

DNA MARKER ASSISTED BREEDING TO DEVELOP ALFALFA VARIETIES FOR A WATER-CHALLENGED FUTURE

Ian Ray¹, Gina Babb and Chris Pierce

ABSTRACT

Large portions of the Great Plains and the western U.S. are regularly plagued by drought and diminishing water resources for irrigation. Alfalfa cultivars adapted to these regions, and that can remain productive under reduced irrigation allotments, are clearly needed. This study evaluated the potential of using DNA marker assisted selection (MAS) technology to improve alfalfa forage productivity in drought-prone and well-watered environments. This process initially involved identifying DNA markers that were associated with alfalfa forage and root biomass production during drought stress. Some of these markers were then transferred into different alfalfa cultivar backgrounds over two generations using DNA MAS. These populations were evaluated in 2011 and 2012 under limited irrigation (LI) and normal irrigation (NI) management field conditions near Las Cruces, NM. In the first-generation MAS populations, selection for high shoot and high root biomass markers, and selection against low shoot and low root biomass markers, benefited forage productivity by 3 to 23% in the LI study. These same populations, however, yielded similarly to each other in the NI study. To produce the second-generation MAS populations, six of the first-generation MAS populations were each mated to three alfalfa cultivars which possessed varying degrees of drought tolerance. Significant forage yield differences were detected among the six MAS hybrids within each cultivar group in both the LI and NI studies. These results suggested that marker assisted selection impacted alfalfa productivity in all three cultivar genetic backgrounds. Some second-generation MAS populations derived from two of the cultivars outperformed their original cultivar parent by 6 to 19% in the LI study, with the greatest improvement occurring in the cultivar that exhibited the greatest sensitivity to drought stress. In the NI study, some second-generation MAS populations derived from each of the three cultivars outperformed their original cultivar parent by 6 to 7%. These observations suggest that DNA marker assisted breeding approaches can be used to develop alfalfa cultivars with improved forage productivity in both drought-prone and well-watered environments.

Key words: alfalfa, yield, DNA markers, drought, irrigation

INTRODUCTION

Approximately two-thirds of the U.S. alfalfa acreage resides within drought-prone environments of the Great Plains and western regions. Consequently, efficient breeding strategies are needed to reduce the impact of water stress on alfalfa forage yield. Previous research has demonstrated that alfalfa forage yield is positively correlated with root biomass production. This association likely reflects that plants with more extensive root systems may be able to better explore soil

¹ Ian Ray (iaray@nmsu.edu), Gina Babb, and Chris Pierce. Department of Plant and Environmental Sciences, MSC3Q, New Mexico State University, Las Cruces, NM 88003; **In:** Proceedings, 2013 Western States Alfalfa and Forage Symposium, Reno, NV, 11-13 December, 2013. UC Cooperative Extension, Plant Sciences Department, University of California, Davis, CA 95616. (See <http://alfalfa.ucdavis.edu> for this and other alfalfa symposium Proceedings.)

profiles for moisture to sustain shoot growth over longer periods of time. Therefore, one potential approach to develop alfalfa cultivars that are less sensitive to drought stress could involve the use of DNA marker technology to identify alfalfa chromosome regions that naturally influence forage yield and/or root biomass production during water-stress. Once these markers are identified, traditional breeding procedures coupled with DNA marker assisted selection (MAS) could be used to transfer targeted biomass markers into commercial cultivar backgrounds. Towards this end, DNA markers associated with forage or root biomass production during water-stress were previously identified in experimental alfalfa populations. Our goal in the current study was transfer some of these biomass markers into three different alfalfa cultivar backgrounds using DNA MAS, and to evaluate their impact on forage yield under water-stressed and well-watered field conditions.

PROCEDURES

Development of first-generation marker assisted selection (MAS) populations: One alfalfa plant that we previously subjected to extensive genetic and forage yield analysis was determined to possess a variety of DNA markers. Some of these markers were associated with either high shoot (HS), high root (HR), low shoot (LS), or low root (LR) biomass production during drought stress. This plant was designated as CW192 and exhibited high fall dormancy and low forage yield when compared to semidormant alfalfa cultivars grown in southern New Mexico. Consequently, to transfer the above DNA markers into a higher yielding alfalfa genetic background we mated the CW192 plant with the cultivar, Malone. Two hundred progeny from this cross were produced and designated as the Cycle 0 (C_0) control population. We subsequently used DNA marker assisted selection to identify specific C_0 plants that either possessed, or lacked, specific combinations of DNA markers. Selected plants within each marker group category were randomly mated to each other to generate 10 Cycle 1 (C_1) MAS populations. Five of these populations were designated as HS1+, HS3+, LS2+, HR2+, and LR2+ to indicate that the population possessed one (1+), two (2+), or three (3+) of the HS, LS, HR, or LR DNA markers. The other five populations were designated as HS1-, HS3-, LS2-, HR2-, and LR2- to indicate that the population did not possess the respective DNA markers.

