Variety Release Application Submitted by Chengci Chen and Chaofu Lu

We recommend that camelina advanced breeding lines MT 144 and MT 229 be licensed releases under Option "b" of the "Variety Release Policy", using the testing requirements for "Specialty Crops", i.e.

b) License Release: New release is protected with PVP under Title V. Variety is licensed under an exclusive (one business) or non-exclusive (more than one business) licenses by the MSU Technology Transfer Office (TTO). MSU royalty fees are collected under license releases and MAES Research Fees are not collected.

INTRODUCTION

Camelina, Camelina sativa L., is native to Eastern Europe and Western Asia (Vollmann & Eynck, 2015). It is characterized as a short-season crop which requires 85 to 100 days to mature and has high adaptability to various climatic and soil conditions, particularly well adapted to production in the temperate climate zone (Hunter & Roth, 2010). Camelina is an annual plant with both spring and winter biotypes. The spring biotype is the most widespread globally and is grown as an early summer annual oilseed crop, but camelina can also be grown as a winter annual in milder climates. Camelina has plant heights of 30-90 cm with branched stems that become woody at maturity, and stems are generally smooth or only sparsely hairy near the base (Hitchcock & Cronquist, 2018). Leaves are arrow-shaped, sharp-pointed, 5 to 8.9 cm long with smooth edges. Camelina produces prolific small, pale yellow or greenish-yellow flowers with four petals. Seeds are contained in pear shaped pods known as silicles resembling flax bolls and have a squared off tip (Klinkenberg, 2008). The seeds have a rough surface and are small, with 1000- seed weight in the range of 0.8- 2.0 g. The oil content of the seed, on a dry weight basis, is typically between 30 and 40 percent, which contains about 64 percent polyunsaturated, 30 percent monounsaturated, and 6 percent saturated fatty acids (McVay & Lamb, 2008a). The oil in camelina seeds is rich in ω -3 (α -linolenic acid; C18:3) and ω -6 acids (linoleic acid; C18:2), phytosterols, and phenolic compounds, which makes it attractive for the production of food and biofuels (Berti et al., 2016).

Camelina has been identified as a multi-purpose crop that provides a source of both oil and protein and enhances farmland's biodiversity and sustainability (Righini et al., 2016). It was recently introduced to North America (Chen et al., 2015; Afshar et al., 2018), primarily as a bioenergy crop in the semi-arid region of the Northern Great Plains and Pacific Northwest (Chen et al., 2015; Obour et al., 2018). Research showed that using camelina oil for aviation biofuel can reduce greenhouse gasses emissions compared with traditional petroleum jet fuels (Belayneh et al., 2015; Kwiatek et al., 2021; Shonnard et al., 2010; Walia et al., 2018; Yang et al., 2016). The increasing demand for camelina oil as sustainable aviation fuel feedstock in recent years requires breeders to develop new varieties that has high seed yield and oil concentration, and can adapt to various environments, especially to marginal land with low nitrogen (N) supply in the Northern Great Plains of USA and other regions, because nitrogen is the major energy input for camelina production, and nitrogen input level significantly impact the energy balance and economic returns in camelina production (Vollmann et al., 2007; Afshar et al., 2016).

BREEDING AND SELECTION PROCEDURE

The lines MT144 and MT229 were bred from the seed collections at Montana State University obtained from various world genebanks. Selections were based upon single plant selections in greenhouse and field trials. A total of 212 spring type *Camelina sativa* accessions were planted in the MSU Plant Growth Center. Seeds were harvested from single plants that were bagged with plastic bags during flowering times to prevent cross pollination.

