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Identifying Promising New Falcata Alfalfa Populations for use in Semiarid Rangelands

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Identifying Promising New *Falcata* Alfalfa Populations for Use in Semiarid Rangelands

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ABSTRACT

Alfalfa (*Medicago sativa* L.) is a valuable crop worldwide. In addition to its use as forage, the nitrogen fixation capabilities of root nodules make it a valuable component for improving soil health. Unfortunately, the semi-arid region of the Northern Great Plains introduces a number of environmental stresses that are detrimental to stand establishment and persistence. The ability to produce adventitious shoots from roots is generally considered a favorable trait for increasing stress resistance. This study aimed to identify alfalfa populations possessing the ability to produce adventitious shoot from root segments. Seven Plant Introductions (PIs), selected from the National Plant Germplasm System, and one commercial cultivar (Persist II) were evaluated. Two 6cm root segments originated at 1cm and 7cm below the cotyledonary node from 1-year-old plants. Root segments were planted in Miracle-Gro® potting soil for 16 weeks. Adventitious shoot emergence was recorded throughout the experimental period. At the end of the experimental period, a number of root segments that produced adventitious shoots, shoot survival, status of flowering and non-shoot-bearing root segment decay were determined. Six of the seven PIs produced adventitious shoots. No shoots produced on Persist II and PI 494662 roots. Among the six populations, frequency of generating adventitious shoots from roots ranged from 3.8% to 57% and frequency of shoot survival varied from 33% to 100%. Speed of regeneration from roots and viability of root segments in soil varied among populations. PI 631678 had the greatest frequency and speed of regeneration from roots, high survival and flowering frequencies. Little difference in adventitious shoot regeneration and survival related to distance below the cotyledonary node within the same populations.

Keywords: alfalfa, adventitious shoot, emergence, root

INTRODUCTION

Alfalfa is a valuable forage crop in North America. High protein content makes it an excellent complement to corn silage, lessening and sometimes eliminating the need for protein supplements. Also possessing relatively high concentrations of calcium and mineral, alfalfa is particularly valuable for dairy cattle (Jennings, 2014). In addition to the nutritive value alfalfa possesses as a forage crop, the nitrogen fixation properties of alfalfa roots make it a valuable component in soil fertility management. In the semi-arid regions of the Northern Great Plains, yellow-flowered alfalfa (YFA) (*Medicago sativa* subsp. *falcata*) holds a particular interest.

YFA has become naturalized to semi-arid climate conditions in the Grand River National Grassland of South Dakota (Xu et al, 2005). Alfalfa has traditionally been plagued by difficulties in seed germination and stand establishment in this region. These difficulties stem from extreme climate variations: drought, extreme cold, and grazing pressures from cattle. Overcoming these difficulties would be beneficial for this valuable forage crop.

Adventitious growth is vegetative reproduction that occurs from a living portion of a parent plant, instead of from a seed. This ability is generally considered favorable for increasing stress resistance in plant populations, particular in environments when seed production is unreliable under grazing, stand establishment from seedlings, and persistence is poor. Adventitious shoots developed on the roots of alfalfa is an uncommon phenomenon. First observation, reported in the literature, was made in South Dakota by Oakley and Garver (1913) on a strain of YFA, originating from Orenburg, Russia and later strains from Semipalatinsk, Siberia (Oakley & Garver, 1917). Inspired by previous observations, Smith (1950) further studied the capability of YFA to produce adventitious shoot growth from root segments. Heinrichs (1954) suggested the adventitious shoot production on the alfalfa is genetically conditioned. If this trait could be cultivated in commercial YFA populations, the stress resistance of this valuable crop could be increased. Alfalfa has been shown to respond to intersubspecific hybridization (Riday & Brummer, 2006), and it is hoped that these difficulties may be overcome by introducing stress resistant traits such as adventitious growth potential.

The objective of this study was to identify Plant Introduction populations of YFA possessing the ability to produce adventitious shoot growth from root segments and to evaluate effects of root segments cut below the cotyledonary node on adventitious shoot regeneration and survival.

MATERIALS & METHODS

Seed Source

The seeds of the eight population entries used in this study were obtained from a commercial Millborn Seeds company (Persist II) as control and seven PIs from National Plant Germplasm System (PI491407, PI494660, PI494661, PI494662, PI631677, PI631678, and PI631682). PIs were selected based upon the origin latitudes and climates similar to South Dakota (Table 1).

For each entry, 100 uniform seeds were selected, scarified, and inoculated with *Rhizobium* before planting in individual 164ml plastic cell containers (Ray Leach "Cone-containers"; Stuewe and Sons, Inc., Tangent, OR) filled with Mircle-Gro® potting soil. Seedlings completed first year dormancy under ambient day and night photoperiod cycle in a greenhouse located in Brookings, S.D.

