Medicinal and Aromatic Plant Research

Objective 4. Double Haploid Trials

2004

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Saskatchwan Agriculture and Food

Medicinal and Aromatic Plant Research
Program Background

Production and processing of spice crops and medicinal herbs represents a potential bright spot in the future of Saskatchewan's agricultural sector. Saskatchewan growers have established a worldwide reputation as cost-competitive suppliers of superior quality medicinal plants and spices. Based on present growth rates, the Saskatchewan Herb and Spice Association (SHSA) projects the value of primary production of Saskatchewan-grown herb and spice products will approach $ 200 million annually by 2010. Numerous opportunities are available to add further value to these crops through processing, blending, preservation, extraction, encapsulation and packaging. A number of Saskatchewan companies have seized these opportunities, resulting in significant employment and economic activity in both urban and small town settings.

The marketplace for medicinals and spices is demanding, rapidly changing and highly competitive. To stay viable, Saskatchewan's herb and spice sector must focus on the best crops, varieties and production practices available. Emerging threats such as disease must be identified and dealt with effectively, but in a manner that does not jeopardise Saskatchewan's reputation for producing a safe, quality product.

This project takes a multi-disciplined approach to address the key production challenges for cumin and milk thistle under the relatively short and cool growing conditions in Saskatchewan. Production of these crops in Saskatchewan is presently limited by challenging growing conditions and specific agronomic problems. This project aims to alleviate these problems by crop improvement and development of superior agronomic practices.

Although improvement of spice/medicinal crops is possible utilizing standard plant breeding methods - the process is slow and consequently expensive. Double haploid technology allows the creation of a genetically homogeneous population without the need for multiple generations of selfing. This has the potential to greatly accelerate progress in improvement of medicinal/aromatic plants.

Program Objectives

1) Introduction/development of new/improved lines of cumin and milk thistle by:

a) accessing and evaluating potentially suitable material from public and private sources.
b) working with breeders from the Crop Development Center of the University of Saskatchewan to further develop adapted crop lines.

2) Pathology support to reduce losses to disease in cumin:

Blossom blight has decimated previous plantings of cumin in Saskatchewan.
a) Agronomically superior lines of cumin identified under Objective #1 will be evaluated for disease sensitivity in a disease nursery previously established by the Dept of Plant Sciences.
b) An integrated disease management approach will be developed, involving selection of resistant lines, identification of preventative production practices and evaluation of chemical control options.

3) Agronomy of Milk Thistle:

a) Time of seeding, seeding rate and row spacing effects on growth, yield and quality characteristics.
b) Optimizing nitrogen and phosphorus application rates.
c) Comparing organic products to standard chemicals for desiccation of the crop.

4) Field performance of new lines of spice/medicinal crops created using double haploid technology:

a) To evaluate lines of dill, fennel, anise, and cowcockle created by PBI/NRC using double haploid technology.
b) to compare the performance of the double haploid lines to parental lines

Objective 4.Double Haploid Trials

Medicinal and aromatic plants share common breeding objectives with most traditional crops - the industry is striving for increased yields and superior quality. Traditional breeding programs are costly and take years to achieve results. This is particularly true in self pollinating species and in situations where extensive backcrossing is required to introduce a new trait into a crops with complex quality and yield expectations.

In the double haploid (DH) technique used by PBI/NRC (Alison Ferrie) immature pollen grains (haploids) are exposed to treatments that result in doubling of the existing genetic material - resulting in homozygous, true breeding material in a single generation. This technique has been used to reduce the length of the crop improvement cycle of crops like canola and wheat by several years. Dr. Ferrie has also applied the DH technique to a wide range of medicinal and nutraceutical plant species.

The objective of this aspect of the program was to evaluate the field performance of the double haploids of various herb and spice crops that have been created by Dr. Ferrie.

2003 Trials

In 2003, seed of double haploids of dill and fennel were obtained from Dr. Alison Ferrie of PBI/NRC. Due to limited seed supplies, the double haploid lines were grown from transplants. Seed was pre-germinated on petri dishes and then transferred to transplant flats held in the Dept of Plant Sciences greenhouses. The transplants were moved into the field when they were 6 weeks old.

The field plots were located at the Department of Plant Sciences, Horticultural Crops Research Station in Saskatoon. The features of the site were previously described in the **Cumin Germplasm Evaluation Section (Objective 2).**

The seedlings were planted out in early June. Rows were spaced 0.5 m apart, with 15 cm between seedlings within a row. The number of plants of each line varied according to the % of the seeds planted that actually germinated. Weeds were controlled by hand removal. The plots were watered as needed. No significant problems with insect pests or disease were observed. Plant heights were recorded at several points during the growing season. The crops were harvested in late September, after the first killing frosts but before significant shattering occurred.

