BREEDING FOR ALFALFA COMPATIBILITY WITH CORN

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ABSTRACT

Alfalfa is the primary forage legume grown in the United States. Research and demonstration projects have shown that alfalfa can be successfully interseeded during its establishment year with corn grown for silage. This practice can increase overall system profitability and sustainability. The alfalfa establishment environment in this system is, however, very challenging due to shading, interspecies competition, and humid conditions under corn and this often results in poor stands of alfalfa during wet growing seasons. Studies carried out during 2015 and 2016 demonstrated considerable differences among alfalfa varieties in their ability to establish under corn. A subsequent study under corn in 2019 found one cycle of selection improved stand density of five alfalfa synthetics by an average of 35% over their parental base germplasms (105 vs. 78 plants m^{-2}). Based on these results further cycles of selection for alfalfa establishment under corn silage were initiated in 2020 and 2022. During 2022, fall ground cover of cycle-2 and cycle-1 selected intercropped alfalfa germplasms after corn harvest averaged 91% and 67% respectively compared 39% for non-selected germplasms. In another study, alfalfa RNA was isolated in July from leaf and root tissue after corn canopy closure from several selected germplasms and their non-selected base germplasms. Using an RNAseq approach comparing selected and non-selected germplasms, 345 differentially expressed genes (DEGs) were identified in leaves and 250 DEGS were identified in roots with 18 DEGs identified in both tissues simultaneously. Based on our results we anticipate further selection gains for alfalfa established under corn silage, possibly accelerated by DNA-based molecular markers. Ideally with enough selection, alfalfa varieties could be developed that successfully establish under corn silage without the need of pesticides or growth regulators. Such varieties would improve the reliability, profitability and sustainability of this intercropping system.

Key Words: alfalfa, corn silage, intercropping, breeding

INTRODUCTION

Alfalfa is the primary forage legume grown in the United States (USDA NASS, 2021). Corn silage in dairy systems is another major feed source that can often compete with alfalfa for dairy feed acreages on dairy farms (Russelle, 2013). Spring established alfalfa has less yield than alfalfa stands in post-establishment years. Fall established alfalfa prevents a full growing season of corn silage or soybeans in northern climates. Therefore, it is of interest to establish alfalfa stands while simultaneously being able to grow a high yielding forage such as corn silage. Extensive research studies and demonstration projects have shown that alfalfa can be successfully interseeded during its

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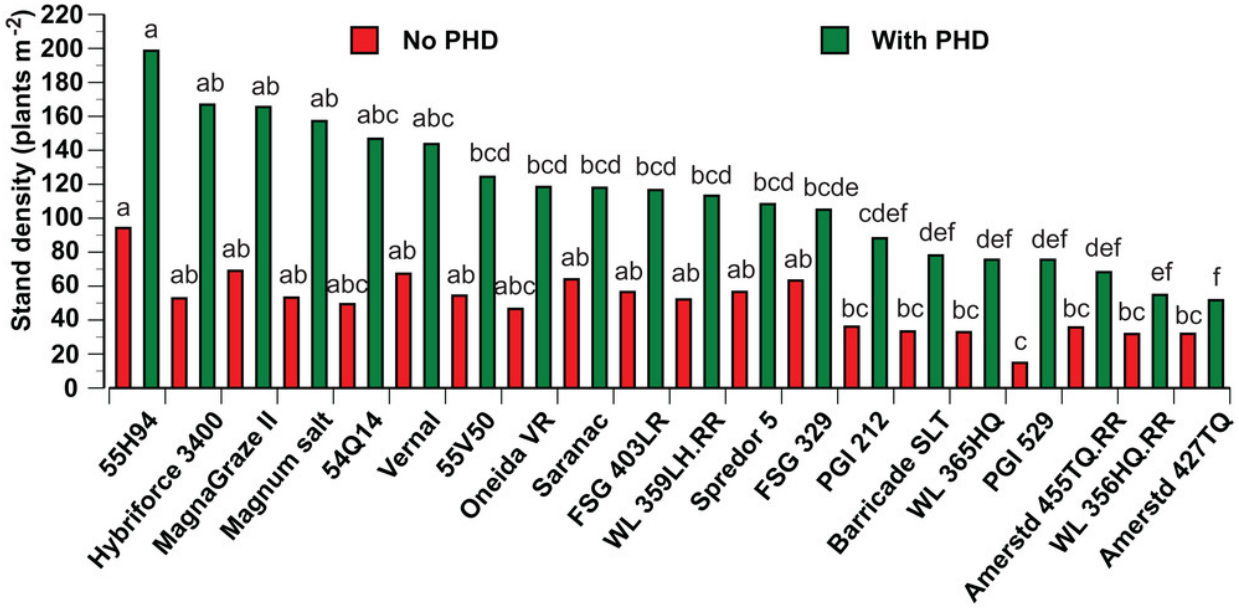

Figure 1. Final stand density of interseeded alfalfa varieties following corn silage harvest in 2015 as influenced by prohexadione (PHD) treatment. (Grabber et al., 2021b; Fig. 4)

establishment year with corn grown for silage and that this practice increases profitability and sustainability of the system (Grabber, 2016; Osterholz et al., 2019; Osterholz et al., 2020; Grabber et al., 2021a). This newer intercropping system maintains corn silage yields during alfalfa establishment while simultaneously producing dense alfalfa stands during alfalfa's establishment year. Keys to this system's success is applying a growth regulator (prohexadione) and fungicides to the alfalfa prior to corn canopy closure. Based on this work Grabber et al. (2021b) planted an alfalfa variety trial in 2015 and evaluated alfalfa stand establishment among varieties when intercropped with corn silage. Among the 20 varieties tested they observed a 282% and 527% performance differential depending on if the alfalfa stands were treated with a growth regulator or not (Fig. 1). These observations were indicative of substantial genetic variation for the alfalfa establishment ability under corn silage trait.