In 2021 and 2022, over two hundred camelina accessions were planted as single-plant plots at MSU-Eastern Agricultural Research Center at Sidney, MT. Single plants were harvested, and plant weights were recorded. Each single plant was threshed to separate biomass and seed. The seed weight, oil and protein concentrations of the seed were measured. Based on the biomass, seed weight, seed oil concentration, fifteen accessions were selected for replicated small plot yield trials in 2022, 2023, and 2024 and a commercial variety, Suneson, was used as check. The field trials were conducted in low and high N environments for the purpose of selecting varieties for adaptation to low N environment. The seeding rate was 5 lb/ac. The seeding and harvesting date are presented in Tables 1-6. The plant biomass and seed yields were recorded in 2022 and 2023, but only seed yield was recorded in 2024. After harvest, seed yield, harvest index, oil

concentration, and oil yield were calculated. During the growing season, plant emergence date and plant density, bolting date, flowering date, maturity date, plant height at maturity, and disease incidence were evaluated. Insects have not been observed in camelina and no insect damage has been noted. In 2023 and 2024, downy mildew was Observed. The disease incidence was scored (from 1-10) based on the percentage of plants infected (Tables 3-6). No significant resistance was observed in all lines tested, but the lines showed some variations in disease severity/damage (not quantified).

After the three years replicated field yield trials, two superior lines, MT 144 and MT 229 were selected for release as varieties. The advantages of these two lines are presented in the following.

CHARACTERISTICS AND SUPERIORITIES OF MT 144 AND MT 229 OVER CHECK VARIETY

1. MT229

Description of MT229:

MT229 was selected from Plant Introductions (PI 650163) originated from the former Soviet Union. Single plants were selected in greenhouse grown with collections of 212 accessions of spring type *Camelina sativa*. The single plant was bagged by plastic bags to prevent cross pollination at the time of flowering. The seed from this single plant was used for single-plant field trial in 2021, and the seed harvested from the single-plant in the field was used to do replicated yield trials in 2022, and the replicated plot trial was repeated in 2023 and 2024.

MT229 was observed to have dark green and wider leaves (darker and wider than Suneson). The plant height was observed 56-78 cm which is similar or slightly taller than Suneson (57-74 cm), and plant height varied among years/environments. The plants flowered in 46 days after planting and matured in 82-87 days, which is slightly later than Suneson (81 days). The distinguish characteristics of MT229 is that the stems and seed pods turned purple in the late growing season, especially under heat and water stress (Figure 1c). The seeds are brown in color, similar to the seeds of Suneson, with 1000 seed weight of 1.06 g (compared to 1.15 g of Suneson) (Fig. 1d).



(a)

(b)



Figure 1. Characteristics of MT229 camelina in rosette (a), flowering (b), and seed filling (c) stages and the picture of the seed of MT229 (d).

In the three years field trial in the low N soil at Sidney, MT229 and Suneson had an average seed yield of 1554 and 1193 kg/ha in 2022, 1971 and 1819 kg/ha in 2023, and 2225 and 1821 kg/ha in 2024, respectively. MT229 consistently produced higher oil concentration than Suneson, i.e., 40.3 vs. 37.6% in 2022, 41.3 vs. 40.2% in 2023, and 40.5 vs. 38.8% in 2024. Therefore, the oil

yield of MT229 and Suneson was 627 and 449 kg/ha in 2022, 814 and 729 kg/ha in 2023, and 901 and 706 kg/ha in 2024, respectively (Tables 1, 3, 5).

In the high N soil environment at Sidney, MT229 and Suneson produced 2104 and 1933 kg/ha in 2022, 2323 and 2146 kg/ha in 2023, and 2605 and 2303 kg/ha in 2024, respectively. MT229 consistently had higher oil concentration than Suneson in all three years, i.e. 41.1 vs. 36.7% in 2022, 41.4% vs. 39.0% in 2023, and 39.6% vs. 37.3% in 2024, respectively. Therefore, the oil yield for MT229 and Suneson was 865 and 712 kg/ha in 2022, 961 and 837 kg/ha in 2023, and 1031 and 860 kg/ha in 2024, respectively (Tables 2, 4, 6).

On average, MT229 had about 14% higher (2130 vs. 1869 kg/ha) seed yield than Suneson, 6.4% higher (40.7 vs. 38.3%) oil concentration, and 21.1% higher (867 vs. 716 kg/ha) oil yield than Suneson (Table 7).

Superiority of MT229 over check variety:

- 1. Consistently higher seed oil concentration than Suneson.
- 2. Consistently higher seed yield than Suneson.
- 3. Averagely, MT229 had about 21% higher oil yield than Suneson.
- 4. Slightly later mature than Suneson that may increase yield in warmer and high moisture environment.

