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Seedling Root Morphology of Six Alfalfa Populations

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ABSTRACT

Seedling root morphology plays a crucial role in seedling survival and stand establishment. Naturalized yellow-flowered alfalfa (YFA) (*Medicago sativa* subsp. *falcata*) has demonstrated adaptation to semiarid conditions of the Northern Great Plains and tolerance to grazing. Seedling stage root morphology is poorly defined. Our objective was to compare morphological traits of seedling roots for six alfalfa populations. Six entries were evaluated: one *M. sativa* population as a control, two *M. falcata* entries, with reported "spreading characteristics" and three naturalized YFA populations. Uniform seeds of each entry were scarified with 320 grade sand paper and inoculated with *rhizobium* before planting. A V-shaped plexiglass rootview growth box (48cm long X 27cm wide X 40cm deep) was divided into six compartments with aluminum foil, each filled with Miracle-Gro potting soil. Ten seeds per population were planted (1.5 cm deep) at 1.5 cm intervals against the Plexiglass wall. The rootview growth box was maintained in a growth chamber (24±3°C; 16h light/8h dark). Soil moisture was maintained with daily misting for 26 days. *M. sativa* and YFA populations had faster primary root emergence (2-3 d) than *M. falcata* populations (8 d). Primary root elongation rate was greater for *M. sativa* (3-4cm/d) than *M. falcata* and YFA populations (1.8-2.6cm/d) for the first 7 days. First lateral root emerged when primary root length reached 10-14 cm for all populations. The first lateral root emerged 2-3cm below the root-stem junction with the exception of one *M. falcata* population (5cm).

Keywords: *Medicago sativa*, *Medicago falcata*, yellow-flowered alfalfa, legume, emergence, seedling root, development, morphology

INTRODUCTION

Alfalfa (*Medicago sativa*) is an important forage crop grown worldwide. But alfalfa has difficulty establishing in arid and semiarid rangelands because of inadequate soil moisture (Ries, 1982) and to persist because of winterkilling and grazing (Oakley and Garver 1917). There has long been a need and demand for cultivars of alfalfa that have the ability to establish and persist in grazing lands of semiarid regions, and to increase forage quality and animal production. Since introduction, over a hundred varieties of alfalfa have been developed but very few have been successfully naturalized in the North America rangelands (Rumbaugh 1982) even though the conditions are similar to its native range up to 64°N in Siberia (Hansen 1909). However, recently it was discovered that a population of predominantly Yellow-flowered alfalfa (YFA) (*Medicago sativa* subsp. *falcata*) has become naturalized in rangeland of northwestern South Dakota (Xu et al. 2005) and persisted under grazing pressure.

Yellow-flowered alfalfa (*Medicago falcata*) has demonstrated unique adaptations for survival under grazing in rangelands of the Northern Great Plains (Berdahl et al. 1989). YFA was first introduced to the United States in 1897 by Niels Ebbesen Hansen who was one of the first agricultural explorers working for the United States Department of Agriculture (Oakley and Garver 1917). During Hansen's exploration of Russia and Siberia, he observed along with other Russian agronomists that *M. falcata* varieties have several desirable traits for semiarid pastures, including grazing tolerance whereas strains of *M. sativa* do not; palatable to cattle, horses, and sheep; greening up early in cold spring; tolerant to severe drought in summer; alkali and salt resistance; winter hardiness; broad and deep-set crowns; and the habit of sprouting by roots proliferation (Hansen 1909). The spreading habit via rhizomes and proliferating roots enabled YFA to endure grazing and trampling to a great extent, provided them self-renovation after the death of the main rootstock (Southworth 1921), further enhanced persistence of stands. The capacity of YFA vegetative reproduction through root or rhizome has significant potential utility for grazing-type alfalfas to improve depleted rangelands, and agricultural and economical implications.

