HME Ryegrass Field Trial 2021

Aim

To test herbage growth and fatty acid content for two HME segregating T2 populations that had been generated via agrobacterium-mediated transformation and had been confirmed to contain a single copy of the HME construct.

Hypothesis was that the proportion of fatty acid increase seen in the hemizygous HME plants would be seen in swards in the field and there would be no significant growth penalty associated with HME.

Trial Design

Two HME segregating populations were used in the field trial. These included RCR6702 (20-25% increased FA) and RCR7106 (16-20% increased FA). Both had been crossed with line E24 from the elite founder mother plants provided by PGGWs.

Each population was treated as an independent experiment where HME+ and Null swards were grown in a randomised alternating arrangement in a row east to west (n=9). Essentially this is the same sward design as used in 2019, but with greater replication.

Each replicate sward consists of 9 central plants arranged with 15 WT border plants and a common WT border between each sward (Figure 1).

Ryegrass0120-6702

Ryegrass0220-7106

For each plant line (6702 and 7106) there will be 9 blocks of transgenic and 9 blocks of null plants. Each block consists of 9 plants arranged in a mini-sward with a WT outer boarder and a common WT boarder between each block (203 WT/transgenic line). Blocks to alternate transgenic block, null block, etc along the row.

= wT
= null or transgenic in a mini-sward arrangement

Figure 1. Schematic of 2020 Field Trial Layout.

40"

After immunoblot analysis to identify transgene status, plants were transplanted into the field in early May.

Harvests occurred on: 11/06/2020; 07/07/2020; 03/08/2020; 02/09/2020; 06/10/2020

Due to the death of several border plants before harvest 1 (coded red in following slide), the WT border of the 9th replicate sward was dismantled and used to fill gaps in the other swards (Figure 2). This replicate was subsequently excluded from analysis (n=8) (Figure 3).

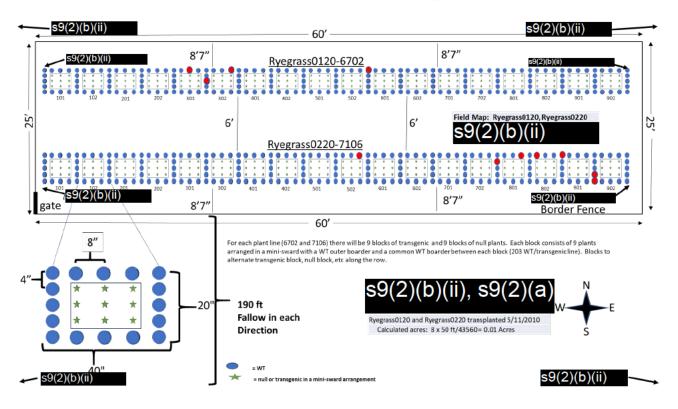


Figure 2. Schematic showing position of boarder plants lost

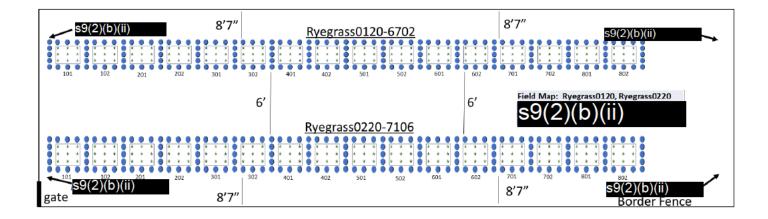


Figure 3. Schematic showing revised field trial layout with 8 replicates.

Analysis

Harvest 1 data was excluded from the analysis, because plants were establishing during this time.

Plants were harvested individually; samples were weighed in the field, oven dried (65°C) and reweighed. Fatty acids were determined for each plant in harvests 3 and 4.

DW data for each sward were converted onto a growth rate per unit ground area basis by dividing by harvest interval and assuming an internal ground are of 0.18 m².

FA content for each sward was determined by averaging the FAMES data.

Data for each sward were then analysed by repeat measures ANOVA, followed by pairwise comparison of means using the Benjamini-Hochberg procedure; statistical outputs are shown below.

Summary

Normal sward growth rates. No significant differences between Null and HME swards. HME swards consistently exhibited +0.6-0.9% FA (25-34% increase over the null); in all cases this was highly significant.

In 2019, very similar FA differences led to +0.3-0.5 kJ/gDW higher GE for HME swards. Herbage GE and N analyses currently underway.