Results

Germination percentages varied greatly between crops and DH lines. In general, the parental lines of dill had a higher germination % than the DH, but some of the DH lines of dill showed excellent seed viability. A few of the DH lines of dill completely failed to germinate. The parental material in the fennel trial had excellent seed viability. This was matched by about 50% of the DH lines; others had a lower % germination and a few DH lines of fennel failed to germinate. .

Plants heights also varied between DH lines and the parental strains. Eight of the nine DH lines of dill tested were substantially shorter than the parental line. However, one line (DH-1) was significantly taller than the parental line. The DH lines of dill were also much more uniform in height from plant to plant than the parental lines. Heights of the DH fennel were either very similar to the parental line **or were nearly double the norm.** There were too few DH anise plants to effectively judge plant heights.

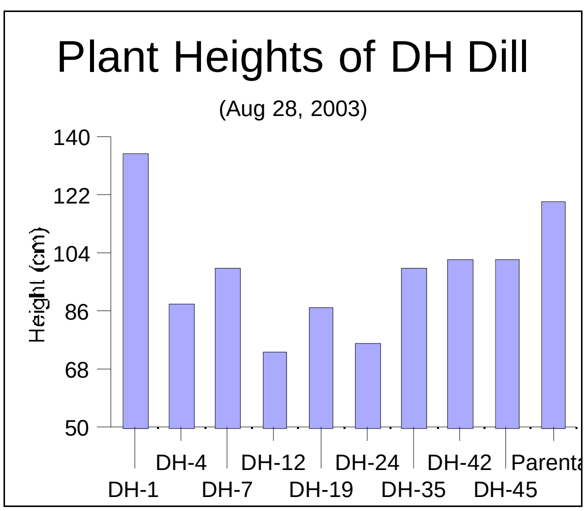

Figure 4.1

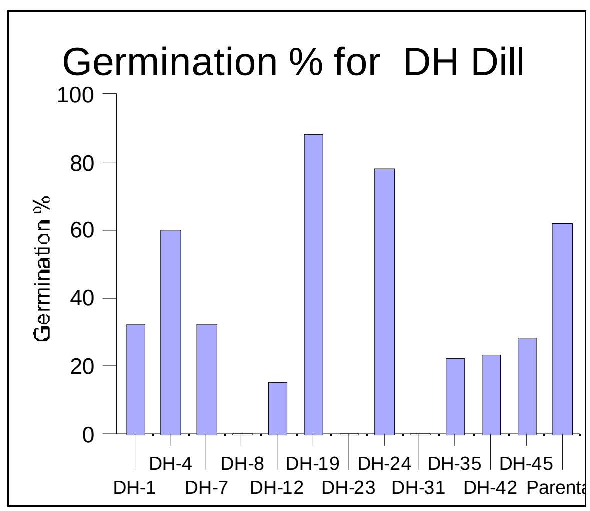

Figure 4.2

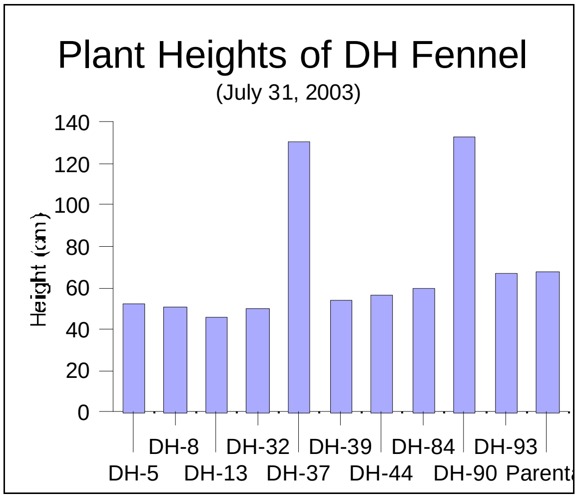

Figure 4.3

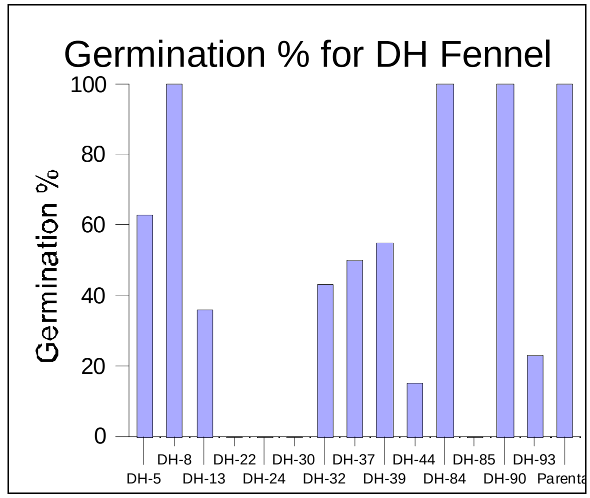

Figure 4.4

Seed yields for a number of the DH lines of dill were substantially greater than yields from the parental lines. **The three most productive DH lines averaged 61% higher yields than the parental line**. There was no apparent relationship between the height of the DH plants and their productivity.

The quantity and quality of the oil produced by the DH dill lines was evaluated by solvent extraction following by gas chromatography.

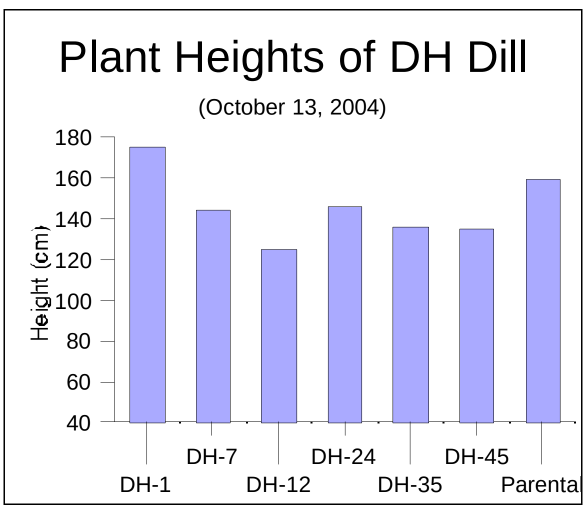

Figure 4.5

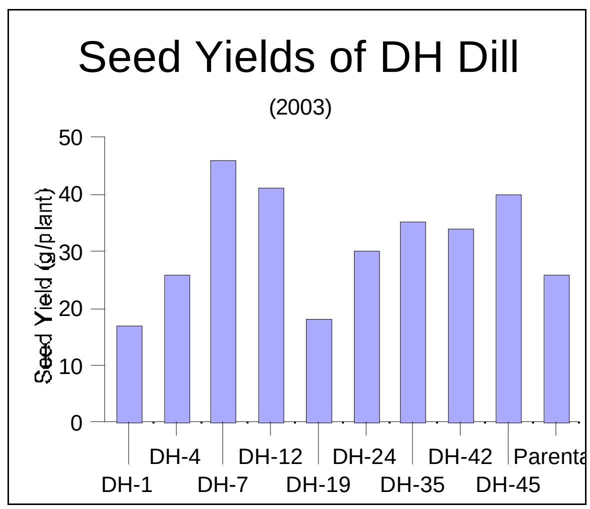

Figure 4.6