Experimental Procedure

From each entry, there were 25 to 50 healthy 1-year-old plants randomly selected and excavated for assessment of the ability of root segments to produce adventitious shoots. After removal of soil and lateral roots, each taproot was cut into two 6cm segments originating at 1cm and 7cm below the cotyledonary node. Three root segments of each entry were planted upright in an 11.5cm dia. X 9.5 depth plastic pot filled with Miracle-Gro® potting soil. Pots were maintained in a greenhouse with 16h light/8h dark photoperiod, 24±3°C temperature, and watered daily by misting.

Table 1: Eight alfalfa populations were evaluated for the ability to produce adventitious shoots from root segments.

Entry	Description	Marketer/Origin
PI491407	*M. sativa* subsp. *falcata, NPGS*	China, Nei Monggol (Inner Mongolia)
PI494660	*M. sativa* subsp. *falcata, NPGS*	Romania, Lat. 46° 46’0” N, Long. 23° 36’0”E
PI494661	*M. sativa* subsp. *falcata, NPGS*	Romania, Lat. 46° 46’0” N, Long. 23° 36’0”E
PI494662	*M. sativa* subsp. *falcata, NPGS*	Romania, Lat. 44° 19’0” N, Long. 23° 48’0”E
PI631677	*M. sativa* subsp. *falcata, NPGS*	Mongolia, Lat. 49° 49’32” N, Long. 92° 03’48”E
PI631678	*M. sativa* subsp. *falcata, NPGS*	Mongolia, Lat. 49° 46’40” N, Long. 91° 53’52”E
PI631682	*M. sativa* subsp. *falcata, NPGS*	Mongolia, Lat. 48° 10’33” N, Long. 91° 45’29”E
Persist II	*M. sativa,* Cultivar, Conventional Hay-Type	Millborn Seeds Inc.

Data Collection

Shoot emergence was recorded daily and tallied weekly. Shoot-bearing root segments were moved to individual pots after one to two weeks of emergence to ascertain the exact root segment had produced the shoot (Fig. 1). Flowering plants were hand-pollinated to evaluate the capability of sexual reproduction. At the end of 16-week period following planting, a number of root segments that produced shoots, shoot survival, and status of flowering on survived shoots were determined. The non-productive samples were excavated to detect whether root segments had decayed over the course of the project or were unproductive.

Data Analysis

Figure 1: Adventitious buds on the roots of PI 63168 (left) and adventitious shoot-bearing root segments are displayed (right).

Frequency of shoot regenerated from root segments was calculated by using the number of root segments producing adventitious shoots that appeared above the soil surface divided by the total root segments planted for each distance below the cotyledonary node, respectively. Percentage of shoot survival was calculated based on the number of shoot-bearing root segments whose shoots survived by the end of the 16-week period. The percentage of surviving shoots that produced flowers was also calculated by the end 16-week study period. Entries of Persist II and PI 494662 were excluded from data analysis because no shoots generated from the root segments. Frequency of generating shoots from root segments was analyzed using PROC FREQ (SAS, 1990). A Chi-square (χ^2) analysis was used to detect the differences ($P<0.05$) among the six populations and among distance below the cotyledonary node.

RESULTS

Of the eight populations of alfalfa used in this study, no shoots generated on PI 494662 and Persist II root segments. Frequency of generating shoots from root segments varied significantly from 11.11% to 57.15% among six PIs for 1cm cut below the cotyledonary

node (*P*=0.0067) and 3.85% to 46.15% for 7cm cut below the cotyledonary node (*P*=0.0144) (Fig. 2). It was the most frequently occurred in PI 631678 (57.15% and 46.15%) for 1cm and 7cm cutting distances below the cotyledonary node, respectively, and least frequently in PI 494660 (11.11% and 3.85%) (Fig. 2).