The critical role of root morphology associated with alfalfa establishment, persistence and productivity have been recognized (Johnson et al 1998). Several researchers reported that the ability of YFA being able to withstand severe conditions of grazing, drought, and cold has been largely associated with belowground morphological traits, such as deep-set and wide crowns, subsurface budding, well-developed rooting rhizomes, numerous branched roots and fibrous root system (Oakley and Graver, 1917, Southworth 1921, Garver 1922, Heinrichs 1963). Root morphologic traits on established 1-year-old alfalfa plants for 1067 plant introductions (PIs) and 110 North American cultivars were evaluated for identifying potential parent material for increasing persistence and productivity (Johnson et al 1998). Seedling survival and establishment are depended on seedling root development and morphology. Rapid root emergence and elongation, and early branching facilitate seedlings to acquire the necessary water and nutrients for survival and growth as well as successfully compete with neighboring plant species. However, little is understood of YFA root morphology at seedling stage. Information on the seedling root morphological characteristic will provide insight of the developmental history of the root system and would be useful for selection of potential parent materials for breeding grazing – type alfalfas in arid and semiarid regions.

This study was designed to test the hypothesis that there is a difference in seedling root morphology among six alfalfa populations. Our objective was to compare seedling root morphological characteristics among six alfalfa populations.

METHODS & MATERIALS

Seed source

Seeds of these six alfalfa populations used in this study were taken from a commercial seed companies (Persist II, Falcata), naturalized populations (SD202, SD203) of alfalfa in the Grand River National Grassland (45º49’N, 102°33’W), and PIs from National Plant Germplasm System (PI494660, PI494661) (Table. 1). Ten uniform seeds from each of the six alfalfa population were carefully selected under a dissecting microscope (10X magnification). Selected seeds were hand scarified (Narem et al. 2009) with 320-grade sandpaper for duration of 5 seconds and repeated 3 times followed by inoculation with *rhizobium* before planting.

Experimental Design

A complete random design was used for this experiment. A V-shaped plexiglass rootview growth box (48cm L x 27 cm W x 40 cm D) was separated into six equal compartments (15 cm L X 13.5 cm W X 40 cm D) using double duty aluminum-foil. Each compartment was filled with Miracle-Gro potting soil. Each seed was planted at a 1.5 cm depth and a 1.5 cm interval against the plexiglass wall using DNA plastic test tubes to secure the seeds in place before covering with a thin top layer of soil. Roots were shielded from light by removable

Table 1. Six alfalfa populations evaluated for seedling root morphology

Entry	Description	Developer/Marketer/Origin
Persist II (*M. sativa*)	Cultivar, Conventional Hay-Type	Millborn Seeds Inc.
Falcata (*M. sativa* subsp. *falcata*)	PYFA developed by N. Smith, Lodgepole. SD, for interseeding rangeland	Wind River Seed Co.
SD202 (*M. sativa* subsp. *falcata*)	PYFA Experimental from feral rangeland in NW, SD, Coiled shaped seed pod	SDSU
SD203 (*M. sativa* subsp. *falcata*)	PYFA experimental from feral rangeland in NW, SD, Sickle shaped seed pod	SDSU
PI494660 (*M. falcata*)	*Medicago sativa* subsp. *falcate* USDA National Plant Germplasm System	Romania, lat. 46º 46’ 0” N, long. 23°36’0” E
PI494661 (*M. falcata*)	*Medicago sativa* subsp. *falcata* USDA National Plant Germplasm System	Romania, lat. 46º54’36” N, long. 23°25’12” E

pieces of sheet aluminum-foil. Immediately after planting, the rootview box was placed into a growth chamber with temperature maintained at 24±3°C and 16 hrs light/8hrs dark photoperiod cycle. Soil was maintained at saturation through daily misting and checked with a soil moisture meter. The rootview box was placed in the controlled environmental growth chamber for 26 days for each trial.