The essential oil content of many of the DH lines was lower than the parental line (3.3%)(Table 4.1). However, DH lines DH-1 and DH-12 had substantially higher essential oil content than the parental line. DH-12 also produced higher yields than the parental line. The oil extracted from dill should contain > 50% carvone. All of the DH lines except for DH-1 met this quality criterion.

Table 4.1. Essential oil content and quality for double haploid lines of dill - 2003.

Sample	% Essential oil	% Carvone
Mammoth (Parental)	3.3	52.8
DH-1	3.9	44.2
DH-4	1.3	57.5
DH-7	2.6	51.8
DH-12	3.8	51.1
DH-19	1.6	51.8
DH-24	1.8	55.7
DH-35	1.9	52.5
DH-42	2.1	51.9
DH-45	1.5	51

2004 Trials

In 2004, the main trial involved direct seeding the DH dill lines tested the previous season using transplants. The trials were run at the Department of Plant Sciences, Horticultural Crops Research Station in Saskatoon. This features of the site were previously described.

The site was prepared by rotovating prior to seeding. The plts were seeded on May 14 using a pushtype precision small plot seeder. The trial was seeded with 5 cm between seeds within a row and 50 cm between rows. Each plot of each line consisted of four 6 m long rows. Weeds were controlled by hand removal. The plots were watered as needed. No significant problems with insects pests or disease were observed. Plant heights were recorded at intervals during the growing season. The crops were harvested on October 13, after the first killing frosts but before significant shattering had occurred. The plots were harvested using a Winter Steiger Nursery Master Elite small plot combine. The harvested material was air dried at 40 C for 5 d, further threshed and then cleaned using a dockage tester. Seed yields and 1000 seed weights were determined at this point.