Recommendation:

We recommend MT229 be released by Montana State University and the Montana Agricultural Experiment Station in 2025 as a licensed variety. Seed increase will be done by the MAES Eastern Research Centers in cooperation with the Montana Foundation Seed program. We recommend the line MT229 be named "Sidney-HO"

Currently, 1700 lbs breeder seeds have been produced at EARC, which is enough for 300 acres foundation seed production.

2. MT144:

Description of MT144:

MT144 was selected from the seed collection CAM226, a breeding line originally named VNJJMK-17 from the former Soviet Union, obtained from the German Federal genebank maintained by the IPK Gatersleben. Single plants were selected in the greenhouse by bagging plants in plastic bags at the time of flowering to prevent from cross pollination. The seed from these individual plants was used for single-plant field trial in 2021, and the seed harvested from the single-plant in the field was used to do replicated yield trials in 2022, and the replicated plot trial was repeated in 2023 and 2024.

MT144 was observed to have dark green and wider leaves (darker and wider than Suneson, but similar to MT229). The plant height is 59-80 cm which is slightly taller than Suneson (57-74 cm), and varied among years/environments. The plants flowered in 46 days after planting and matured in 82-88 days, which is slightly later than Suneson (81 days). The seeds are brown color, similar to the seeds of Suneson. The 1000-seed weight is 1.12 g compared to Suneson seed of 1.15 g per 1000 seeds (Fig. 2d). The characteristics of MT144 are similar to MT229, except that MT144 doesn't show purple seed pod and stems in late season.



(a)

(b)

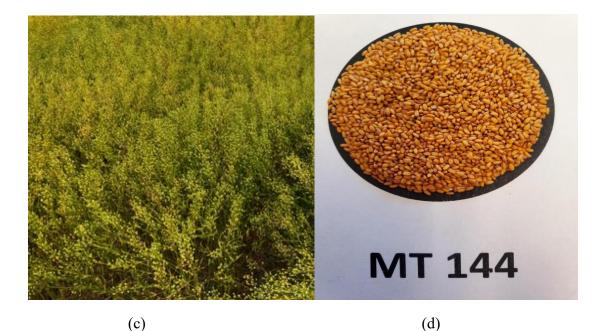


Figure 2. Characteristics of MT144 camelina in rosette (a), flowering (b), and seed filling (c) stages and the picture of the seed of MT144 (d).

In the three years field trial in low N soil at Sidney, MT144 and Suneson had an average seed yield of 1565 and 1193 kg/ha in 2022, 1837 and 1819 kg/ha in 2023, and 1740 and 1821 kg/ha in 2024, respectively. MT144 consistently produced higher oil concentration than Suneson, i.e., 40.1 vs. 37.6% in 2022, 42.9 vs. 40.2% in 2023, and 40.6 vs. 38.8% in 2024. Therefore, the oil yield of MT144 and Suneson was 628 and 449 kg/ha in 2022, 787 and 729 kg/ha in 2023, and 706 kg/ha in 2024, respectively (Tables 1, 3, 5).

In the high N soil environment, MT144 and Suneson produced 1809 and 1933 kg/ha in 2022, 2265 and 2146 kg/ha in 2023, and 2070 and 2303 kg/ha in 2024, respectively. MT144 consistently had higher oil concentration than Suneson in all three years, i.e. 40.1 vs. 36.7% in 2022, 41.1% vs. 39.0% in 2023, and 39.6% vs. 37.3% in 2024. Therefore, the oil yield for MT144 and Suneson was 727 and 712 kg/ha in 2022, 931 and 837 kg/ha in 2023, and 820 and 860 kg/ha in 2024, respectively (Tables 2, 4, 6).

On average, MT144 had about the same seed yield as Suneson but had 6.4% higher (40.7 vs. 38.3%) oil concentration and 7.1% higher (767 vs. 716 kg/ha) oil yield than Suneson (Table 7).