DATA COLLECTION

The dates when the primary root, which arises from the radicle, emerged from seeds after planting were recorded. The primary root growth, emergence date of first lateral root, and the number of lateral roots were recorded daily for 26 days total for each trial or when the primary root reached the bottom of the rootview box. The distance of the first lateral root from the junction between root and stem on the primary root was measured after each seedling was excavated at the end of trial period. There was two trials.

DATA ANALYSIS

Primary root elongation rate was calculated using the equation $(L_2\text{-}L_1)/(T_2\text{-}T_1)$, where L_1 and L_2 are root lengths at sampling times T_1 and T_2, respectively (Pan et al. 2001). Cumulative primary root length and the primary root length at first lateral root emergence was calculated based on daily growth measurements. Average number of lateral roots production after primary root emergence was calculated. A one-way variance analysis was used to analyze each morphological trait variable among six populations at $\alpha=0.05$. A Duncan Multiple Comparison test was used when $p<0.05$ (SAS 1990).

RESULTS

Persist II (*M. sativa*) and YFA populations (Falcata, SD202, SD203) had rapid primary root emergence (2-3 d) compared to PI494660 and PI494661 *(M. falcata*) (8 d) ($p<0.0001$) (Fig.

1). Primary root elongation rate was greater for Persist II (*M. sativa*) (3-4cm/d) than

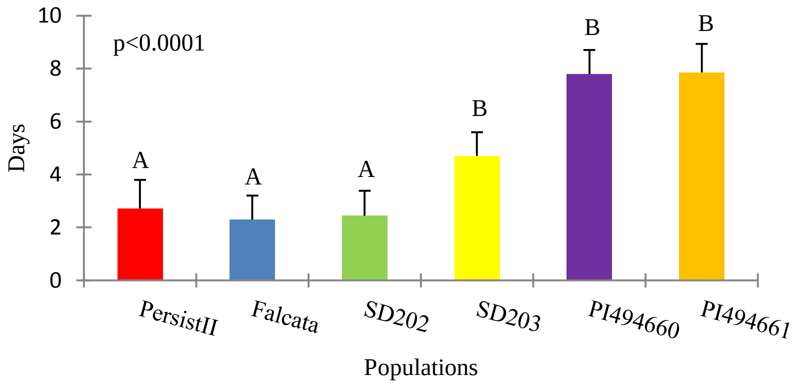

Figure 1. Days of primary root emergence after planting of six alfalfa populations (Trial 1).

PI494660 and PI494661 (*M. falcata*) and YFA populations (1.8-2.6cm/d) for the first 7 days (Fig. 2). The first lateral roots emerged when the primary root length reached a depth of 10-14cm (p=0.5356) showing no difference for all six YFA populations (Fig. 3). The first lateral root emerged 2-3cm below the root-stem junction with the exception of PI494660 population at 5cm (Fig. 4). By day 8 after emergence, Persist II (*M. sativa*) had the longest primary root length and PI494660 (*M. falcata*) had the shortest primary root

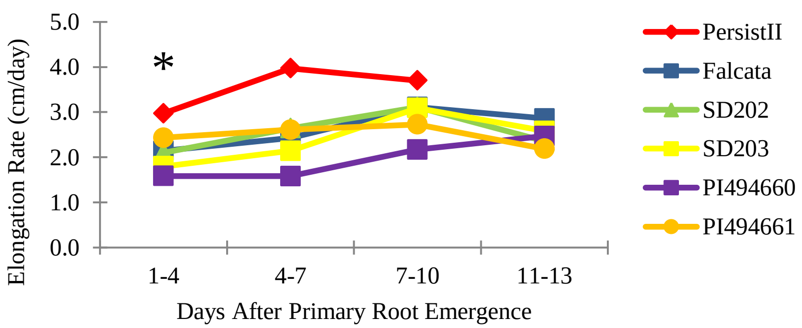

Figure 2. Primary root elongation rate (cm/day) of the six alfalfa populations. * indicates the statistically significant difference at P<0.05 (Trial 1).

length (Fig. 5. A). Persist II (*M. sativa*) lateral roots emerged on day 3 and PI494660 (*M. falcata*) lateral roots emerged much later at day 7 (Fig. 5. B). But all six alfalfa populations lateral root emergence did not begin until the cumulative primary root length reached a depth of 10-14 (cm) (Fig. 5. B, and Fig. 3).

