DEVELOPING ALFALFA VARIETIES FOR A WATER-CHALLENGED FUTURE

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ABSTRACT

Large portions of the central and southern 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 paper summarizes some of the work that has been conducted to develop alfalfa cultivars that are less sensitive to drought stress. Such efforts include field evaluation of populations for their ability to remain productive under reduced irrigation allotments. Results of some physiology studies tentatively suggest that such populations may possess more extensive root systems. Molecular research has identified DNA markers that are potentially associated with alfalfa drought tolerance. These DNA markers have recently been used to move putative drought tolerance genes into standard and high forage quality type alfalfa cultivars. Fields studies evaluating the impact of these DNA marker alleles on forage productivity and nutritional quality, in these cultivar backgrounds, are in progress under well-watered and water-deficit conditions.

Key Words: alfalfa, irrigation, water-use efficiency, drought, DNA markers.

INTRODUCTION

Improving alfalfa productivity in water-limited environments is a major goal of the New Mexico alfalfa breeding program. Conventional methods of selecting for seedling survivability and mature plant vigor under deficit irrigation treatments have proved to be useful for developing cultivars that are less sensitive to drought stress. Such approaches have also been used to identify additional alfalfa populations that may provide useful alleles to improve drought tolerance in the future. Preliminary evidence suggests that development of plants with more extensive root systems may provide one mechanism to improve alfalfa productivity under water-deficit conditions – particularly in flood irrigated environments. Such plants may be able to more thoroughly extract available soil moisture over extended periods of time. To better understand the genetic mechanisms responsible for drought tolerance, genetically defined alfalfa populations have been used to identify specific DNA marker regions on alfalfa chromosomes that influence alfalfa forage and root biomass production under deficit flood irrigated field conditions. Efforts are currently underway to determine if some of these “drought tolerance” DNA markers can be used to improve the performance of elite cultivars under normal & deficit flood irrigation management.

In addition to high forage yield, the economics of alfalfa production requires that the herbage possess high nutritional value. Since alfalfa leaves have significantly higher nutritive value than stems, one approach to improve forage quality has been to develop cultivars which possess a

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greater proportion of leaves in their forage (i.e. a greater leaf-to-stem ratio, LSR). Some evidence suggests, however, that alfalfa populations possessing greater LSR may be more sensitive to deficit irrigation management (Ray et al. 1999a). This observation could reflect that high LSR populations may transpire water more quickly resulting in rapid soil moisture depletion, and consequently, early termination of shoot growth. If populations possessing high LSR are grown in water-limited environments, therefore, they may experience a more rapid deterioration of forage yield and quality resulting from stress-induced leaf and shoot senescence. Given this consideration, our current field studies are evaluating the impact of "drought tolerance" DNA markers in one high LSR and two standard type alfalfa cultivars.

PROCEDURES

Conventional Selection Methods

Identifying alfalfa populations from around the world that potentially possess drought and salt tolerance: We used a two-step procedure to identify alfalfa populations that might perform well in irrigated environments. We first utilized the USDA Germplasm Resource Information Network (GRIN) database to screen about 1300 alfalfa populations, which were previously collected from around the world and are currently maintained by the USDA National Plant Germplasm System. These populations were previously subjected to salt stress at the seedling and/or mature plant level stage at Utah State University and the University of Arizona. We used this inital approach because all irrigation water contains salts. Consequently, increased soil salinity and its detrimental impact on crop productivity frequently accompany irrigated agriculture. Based on our search, we identified 303 populations that appeared to exhibit some degree of seedling and/or mature plant salt tolerance. Then, based on the GRIN fall dormancy response data for each population, these materials were divided into four groups: very dormant (80 entries), dormant (97 entries), mildly-dormant (80 entries), and nondormant (46 entries). Each dormancy group was planted in a separate field nursery near Las Cruces, NM to facilitate irrigation and harvest management. In the second step of our screening process, each population was subsequently evaluated for biomass productivity in a non-replicated field experiment over two years under deficit irrigation conditions (i.e. 28-day flood irrigation interval=50% of normal irrigation frequency). For comparison purposes, plots of the drought tolerant alfalfa cultivar, 'Wilson', were planted adjacent to each population.

'Wilson' Cultivar Development: 'Wilson' alfalfa was developed at New Mexico State University (NMSU) using the field stress procedure described by Melton (1989b). In this procedure, three irrigations were used to establish alfalfa seedlings in early April with no subsequent irrigations applied. As monsoon rains arrived in mid-summer, more than 99% of the plants in the nursery appeared dead. The most vigorous surviving plants, that were revived by the rainfall, were selected and intermated for seed production.

