Diploid *Medicago falcata* Accession 'DON': Seedling and crown in the first growing season

Edwin T. Bingham and Thomas J. Haas
ebingham@wisc.edu tjhaas@wisc.edu

Objectives: The main objective was to study the diploid *M. falcata* accession named DON after the Don River Region of Russia, where it was collected by N. E. Hansen in the early 1900s. Hansen brought DON to South Dakota, and also placed it in the Plant Introduction Collection. Following its introduction, DON was used by several alfalfa breeders in the USA and Canada. Reports by Murphy, Rumbaugh, and Adams in Volume 1 of MGR on this web site provide more information about DON, and about Hansen, as do other publications that will be reviewed as this study continues. It is well established that the alfalfa cultivar 'Narragansett' contained germ plasm from DON, and that Narragansett was used in the development of 'Iroquois', and its derivatives, in' Multileaf', and in several other cultivars. Moreover, it now appears that DON was the diploid M. falcata that was used in the development of 'Vernal' alfalfa. A report will be devoted to this in the future. Vernal and its derivatives are included in ca 80% of the pedigrees of over 1000 alfalfa cultivars certified in the USA. Hence, DON is a very important accession, and we would like to understand more about its morphology and genetics.

Another objective was to study contractile growth and seedling characteristics of a sample of dormant and non-dormant alfalfa cultivars. Lastly, diploid DON was crossed with tetraploid M. falcata WISFAL to begin the process of developing tetraploid DON via sexual polyploidy. Information about WISFAL can be found in the first report in Volume 4 of this web site.

Methods: Seed of the original plant introduction of DON no longer is available. The seed of DON that was used in this study was from seed increases made by Melvin D. Rumbaugh that are stored at Utah State University, and were obtained from Kay Asay about 1999. We are currently in contact with Michael Peel, USDA-ARS at Utah State, regarding DON. Other seed lots were obtained from our seed inventory. They were of different ages, and germination percentage. Seeds were scarified with medium sandpaper, and planted in Jiffy-7 peat pellets, two seeds per pellet in three rows of seven pellets in trays. The first measurement was on all seedlings, including doubles in some pellets. The second measurement was after seedlings were thinned to one per pellet.

Conclusions: The first measurement on April 26, 2005 was about seven or eight days post emergence of the seedlings (see pictures below). The cotyledon node of most DON seedlings was at or below the soil surface. The same was true for CADL 98, and

a few seedlings of 2x and 4x WISFAL, PRS Travois, and Alfagraze. The cotyledon nodes of other entries were all above the ground, and they were all less dormant.

The second measurement on June 2, 2005 was after the seedlings had undergone contractile growth. The extent of contractile growth can be seen in the table of measurements below. Importantly, all of the entries showed contractile growth, including the non-dormant ones. Moreover, contractile growth was in the range of 5-6 mm across all entries. The eventual depth of the cotyledon node was a function of the initial height of the node in relation to the soil surface, and subsequent contractile growth. This placed the cotyledon node at or above ground on most seedlings of non-dormant cultivars.

Most of the entries were seeded in a field nursery in June, with the intention of making a larger number of measurements per entry under field conditions. This proved more difficult than expected because of large errors in measurement in the field (see pictures below). Hence, we decided to wait until October and measure the depth of the crowns of the entries. In October, plants to be measured were trimmed to the soil surface with scissors. There still were large errors in measurement; hence, only sample photographs of materials are presented below. In the case of DON, we were interested in whether the deep cotyledon node would translate to a deeper crown, but a deeper crown was not observed in the first growing season. It can be concluded that the cotyledon node of DON seedlings is deeper than any *Medicago* in this study, but crown depth appeared the same as 2x WISFAL.