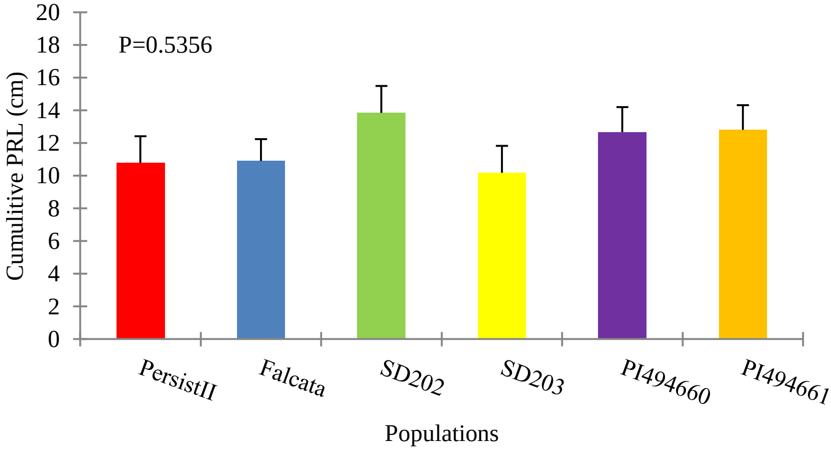

Figure 3. Cumulative primary root length when its first lateral root emerges (trial 1).

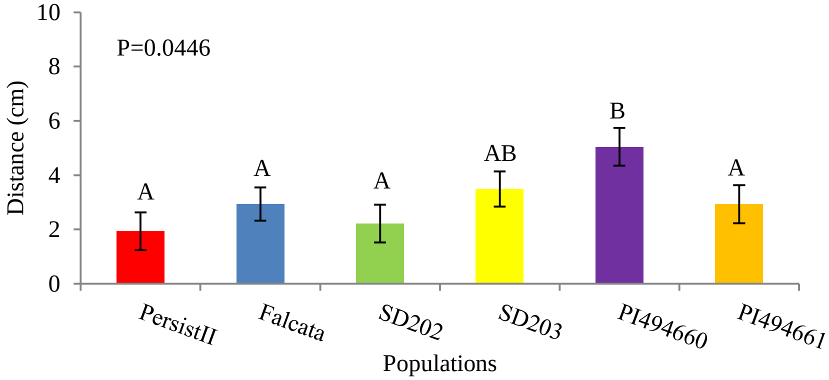

Figure 4. The distance of first lateral root from the junction between root and stem on the primary root (Trial 1).