Results

Direct seeded dill trial.
As the trial was seeded quite heavily, all lines produced a complete stand. Line DH-12 emerged exceptionally quickly, established a very strong stand and was the first line to begin flowering. At the time of evaluation in late July, DH-12 was the tallest line and was furthest advanced in terms of flower development (Figure and Table 4.2). Line DH-12 also showed great uniformity of plant height at that time. However, by the final harvest line DH-12 was the shortest line but its seed yields exceeded all others, including the parental line **by a factor of 300 % (Figure and Table 4.2).** The precocious characteristics exhibited by DH-12 appeared highly beneficial in the relatively cool and short 2004 growing season. None of the lines were actually very mature by the time of harvest, but line DH-12 was more advanced than the others. Seed size for DH-12 was comparable to the parental line. None of the other DH lines produced seed yields that exceed the parental line. Although line DH-7 had yielded well in the 2003 trial, it performed poorly in 2004. Line DH-1 was slowest to develop but by the en d of the growing season it was substantially taller than the parental line. Seed yields from DH-1 were poor as it is slow to develop, however the seeds that it did produce were exceptionally large (Table 4.2). The unusually large size of the DH-1 plants may make it desirable for production of dill oil which can be extracted from both the seed and the herbage.

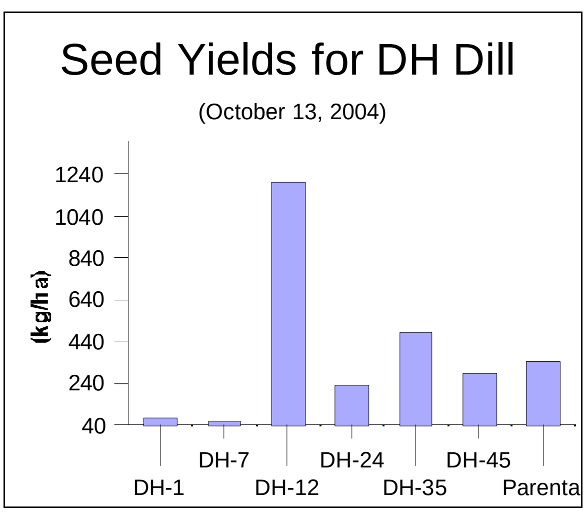

Figure 4.7

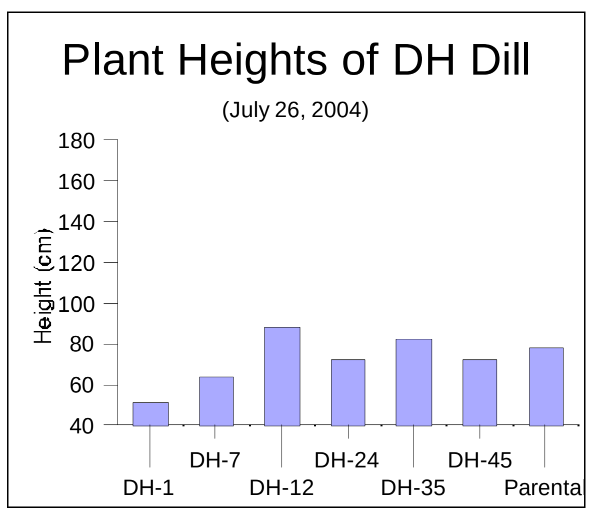

Figure 4.8

Table 4.2 Plant heights, yields and seed size for various lines of direct seeded double haploid dill in 2004.

	July 26			**Final Harvest**			
	Height (cm)	**Height (cv %)**	**Stage**	**Height (cm)**	**Height (cv %)**	**Seed Yield (kg/ha)**	**1000 seed weight (g)**
DH 1	51 e	22	0	175 a	29	76 c	3.55 a
DH 7	64 d	21	1.5	144 cd	29	54 c	2.67 ab
DH 12	88 a	5	5	125 e	29	1205 a	1.94 b
DH 24	72 c	9	2	146 c	29	230 bc	2.12 b
DH 35	82 b	12	2	136 cd	30	481 b	1.70 b
DH 45	72 c	14	1.5	135 de	30	286 bc	2.09 b
Parental	78 b	9	2	159 b	30	344 bc	2.02 b

Values within columns followed by the same letter are not significantly different p = 0.05

Table 4.4. Essential oil content and quality for double haploid lines of dill - 2004.