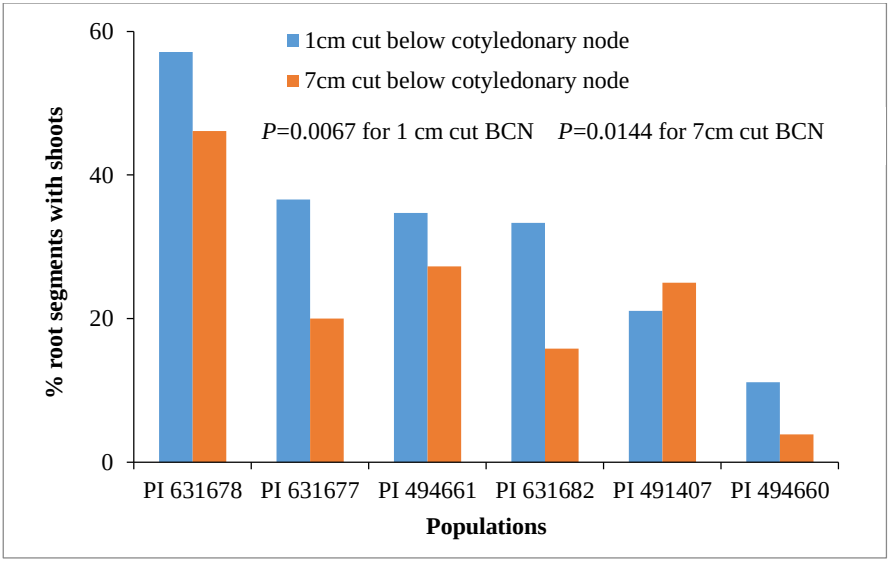

Figure 2: Among the six PIs, the frequency of generating shoots from roots ranged from 11.11% to 57.15% for 1cm cut below the cotyledonary node (*P*=0.0067) and 3.85% to 46.15% for 7cm cut below the cotyledonary node (*P*=0.0144).

Once adventitious shoots had appeared above the soil surface, survival rate were similar among the productive populations except for PI 631682 (Fig. 3). Furthermore, most surviving plants produced flowers (Fig. 4). If the flowers were hand-pollinated they formed pods and produced seeds (data not shown in the paper). The speed of emergence of shoots had a great variation among the six populations. PIs from Mongolia (PI 631678, PI 631677 and PI 631682) had faster emergence, as early as the first week following planting. Of the root segments with shoots, 80% to 100% appeared by the eighth week. While PIs from Romanian (PI 494661 and PI 494660) shoots emerged much slower and first emergence appeared at the seventh week and 75% to 96% shoot-bearing roots occurred between the seventh and fourteenth week. PIs from Inner Mongolia of China (PI 491407),

displayed 76% shoot production generated during between the fourth and eighth week. The highest occurrence of regeneration occurred across the six PIs during the seventh week. PI 631678 had the greatest speed of adventitious shoot production. In addition, it produced adventitious growth longer than any other populations (Fig. 5). Viability of root segments in soils varied among the populations (Fig. 5). Viability of PI 494661 root segments lasted for 16 weeks (Fig. 5) and displayed a low percentage of non-shoot bearing root segment decay (37%) compared to other populations (52% to 87%) except for PI 494662.

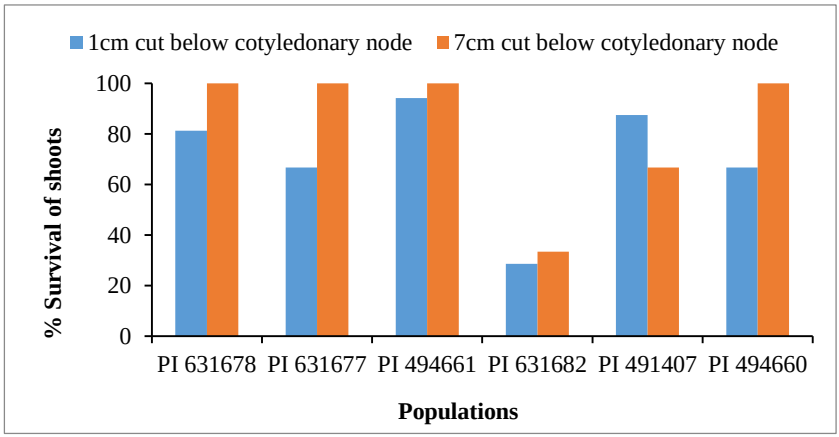

Figure 3. Six PIs showed similar survival rate of emerged shoots, with the exception of PI 631682, for both cutting distances below the cotyledonary node.

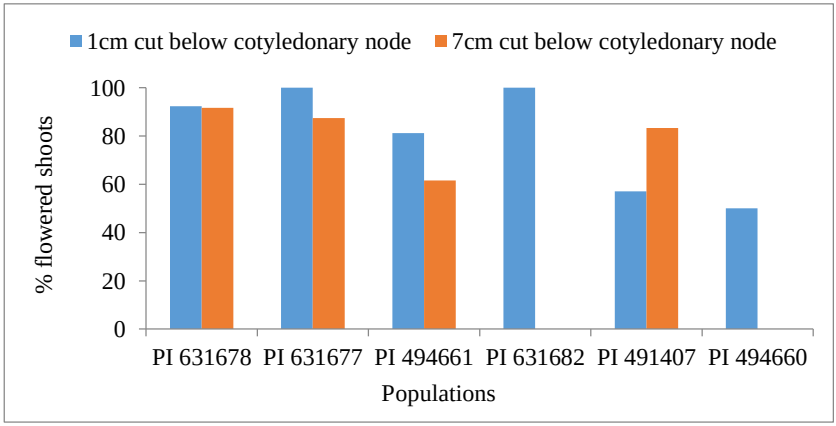

Figure 4: Most surviving plants produced flowers across all six PIs, with the exception of PI 631682 and PI 494660 on the 7cm cutting distance.