Development of second-generation MAS populations: In order to continue transferring these DNA markers into commercially useful alfalfa genetic backgrounds, we mated three cultivars with the C_0 population and five C_1 populations that possessed different biomass markers (i.e., HS1+, HS3+, LS2+, HR2+, and LR2+). Two of the cultivars, 'Malone' and 'NuMex Bill Melton' (designated as 'Melton'), were developed at New Mexico State University. The third cultivar (designated as 'Multileaf') was developed by private industry and exhibited a high degree of multifoliate leaves which provided it with high forage quality. The second-generation hybrids produced by mating the Malone cultivar with the C_0 and C_1 MAS populations were designated as Malone C_0 , MaloneHS3+, MaloneHS1+, MaloneLS2+, MaloneHR2+, and MaloneLR2+. Similar designations were used for the MAS-derived hybrids produced from the Melton and Multileaf cultivars (e.g. MeltonHS3+, MultileafHS3+, etc.).

The first-generation C_0 and 10 C_1 populations, the 18 second-generation cultivar-MAS hybrids, and the Malone, Melton, and Multileaf cultivars were evaluated in two adjacent field studies near Las Cruces, NM during 2011 and 2012. One study received 14-day normal irrigation (NI)

interval management and the other received 28-day limited irrigation (LI) interval management. Forage yield data were collected over six harvests in each year for both studies.

RESULTS

General observations: Forage yields of the populations in the LI study averaged 40% less than the NI study over two years indicating that substantial water stress occurred in the LI study. The Melton cultivar yielded 6.53 tons/acre (t/a) and was the most productive cultivar in the LI study, followed by Malone (5.96 t/a), and Multileaf (5.38 t/a). This ranking is reasonable given that Melton and Malone were developed based on selection within New Mexico environments. Furthermore, Melton experienced selection pressure for improved productivity in water-limited environments during its development, while Malone and Multileaf did not. In the LI study, the second-generation Melton-MAS hybrids tended to yield more than the Multileaf-MAS hybrids, which tended to outperform the Malone-MAS hybrids.

In the NI study, the three cultivars yielded similarly to each other, with Melton ranking the highest at 9.81 t/a, followed by Multileaf (9.54 t/a), and then Malone (9.3 t/a). As was observed in the LI study, the Melton-MAS hybrids tended to yield more than the Multileaf-MAS hybrids, which tended to outperform the Malone-MAS hybrids.

Performance of the three cultivars in the NI and LI studies indicated that drought stress reduced the forage yields of Melton, Malone, and Multileaf by 33%, 36%, and 44%, respectively. Thus, Melton was the least sensitive to drought stress while Multileaf was the most sensitive. These outcomes suggest that the greater proportion of leaves in the Multileaf cultivar may have resulted in higher transpiration rates which depleted available soil moisture more rapidly. Consequently, drought-induced termination of forage regrowth may have occurred earlier in Multileaf than in the other two cultivars.

In both the NI and LI studies, all three cultivars significantly outperformed the C_0 base population. In addition, most first-generation MAS-derived C_1 populations yielded less than the three cultivars and the 18 second-generation cultivar-MAS hybrids. The relatively low yield of the C_0 and C_1 populations reflected that they still possessed 50% of the genetic background from the low yielding CW192 parent plant. As a reminder, CW192 donated the biomass markers that we were investigating. The overall higher yields of the second-generation MAS-derived hybrids, which were produced by mating the selected C_0 and C_1 populations with the three cultivars, reflected that they only possessed 25% of the genetic background from the low yielding CW192 parent plant.

Yield of first-generation MAS-derived populations: Selection for high shoot and high root biomass markers, and selection against low shoot and low root biomass markers, benefited alfalfa forage productivity by 3 to 23% among the 10 MAS-derived C_1 populations in the LI study (Table 1). These results were consistent with those obtained from a previous drought-stressed field study where we initially identified the HS, LS, HR, and LR DNA markers in the CW192 plant. These same C_1 populations, however, yielded similarly to each other under NI management (data not shown). We also observed that yield differences between the highest and lowest performing C_1 populations were substantially less in the NI study (a 12% difference), as

compared to the LI study (a 42% difference). Collectively, these outcomes indicate that the impact of the DNA markers was greater under drought-stressed than well-watered conditions.