Rumbaugh has noted in his writings that diploid DON can be used as the pistillate parent in crosses with tetraploids to elevate diploid DON germplasm to the tetraploid level. This was done in open pollination in the case of Narragansett, and by hand crosses in the case of Vernal. We decided to use this concept in open pollination with tetraploid WISFAL in the summer of 2005. To do this, ten DON plants about one year old were transplanted into the center of a half-acre nursery of tetraploid WISFAL and allowed to produce open-pollinated seed. Brown pods on the DON plants were hand harvested about August 1, 2005, allowed to dry for about three weeks, then threshed, scarified, and sown in the field in late August. A picture below taken in mid-October 2005, illustrates a larger plant in a row with smaller DON plants. From about 5 grams of scarified OP seed sown in the field, there are at least 12 large, presumably tetraploid progeny of DON X WISFAL. These tetraploids will be confirmed and then backcrossed to diploid DON. The process will be repeated about twice to produce an experimental that is over 90% DON at the tetraploid level. Yellow flower color ensures purity of the plants in the backcrosses.

Any researcher with information about DON or related issues is welcome to contribute.

Table 1: Position of cotyledon node in relation to the soil surface, in millimeters (mm)
Planted April 14, 2005
Number of plants n, at mm below or above the soil surface

Measured April 26 2005

	-10	-9	-8	-7	-6	-5	-4	-3	-2	-1	0	1	2	3	4	5	6	7	8	9	10	**Avg**
2x DON						1	1	2	2	2	4		1									-1.5
2x WISFAL										1	3	2	1	1								0.8
CADL 98								1	1	1	3	1	1									-0.4
PRS**Travois										1		1	2	2	2		1		1			3.2
WISFAL 91 (4x)***									1	1	4	1	1	2	1							0.9
Vernal '56												1	2	3	2	1	1					3.3
Alfagraze											1	2	3	2		1						2.1
P5454												1	1	2	3	3	1	1		2	1	5.1
Hayden												1	1	1	1	1	1	2	3	3	2	6.6
Sequel													1	2	3	4	3	2	2	1	1	5.6
Peruvian												1	1	1	1	2	3	4	3	2	2	6.4
Wadi-Qurat												1	1	1	1	2	3	4	3	3	2	6.5

Measured June 2 2005 *

	-10	-9	-8	-7	-6	-5	-4	-3	-2	-1	0	1	2	3	4	5	6	7	8	9	10	**Avg**
2x DON	1	1	2	3	3	1	1															-6.9
2x WISFAL					1	2	2	1														-4.5
CADL 98						1	2	3	1													-3.4
PRS**Travois	1	1	2	1	2	1	1	1														-6.6
WISFAL 91 (4x)***			1	1	1	2	3	1	1	1												-4.5
Vernal					1	1	2	2		2	1											-3.0
Alfagraze							1	2		2	2	2										-1.1
5454							1	1	1	2	3	2	1									-0.6
Hayden									1	3	4	2	2									0.1
Sequel								1	2	2	3	2	1									-0.5
Peruvian										1	4	4	3	1								0.8
Wadi-Qurat										1	4	4	3	1								0.8

**Reduction in number of plants on this date was due to roguing to one seedling per peat pellet.*
***PRS=Powder River Seeds Travois purchased 2005.*
****Information about 2x and 4x WISFAL, and references to their development can be found in Vol. 1, Report 1 of this web site.*

Figure 1: DON seedlings with cotyledonary node below soil surface.

Figure 2: Travois seedlings with cotyledonary node above soil surface.

Figures 3 – 8: Examples of seedlings in the field, two to three weeks post-emergence with various degrees of contractile growth to place the cotyledonary node at or below the soil surface.

Figure 9: Crowns of 2x DON and 2x WISFAL are similar.

Figure 10: Crowns of CADL and *M. coerulea* (both diploids) are similar, except *M. coerulea* is prostrate.

Figure 11: Crowns of *M. truncatula* (for comparison), *M. sativa*, non-dormant and dormant. Non-dormant crowns are relatively narrow.

Figure 12: Diploid DON and a large plant from sexual polyploidization (white stake).