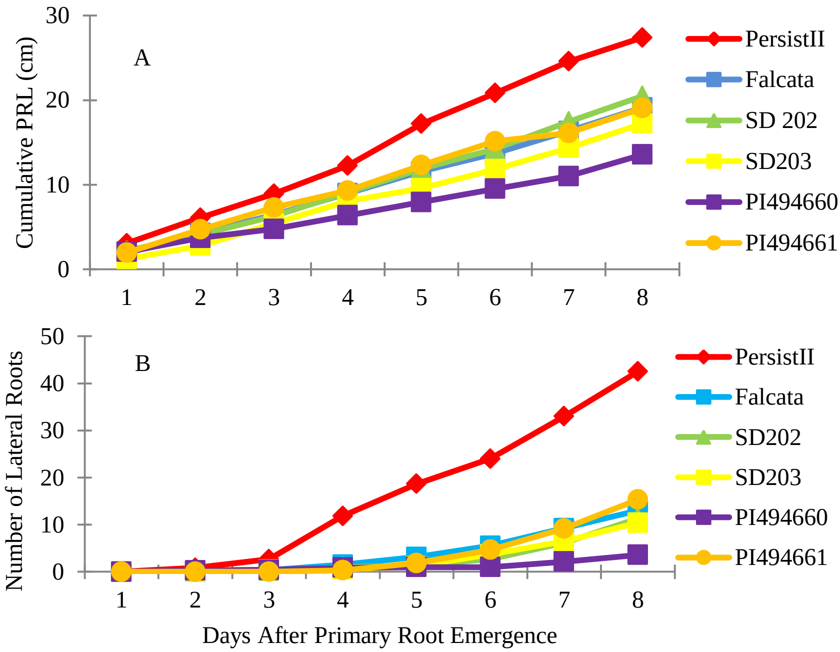

Figure 5. The cumulative primary root growth (cm) of the six alfalfa populations over 8 days after primary root emergence (A), the average number of lateral roots per primary root over 8 days after primary root emergence (Trial 1) (B). Day 1 was defined as the day when primary root emerged.

DISCUSSION

Persist II (*M. sativa*) demonstrated the fastest root emergence (2 – 3 days after planting), the fastest primary root elongation rate (3-4cm/day), and the earliest lateral root initiation, and more lateral root production for the first 8 days due to its quick primary root elongation rate compared to the other five alfalfa populations. This indicates that Persist II (*M. sativa*) may have the potential for the greatest establishment among the six alfalfa populations under ideal environmental conditions. Speed of root emergence is crucial for initial establishments of plant species, especially in semiarid environments where conditions are harsh. The elongation rate of the primary root is equally important after emergence for fast establishment leading to root penetration and exploration in soils for needed resources, such as water and nutrients. However, plant species cannot rely solely on their primary roots for nutrient uptake. The increase in root surface area by produced lateral roots and enhanced nutrient uptake gives plants higher survivability (Thornley 1971). Plants with more branched root systems may enable to persist under grazing and tolerance to drought and cold (Daday1962, Garver 1922).

Results from this study also demonstrate *M. falcata* (PI494660 and PI494661) populations are conservative in primary root emergence and slow primary root elongation rate (8 d and 1.8-2.6 cm/d) of the six YFA populations under observation. These phenomena may be a reflection of *M. falcata* origin (Table 1). These characteristics imply natural selection and adaptation to very harsh and unpredictable environments, but further research is needed to confirm this suggestion. It is speculated that slow emergence and elongation of the primary root is, in fact, advantage in environments with unpredictable spring to avoid the frost killing of seedlings, and germination occurred only after ideal environmental conditions have been reached.

M. falcata is purported by several researchers to posses the “spreading root” characteristics near the crown (Hansen 1927, Heinrichs 1963) but we did not observe such trait in PI494660 and PI494661 (*M. falcata*) at the seedling stage. It is very probably developed in the later stage.

It must be restated that the objective of this experiment was to compare the root morphological characteristic at seedling development stage of six alfalfa populations about 26 days and the results may vary in adult alfalfa populations. The results presented here were from Trial 1, but the same trends and patterns were confirmed by the Trial 2.

The practical applications of this study would be used to help determine the YFA seedling root morphology variations, and to provide useful information for selecting potential parental materials to develop grazing-type alfalfa for semiarid regions.

LIMITATIONS

This study was conducted between the months of August to December of 2011. Therefore, the main limitation of this study was time. Due to this time constraint, the study of morphological root traits in YFA was limited to 26-day old seedling stage only. The second limitation was space. This study was conducted under a well controlled environmental condition a growth chamber, due to limited space in the growth chamber, only six alfalfa populations were evaluated for their seedling root morphology for total two trials with 26 days per trial. The limitation of time and space prevented more populations to be evaluated and more trials and long duration for each trial to be conducted.

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