Table 1. Comparison of seasonal forage yields (tons/acre) for 10 marker assisted selection Cycle 1 alfalfa populations evaluated under water-deficit field conditions over six harvests each of two years (2011 and 2012) at Las Cruces, NM.

Population comparison ^a	2-Yr Avg. Yield	Yield effect of DNA markers
HS3+ vs. HS3-	5.2 vs 4.2*	+23%
HS1+ vs. HS1-	4.3 vs 3.7	+18%
LS2+ vs. LS2-	4.8 vs 4.9	-3%
HR2+ vs. HR2-	4.6 vs 3.8*	+20%
LR2+ vs. LR2-	4.4 vs 5.2*	-15%

* Significantly different forage yield based on $LSD_{0.1}=0.67$.

^aPopulation names indicate that plants were selected for the presence (+), or absence (-), of three high shoot biomass markers (HS3); one high shoot biomass marker (HS1), two low shoot biomass markers (LS2), two high root biomass markers (HR2), or two low root biomass markers (LR2).

Yield of second-generation MAS-derived populations: Significant forage yield differences were detected in both field studies among the six hybrids that were each derived from mating the Melton, Malone, and Multileaf cultivars with the C0, HS3+, HS1+, LS2+, HR2+, and LR2+ populations (Tables 2, 3, and 4). These results suggest that marker assisted selection impacted alfalfa productivity in all three cultivar genetic backgrounds.

Table 2. Malone-MAS hybrids: Seasonal forage yield (tons/acre) over six harvests in each of two years (2011 and 2012) under drought-stressed and well-watered conditions at Las Cruces, NM.

Limited Irrigation ^a		Normal Irrigation ^a	
Population	2-Yr Avg. Yield	Population	2-Yr Avg. Yield
MaloneHS3+	6.34 a	MaloneHS3+	9.86 a
Malone	5.96 ab	MaloneHR2+	9.47 ab
MaloneLS2+	5.72 abc	MaloneC0	9.45 ab
MaloneLR2+	5.40 bcd	MaloneHS1+	9.44 ab
MaloneHR2+	5.01 cde	Malone	9.30 ab
MaloneHS1+	4.96 cde	MaloneLR2+	8.83 b
MaloneC0	4.74 de	MaloneLS2+	8.74 b
C0	4.22 e	C0	7.86 c

^a Limited irrigation treatment was flood irrigated every 28 days while the normal irrigation treatment was irrigated every 14 days. Values followed by same letter are not significantly different at $P \leq 0.10$.

HS1+ and HS3+, one or three high shoot biomass markers present, respectively. LS2+, two low shoot biomass markers present. HR2+, two high root biomass markers present. LR2+, two low root biomass markers present.

Table 3. Melton-MAS Hybrids: Seasonal forage yield (tons/acre) over six harvests in each of two years (2011 and 2012) under drought-stressed and well-watered conditions at Las Cruces, NM.

Limited Irrigation ^a		Normal Irrigation ^a	
Population	2-Yr Avg. Yield	Population	2-Yr Avg. Yield
Melton	6.53 a	MeltonHS1+	10.55 a
MeltonHS3+	6.40 ab	MeltonC0	10.19 ab
MeltonHS1+	6.36 ab	MeltonLR2+	9.95 ab
MeltonHR2+	6.23 abc	Melton	9.81 ab
MeltonC0	6.05 abc	MeltonHR2+	9.77 ab
MeltonLS2+	5.43 bc	MeltonLS2+	9.57 b
MeltonLR2+	5.37 c	MeltonHS3+	9.42 b
C0	4.22 d	C0	7.86 c

^a Limited irrigation treatment was flood irrigated every 28 days while the normal irrigation treatment was irrigated every 14 days. Values followed by same letter are not significantly different at $P \leq 0.10$. HS1+ and HS3+, one or three high shoot biomass markers present, respectively. LS2+, two low shoot biomass markers present. HR2+, two high root biomass markers present. LR2+, two low root biomass markers present.

Table 4. Multileaf-MAS Hybrids: Seasonal forage yield (tons/acre) over six harvests in each of two years (2011 and 2012) under drought-stressed and well-watered conditions at Las Cruces, NM.

