run in R version 3.0.2 (R Development Core Team, 2015). The same software was used to generate the PCA biplot.

Root physical traits

Because of the low number of replication, the data related to the root physical traits was not normally distributed. Statistical differences in F_t and in R_e were assessed performing a Kruskal-Wallis test in R v. 3.0.2 using the function *kruskal.test( )*.

Results

Root architectural properties

The PCA output (Figure 2) suggested that domestication impacts root architecture. Even with the low number of replicates, especially for WT, the samples clustered by their breeding history and it appeared that domesticated *M. stipoides* tends to have more complex root architecture than its wild relative.

Root physical traits

Tensile force F_t applied to break the roots of domesticated plant was significantly higher (3.428 N mm^{-1} ± 0.580 SEM) than the force needed to tear roots of the wild type apart (1.687 N mm^{-1} ± 0.475 SEM) (Figure 3, Kruskal-Wallis, $\chi^2 = 5.722$, df = 1, p = 0.017).

The root elongation R_e between the domesticated and wild plants were marginally significantly different (Figure 4, Kruskal-Wallis, $\chi^2 = 3.013$, df = 1, p = 0.083); R_e measured with wild plants tended to be lower (1.519 mm mm^{-1} ± 0.272 SEM) than R_e recorded with the domesticated roots (1.980 mm mm^{-1} ± 0.150 SEM).

Figure 2.
Principal Component Analysis biplot.

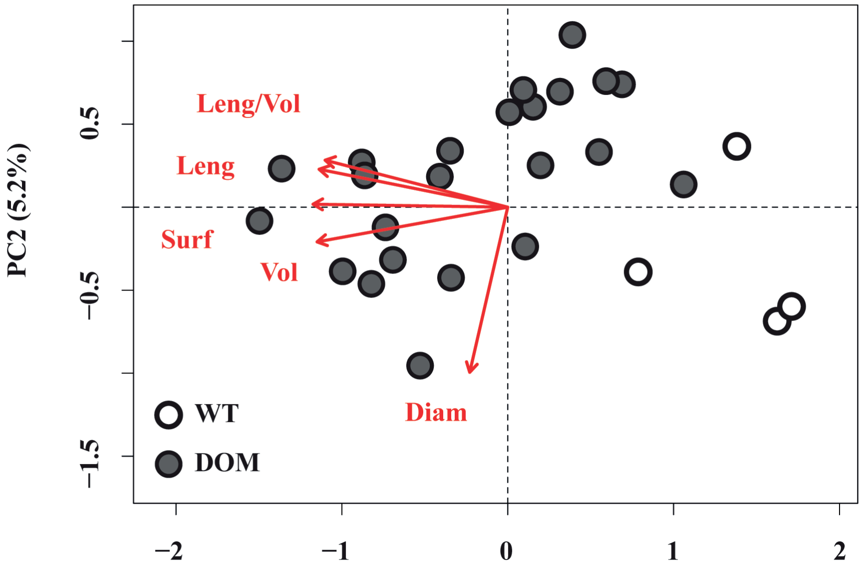


Figure 3.
Average tensile force applied to tear the roots of the domesticated (DOM, dark grey) and wild (WT, white) *Microlaena stipoides*. Bars indicate SEM. * indicates significant difference ($0.01 < p\text{-value} < 0.05$).

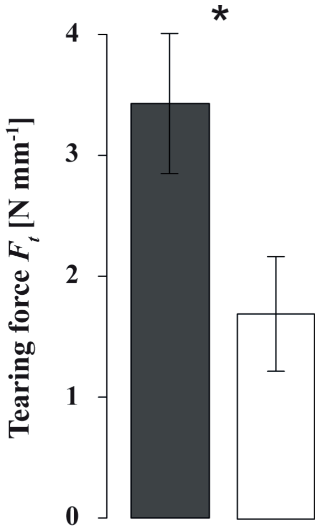
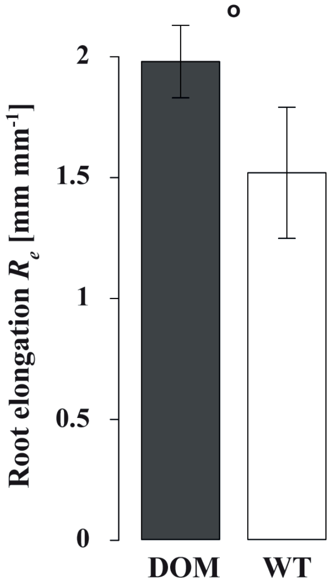


Figure 4.
Average root elongation before the roots of the domesticated (DOM, dark grey) and wild (WT, white) *Microlaena stipoides* were pulled apart. Bars indicate SEM. ° indicates marginally significant difference.



Discussion

This preliminary dataset shows the effect of domestication of Australian native grass species can have an impact on root architecture. After ca. 10 years of breeding, the root architecture of domesticated *M. stipoides* is more complex and the domesticated grass roots are harder. This could endow the domesticated grass with more resistance to abiotic stress as drought or soil compaction over the wild type. Tougher roots can result in a better resistance to insect herbivory (Johnson et al., 2010) rendering the domesticated cultivar more resistant to root herbivory, potentially resulting in a higher yield in case of high herbivore pressure. Both architectural complexity and root strength impact the anchorage of grass in the ground (Ennos, 1991), therefore the current domesticated bred could be more resilient to mammalian grazing above ground.

Root architecture can have a significant impact on natural enemies of insect pests in the rhizosphere. For instance, entomopathogenic nematodes (EPNs) move in the vicinity of the roots (Ennis et al., 2010; Demarta et al., 2014). In the absence of cue, an increase in the root architectural complexity decreases the ability of the EPNs to locate and kill their insect host (Demarta et al., 2014). Therefore, the increased complexity of the root architecture of domesticated *M. stipoides* could impact upper trophic levels in the event of insect root herbivory.

More replicates and analyses (i.e., chemical defences) should be considered to draw truthful conclusions on the impact of domestication of *M. stipoides* roots. But the current preliminary data strongly suggest that, similarly to crop plants (Chen et al., 2015), the breeding of pasture plants can result in the selection of an unexpected trait, for the good or the bad.

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