'NuMex Bill Melton' Cultivar Development: 'NuMex Bill Melton' alfalfa (released in 2008) was developed at NMSU by subjecting the cultivar Wilson to 4 cycles of selection for resistance to the diseases, Phytopthora root rot and anthracnose (race 1). Resistant plants from this material were intermated, and their seed was planted in a field trial that received deficit irrigation (i.e. 28-day flood irrigation interval=50% of normal) for two years. Early in the spring of the third production year selection was imposed for forage production on surviving plants. The selected plants were intermated to produce breeder seed of NuMex Bill Melton (Ray et al. 2008).

Alfalfa Physiology Studies

Three replicated field studies, which evaluated 30 to 45 alfalfa populations eachunder normal and/or deficit irrigation management over two years, were conducted during 1995-1999 near Las Cruces, NM (Ray et al. 1999a, 1999b, 2004). Our goal was to determine genetic associations between forage yield and morphological/physiological traits that might impact alfalfa water-use efficiency (WUE). Some of the traits evaluated included: leaf-to-stem ratio, leaf thickness, and canopy temperature. Shoot carbon isotope discrimination was also measured as an efficient surrogate method to determine gas exchange WUE (i.e. amount of carbon fixed per amount of water transpired by the plant).

DNA Marker Assisted Selection Methods

A five-year collaborative field and laboratory study (2004-2009) between NMSU and the Samuel Roberts Noble Foundation (Ardmore, OK) was funded by the USDA to screen 600 DNA markers for their association with alfalfa forage productivity during drought stress. In this study, 176 genetically defined alfalfa populations were evaluated under drought-stressed field conditions over two harvests in each of three years. Each population was characterized for herbage yield and the presence or absence of the above DNA markers. Results of this study identified 10 alfalfa DNA/chromosomal regions that influenced forage yield during drought. When the field study was completed in fall 2007, all plots were excavated to a 10-inch soil depth and crown/root biomass was measured. DNA markers in five chromosome regions were identified that influenced root/crown biomass.

We are currently validating the usefulness of some of these DNA markers to improve alfalfa cultivar performance in water-stressed environments. To accomplish this goal, a plant from our previous experiment that possessed certain DNA markers was crossed to one plant from the NMSU cultivar 'Malone' (Melton, 1989a). DNA of 200 seedlings derived from that cross were screened with the previously identified shoot and root biomass DNA markers. Four groups of plants possessing different combinations of DNA markers were identified. These included plants possessing high shoot biomass markers, low shoot biomass markers, high root biomass markers, or low root biomass markers. These four groups of Marker-Assisted-Selection (MAS) plants were then intermated with three alfalfa cultivars. These included the standard type variety (Malone), a drought tolerant variety (NuMex Bill Melton), and one proprietary variety with high leaf-to-stem ratio (LSR).

In October 2010, seed representing the above 12 MAS-cultivar hybrid populations, and their original parent cultivars, were planted near Las Cruces, NM in a replicated field study. During 2011, 2012, and 2013 the populations will be evaluated for forage yield productivity under standard and deficit irrigation management (i.e. 14-day and 28-day irrigation intervals, respectively). The field plots will also be sampled during the first three harvests of each year to determine forage quality parameters. Each sample will separated into leaves and stems to determine the leaf-to-stem ratio. The leaf and stem subsample components for each population will be subjected to Near Infra-Red Reflectance Spectroscopy analysis to determine their neutral detergent fiber, acid detergent fiber, crude protein, digestibility and lignin content (Marten et al. 1988). When the forage yield/nutrition study is completed in the fall of 2013 all field plots will be excavated to a 10-inch soil depth and crown/root biomass will be measured.

RESULTS AND DISCUSSION

Conventional selection methods: The two-year deficit irrigation study of alfalfa populations from around the world indicated the following. Mildly dormant (fall dormancy 5-7) and nondormant (fall dormany 8-9) populations originating from regions in geographic proximity to Uzbekistan appeared to offer the greatest potential for drought tolerance. This conclusion was based on the observation that their forage productivity often equaled or exceeded that of the drought tolerant cultivar Wilson, which was grown in a plot adjacent to each experimental population. Germplasm from Peru also appeared to exhibit some degree of drought tolerance in our New Mexico field environment. The results indicated that incorporation of Uzbekistan type germplasm in alfalfa breeding programs may provide useful genetic diversity for improving alfalfa drought tolerance in irrigated western U.S. environments.

The NMSU alfalfa cultivar, 'Wilson', exhibited its best performance when grown on well-drained soils. Relative to other cultivars, it performed particularly well on such soils under deficit irrigation management. Unfortunately, Wilson was susceptible to the disease, Phytophthora root rot. Consequently, this variety was not able to persist under standard flood irrigation management practices on heavy textured soils. In addition, this variety was susceptible to Anthracnose, which caused significant problems under sprinkler irrigation, or during periods of high humidity that accompanied monsoon rains in late summer.