Limited Irrigation ^a		Normal Irrigation ^a	
Population	2-Yr Avg. Yield	Population	2-Yr Avg. Yield
MultileafLS2+	6.42 a	MultileafLS2+	10.21 a
MultileafHS1+	5.93 ab	MultileafHR2+	9.73 ab
MultileafLR2+	5.92 ab	MultileafHS3+	9.66 ab
MultileafHR2+	5.65 ab	MultileafHS1+	9.62 ab
MultileafHS3+	5.46 ab	Multileaf	9.54 ab
Multileaf	5.38 b	MultileafC0	9.14 b
MultileafC0	5.25 b	MultileafLR2+	8.90 b
C0	4.22 c	C0	7.86 c

^a Limited irrigation treatment was flood irrigated every 28 days while the normal irrigation treatment was irrigated every 14 days. Values followed by same letter are not significantly different at $P \leq 0.10$. HS1+ and HS3+, one or three high shoot biomass markers present, respectively. LS2+, two low shoot biomass markers present. HR2+, two high root biomass markers present. LR2+, two low root biomass markers present.

Three second-generation MAS-derived hybrids, and the Melton cultivar, were consistently identified among the 20% highest yielding entries in both the NI and LI studies. The three hybrids included MaloneHS3+, MeltonHS1+, and MultileafLS2+. These three populations were also the only ones to significantly outyield one or more other hybrids, within their respective cultivar-hybrid groupings, in both field studies (Tables 2, 3, and 4). Other features associated with these three hybrids are described below.

In both studies, MaloneHS3+, which possessed three high shoot biomass markers, was the highest yielding Malone-derived hybrid. This second-generation hybrid also demonstrated a non-significant 6% yield advantage over the Malone cultivar in both studies (Table 2). The MeltonHS1+ hybrid, which possessed one high shoot biomass marker, was the second highest yielding Melton-derived hybrid in the LI study. Although it yielded 2% less than the drought tolerant Melton cultivar under drought stress (Table 3), MeltonHS1+ was the highest yielding Melton-derived hybrid in the NI study where it demonstrated a non-significant 7% yield advantage over the Melton cultivar. Surprisingly, the highest yielding Multileaf-derived hybrid in both the LI and NI studies was MultileafLS2+, which possessed two low shoot biomass markers (Table 4). This hybrid demonstrated a significant 19% yield increase over the Multileaf cultivar in the LI study, and a nonsignificant 7% yield advantage over Multileaf in the NI study.

An explanation for the unanticipated yield benefit associated with the LS2+ markers in the Multileaf cultivar has potentially been provided based on additional research. Those results indicate that both markers are strongly associated with increased gas-exchange water-use efficiency (i.e. higher amounts of carbon fixed from the atmosphere per amount of water transpired by the plant). We have also demonstrated in limited irrigation field environments that increased gas-exchange water-use efficiency tends to reduce forage yield in alfalfa cultivars which possess trifoliolate leaves (i.e. three leaflets per leaf). Those results agree with our observation in the current MAS study, where the LS markers tended to reduce forage yield in the Malone and Melton derived hybrids which possessed only trifoliolate leaves. In the Multileaf cultivar background (which possessed three to seven leaflets per leaf), however, we speculate that these markers may be improving the ability of MultileafLS2+ to regulate stomatal opening and closing to reduce excessive loss of water through its leaves during carbon fixation. Consequently, soil moisture may be available for a longer period of time to sustain forage regrowth in the MultileafLS2+ hybrid.

CONCLUSIONS

Results of this study suggest that marker assisted selection improved forage yield of the Malone and Multileaf cultivars under water-limited conditions by 6 and 19%, respectively. Neither of these cultivars had previously experienced selection for drought tolerance. The greatest improvement in drought tolerance was observed in the most drought-sensitive cultivar, Multileaf. Marker assisted selection did not benefit drought productivity of the Melton cultivar, which had previously experienced selection pressure for drought tolerance. Under well-watered conditions, marker assisted selection appeared to benefit the productivity of all three cultivars by 6 to 7%. It is worth noting that only 50% of the plants in the second-generation cultivar-derived hybrids possessed any given marker. Consequently, additional selection within these populations to increase marker frequencies may provide additional forage yield improvement. These studies also appeared to indicate that the yield effects of the DNA marker regions were influenced by the cultivar genetic background in which they resided. Collectively, the results suggest that the transfer some DNA markers into other cultivars via MAS may simultaneously improve their forage productivity in water-limited and well-watered environments. To determine if any of the populations developed in this study are suitable for release as cultivars, seed increase of the highest-performing second-generation hybrids will occur in 2014 followed by additional yield testing in multiple New Mexico and California field environments.