INITIAL SELECTION STUDY

Based on the results of the alfalfa variety trials (Grabber et al., 2021b) we decided to conduct a selection study for the alfalfa establishment ability under corn silage trait. In autumn 2017 parents of five synthetics were selected out the surviving alfalfa plants from variety trials established in 2015 and 2016 as part of the Grabber et al. (2021b) study. During 2017 and 2018 seed of the five synthetics was produced. The five synthetics were designated S&W-1 (109 parent), Alforex-1 (40 parent), Alforex-2 (47 parent), DFRC-1 (54 parent), and DFRC-2 (68 parent). In May of 2019 the five synthetics selected for increased alfalfa establishment ability under corn silage were planted along with each of the five synthetic's unselected parental base germplasms. The ten entries were planted

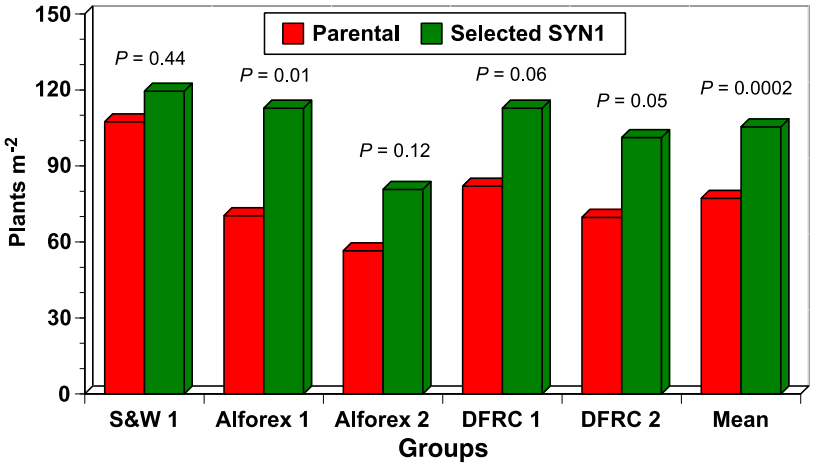

Figure 2. Final stand density of five selected alfalfa synthetics compared to their unselected parental base germplasms following intercropped corn silage harvest in 2019.

in replicated trials along with a corn silage companion. In the study only alfalfa plots that were treated with the growth regulator prohexadione and fungicides had sufficient alfalfa stand for analysis. On average the five selected alfalfa synthetics had 35% greater alfalfa plant establishment density compared to the average of the unselected parental base germplasms (105 vs. 78 plants m^{-2}) (Fig. 2).

CONTINUING BREEDING EFFORTS

After observing consistent and marked selection gains for the alfalfa establishment ability under corn silage trait we decided to conduct additional selection for this trait. A second round of selection was made in spring of 2020 from alfalfa plots that had been planted in 2019 and intercropped with corn silage. These plots had very few surviving plants and 26 plants mostly DFRC-1 plants were recovered and intermated during summer of 2020 to form IntAlf20 syn1 which is a cycle-2 population. During summer of 2021 syn2 seed was increased on IntAlf20. In spring 2022 IntAlf20 (cycle 2), DFRC-1 (cycle 1), DFRC-2 (cycle 1), and 11 unselected germplasms were planted intercropped with corn silage. No growth regulator or fungicide was applied to the plots to maximize selection pressure. After corn silage was harvested in October 2022 plots were visually evaluated for percent ground cover. IntAlf20 (cycle 2) had 91% ground cover, while the cycle 1 entries averaged 67% ground cover, and the unselected germplasms averaged 39% ground cover. These observation show that repeated rounds of selection improve the alfalfa establishment ability under corn silage trait. Surviving superior alfalfa plants were selected out of the 2022 plots to further improve this alfalfa establishment trait.

RNAseq ANALYSIS TO IDENTIFY ALFALFA INTERCROPPING GENES

After determining that alfalfa's establishment ability under corn silage had a genetic component we conducted a study to identify potential genes associated with this trait. Using the 2019 study we chose two selected and unselected-base germplasm pairs from among the five germplasm pairs, one pair's base germplasm had superior alfalfa establishment ability under corn silage while the other had inferior establishment ability. In July 2019 alfalfa RNA was isolated from leaf and root tissue after corn canopy closure at the onset of increased alfalfa plant stress. Comparing selected and non-selected germplasms, 345 differentially expressed genes (DEGs) were identified in leaves and 250 DEGs were identified in roots with another 18 DEGs identified in both tissues simultaneously.

CONCLUSIONS

We anticipate further selection gains for alfalfa established under corn silage, possibly accelerated by DNA-based molecular markers. Ideally with enough selection, alfalfa varieties could be

developed that successfully establish under corn silage without the need for pesticides or growth regulators. Such varieties would improve the reliability, profitability and sustainability of this intercropping system. Grabber et al. (2021a) found that an alfalfa stand's first autumn plant density should be around 200 plants m^{-2} or more to have good stands the following years. We anticipate with repeated rounds of selection that this target plant density will be achieved.

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