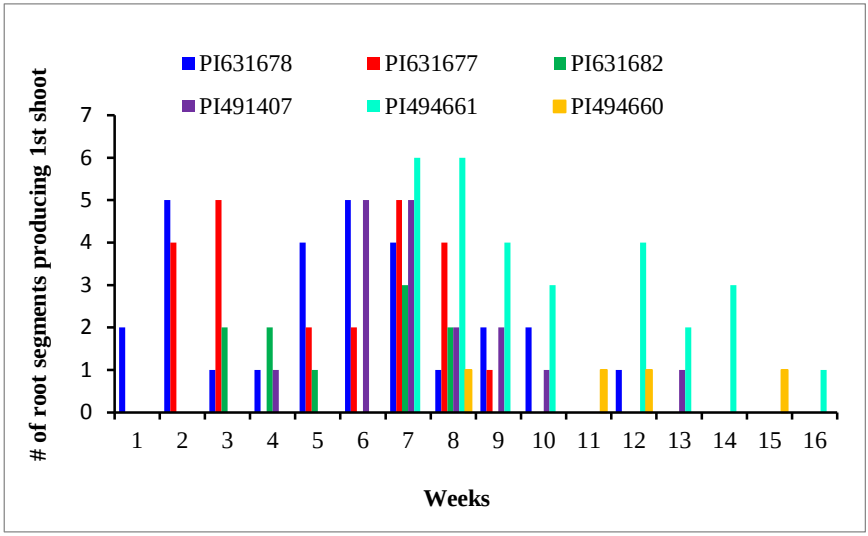

Figure 5: Speed of adventitious shoot development varied among the populations. PI 631678 produced shoots during the first week after planting and lasted for 13 weeks. While PI 494661 shoot production was delayed until the seventh week. The highest occurrence of regeneration across the six populations was the seventh week. Viability of root segments in soil varied among the populations.

DISCUSSION

Improving yellow-flowered alfalfa's persistence in South Dakota's climate will result in significant economic benefits for livestock producers. Today, this valuable species is limited by poor stress resistance and stand persistence. The first step in increasing stress resistance of the commercial strains is by identifying populations that exhibit the desired traits. This study was the first step in that process.

In order to determine populations that would excel under South Dakota weather, it is important to start looking in areas with a similar environment. Seven populations with latitudes and climates similar to South Dakota were selected. The results of this study suggested that shoot emergence is the most limiting factor for this stress-resistant trait.

Once adventitious growth begins, survival and flowering rates appear to be consistent across the populations (Fig. 3, Fig. 4).

Of these populations, the Mongolian strains seemed to possess higher potential for adventitious reproduction (Fig. 2). Among the examined populations, PI 631678 exhibited the highest frequent production of adventitious shoot, followed by PI 631677 and PI 494661. Both PI 631677 and PI 494661 had similar but less frequent generating shoots from roots segments compared to PI 631678.

In addition to the ability to produce adventitious shoots, one should also consider when this growth begins and how long it lasts (Fig. 5). PI 631678 was the most productive PI in this study, and also exhibited the fastest production (began during the first week) and longest production period of the study (about 12 weeks). Meanwhile, PI 494661 root segments demonstrated the longest viability in soil compared to other populations. From a management standpoint, these traits could have profound implications. In South Dakota, weather is seasonally unpredictable, experiencing late frosts, early thaws, and a wide variability of seasonal temperature and rainfall (Spuhler, 1971). A strong and long production period in addition to long viability in soil could increase the ability of the cultivar to resist the seasonal stresses that South Dakota is capable of producing.

The presented preliminary results suggest that Mongolian strains like PI 631678 and Romania strains like PI 494661 may have a higher potential for adventitious reproduction than PIs examined in the study (Table 1). The results support the value of testing to identify additional populations in the USDA Plant Introduction Collection which express these traits. Future projects may use the information from this study as a starting point for parental material selection. This study is currently being repeated to determine the true vegetative reproductive capability and consistency of these populations.

Once populations have been successfully identified as possessing the ability to produce adventitious shoots on roots, plant breeding programs can begin to integrate the desired traits into commercially available cultivars. By reducing the need for new seed purchase

every year, the value of the alfalfa forage only increases. This translates into increased value for farmers, ranchers, and anyone who has decided to use alfalfa as a forage crop.

LIMITATIONS

This work was conducted under greenhouse conditions. Greenhouse provides a better controlled environmental condition to evaluate the possibility of these phenotypic traits. This study was also done with a relatively small sample size for populations. Future work will entail a larger sample size for each population and a more elaborate experimental design will be employed. Although the results support the value of testing to identify additional populations in the USDA Plant Introduction Collection which express these traits, evaluation under field conditions should precede any selection effort.

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