Sample	**% Essential oil**	**% Carvone**	**% Limonene**	**Oil yield (kg/ha)**
Mammoth (Parental)	**2.91**	**57.4**	**38.5**	**1001**
DH-1	**2.38**	**55.0**	**40.7**	**180**
DH-7	**2.48**	**51.8**	**40.0**	**133**
DH-12	**2.63**	**53.4**	**43.9**	**3169**
DH-24	**2.38**	**51.2**	**44.1**	**547**
DH-35	**2.12**	**49.6**	**45.8**	**1019**
DH-45	**2.45**	**59.0**	**46.4**	**700**
DH-47 **	**2.51**	**46.8**	**46.3**	**8117**
DH-53 **	**3.11**	**52.9**	**43.3**	**7501**

** = transplanted single row plots

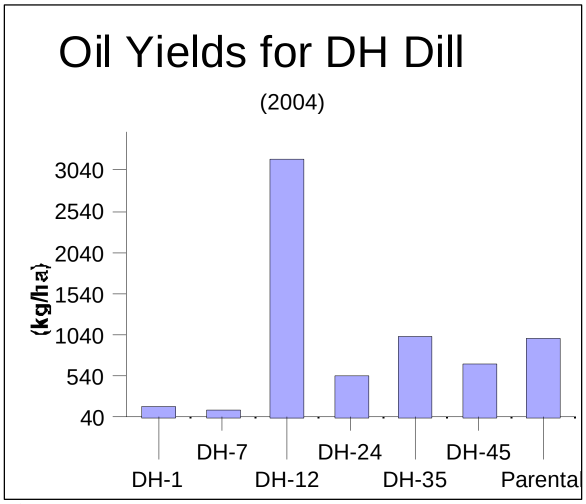

Figure 4.9

On average, essential oil content in 2004 was somewhat lower than in 2003; this likely reflects differences in the growing season. In 2003, several of the DH lines had a higher essential oil content than the parental line - but in 2004 the parental line had the highest oil content. Most lines met quality standards for % carvone (50%). **Oil yield (kg/ha) for line DH 12 was 300% of the parental line.** This reflects both its high seed yield and relatively high oil content. Oil yields for lines DH 47 and DH 53 were both exceptionally high - but as previously mentioned, this may reflect the advantage provided by transplanting and wide row spacings.

Fennel
As in the 2003 trial, the germination % of the DH fennel lines was quite variable. Many lines appeared to be non-viable while a couple showed excellent vigor.

Plant heights for the DH fennel lines in 2004 did not show the extreme line to line variability that was observed in the 2003 trial. Due to the short, cool growing season, no mature seed was harvested from this trial.

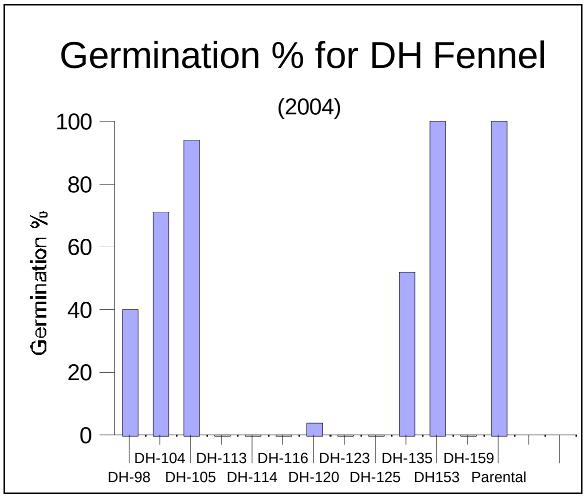

Figure 4.10

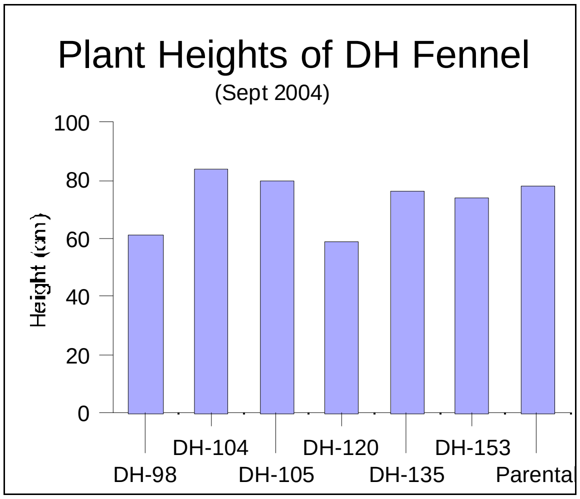

Figure 4.11