Studies involving the Wilson cultivar and other alfalfa populations were conducted under standard and/or deficit flood irrigation management (i.e. 14-day and 28-day irrigation intervals, respectively). Our goal was to determine genetic associations between forage yield and morphological/physiological traits that could potentially influence water-use efficiency . The shoot carbon isotope discrimination trait, which provided an estimate of gas exchange water-use efficiency , showed a strong positive correlation with forage yield. This result, in combination with canopy temperature data, indicated that populations exhibiting the highest yields under both irrigation treatments did NOT appear to obtain their yield advantage from improved gas exchange water-use efficiency (Ray et al. 1999a, 1999b, 2004). That is, higher yielding populations under both irrigation treatments did NOT appear to fix more grams of carbon per unit of water transpired. Rather, our results in conjunction with those published by Johnson and Tieszen (1994), suggest that the higher yielding populations may develop more extensive crown/root systems. Such root systems could allow these populations to more thoroughly explore the soil profile for available moisture to support forage biomass production over an extended period of time. This could be particularly useful on deficit flood irrigated soils.

To improve the performance of the Wilson cultivar we imposed selection for resistance to Phytophtora root rot and Anthracnose, while also selecting for early spring plant vigor under deficit irrigation management. The improved version of 'Wilson' was released in 2008 under the name, 'NuMex Bill Melton'. In state-wide variety trials conducted at Artesia, Farmington, Las Cruces, and Clovis NM under standard flood or sprinkler irrigation management, NuMex Bill

Melton ranked among the top yielding varieties in 11 of 14 evaluation environments (76 harvests). Forage yields of NuMex Bill Melton at the above locations averaged 13%, 10%, and 5% higher than the Wilson, Dona Ana, and Pioneer Hi-Bred 56S82 check cultivars. Under 28-day deficit flood irrigation management at Las Cruces and Artesia NM, forage yields of NuMex Bill Melton averaged 9% and 13% higher than the Wilson and Dona Ana check cultivars, respectively. In three of the above evaluation environments NuMex Bill Melton did not initially perform as well as the top varieties. In these few cases we observed that inferior performance always occurred during the year of establishment. If our deep rooting hypothesis is correct (see discussion above), we suspect that NuMex Bill Melton may initially use significant amounts of carbon to develop a strong root system during the establishment year – occasionally and temporarily – at the expense of shoot biomass production. In subsequent years, however, a more extensive root system may allow it to support forage biomass production over an extended period of time, and consequently, provide a yield advantage.

DNA marker-assisted-selection methods: In order to more effectively improve alfalfa's ability to remain productive in water-challenged environments, we have been attempting to identify regions in the DNA of alfalfa that influence applied water-use efficiency (i.e. biomass production per unit of water applied to the soil). Ultimately, we hope to be able to narrow these DNA regions down to a point where we can identify the individual genes that are responsible for drought tolerance. Towards this end, we have identified DNA markers belonging to 10 different regions on alfalfa chromosomes that influence biomass productivity (Ray et al. 2009). Results obtained from our 176 genetically defined experimental populations indicated that selection for specific DNA marker combinations was potentially capable of improving yield performance by 3 to 14% under drought stress. We also identified five DNA marker regions that influenced alfalfa root biomass production. Selection for some of these root biomass DNA markers may benefit forage production given the data indicated that alfalfa root and shoot biomass were positively correlated ($r=0.62$; $P<0.01$).

To validate the usefulness of the above DNA markers, field studies were planted in October 2010 to evaluate their impact on shoot and root biomass productivity in three alfalfa cultivars. As previously described, these three cultivars have different leaf-to-stem ratios (LSR). Since alfalfa leaves are more nutritious than stems we anticipate that these varieties will possess different herbage nutritional quality, with the high LSR cultivar exhibiting the highest quality under standard irrigation management practices. In water-limited environments, however, we hypothesize that increased leafiness may result in greater transpirational water loss, rapid soil moisture depletion, and consequently, early termination of shoot growth. In water-limited environments, therefore, we anticipate that high LSR cultivars may experience a more rapid deterioration of forage yield and quality resulting from stress-induced leaf and shoot senescence. Under such scenarios, standard type cultivars and/or drought tolerant cultivars may end up exhibiting higher yields and higher forage quality relative to high LSR cultivars. If the high LSR cultivar is more drought sensitive, our experiment will determine if drought tolerance DNA marker-assisted-selection can be used to improve its productivity in water-limited environments. We look forward to generating and analyzing these data over the next three years as they will allow us to test the above hypotheses, while providing us with important information about the influence of alfalfa canopy morphology (i.e. LSR) on forage productivity and nutritive value under well-watered and water-deficit field conditions.

Future plans: We hope to secure funding to begin screening many alfalfa cultivars/germplasm from around the world to identify new genetic diversity in the 10 DNA marker regions that we have determined influence biomass productivity under drought stress. Such work should allow us to identify new and superior genes/alleles in these regions that can be used by plant breeders to further improve alfalfa's ability to remain productive in water-challenged environments.

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