Superiority of MT144 over check variety:

1. Consistently higher seed oil concentration than Suneson.

2. Similar seed yield compared to Suneson.

3. Averagely, MT144 had about 7% higher oil yield than Suneson.

4. Slightly later mature than Suneson that may increase yield in warmer and high moisture environment.

Recommendation:

We recommend MT144 be released by Montana State University and the Montana Agricultural Experiment Station in 2025 as a licensed variety or germplasm. Seed increase will be done by the MAES Eastern Agricultural Research Center in cooperation with the Montana Foundation Seed program.

Currently, 1100 breeder seed has been produced, which is enough for 200 acres of foundation seed production.

REFERENCES

- Afshar RK, Chen C, Lin R, Mohammed AM. Agronomic effects of urease and nitrification inhibitors on ammonia volatilization and nitrogen utilization in a dryland farming system: field and laboratory investigation. J Clean Prod. 2018; 172: 4130–4139. https://doi.org/10.1016/j.jclepro.2017.01.105.
- Afshar R K, Mohammed AY, Chen C. Enhanced efficiency nitrogen fertilizer effect on camelina production under conventional and conservation tillage practices. Ind. Crops Prod. 2016; 94: 783–789. https://doi: 10.1016/j.indcrop.2016.09.043.
- Berti M, Gesch R, Eynck C, Anderson J, Cermak S. Camelina uses, genetics, genomics, production, and management. *Industrial crops and products*, 2016; 94: 690-710.
- Chen C, Bekkerman A, Afshar R K, Neill K. Intensification of dryland cropping systems for biofeedstock production: evaluation of agronomic and economic benefits of Camelina sativa. Ind. Crop. Prod. 2015; 71:114–121. <u>https://doi.org/10.1016/j.indcrop.2015.02.065</u>.
- Hitchcock CL, Cronquist, A. (2018). *Flora of the Pacific Northwest: an illustrated manual*. University of Washington Press.
- Hunter J, Roth G. Camelina production and potential in Pennsylvania. *Penn State College of Agricultural Sciences*. 2010.
- Klinkenberg B. E-Flora BC: Electronic Atlas of the Plants of British Columbia [www. eflora. bc. ca]. Lab for Advanced Spatial Analysis, Department of Geography, University of British Columbia. In: Vancouver. 2008.
- McVay, K., & Lamb, P. (2008a). Camelina production in Montana. *Montana State Univ. Ext. Publ.* 200701AG. Revised, 3(08).

Obour AK, Chen C, Sintim HY, McVay K, Lamb P, Obeng E, Mohammed YA, Khan Q, Afshar R K, Zheljazkov V D. Camelina sativa as a fallow replacement crop in wheat-based crop production systems in the US Great Plains. Industrial crops and products. 2018; 111: 22-

Vollmann J, Moritz T, Kargl C, Baumgartner S, Wagentristl H. Agronomic evaluation of camelina genotypes selected for seed quality characteristics. Industrial Crops and Products. 2007; 26: 270–277. https://doi.org/10.1016/j.indcrop.2007.03.017.

- Righini D, Zanetti F, Monti A. The bio-based economy can serve as the springboard for camelina and crambe to quit the limbo. OCL 2016; 23(5): D504. https://doi.org/10.1051/ocl/2016021.
- Vollmann J, Eynck C. Camelina as a sustainable oilseed crop: Contributions of plant breeding and genetic engineering. *Biotechnology Journal*. (2015); *10*(4): 525-535. https://onlinelibrary.wiley.com/doi/10.1002/biot.201400200

Variety	Planting date	Harvest date	Height (cm)	Biomass (kg/ha)	Yield (kg/ha)	HI	Oil (%)	Oil Yield (kg/ha)
MT144	5/16/2022	8/5/2022	80.0	6226.0	1565.0	0.3	40.1	627.6
MT229	5/16/2022	8/5/2022	76.5	6993.0	1554.1	0.2	40.3	627.1
Suneson	5/16/2022	8/1/2022	73.8	6344.0	1193.3	0.2	37.6	448.9
Mean			76.8	6521.0	1452.1	0.2	39.3	574.3
P-value			0.2603	0.6477	0.0692	0.1305	< 0.0001	0.0354
CV (%)			6.4	18.8	16.3	28.0	1.2	17.1
LSD								
(0.05)			NS	NS	NS	NS	0.8	156.7

Table 1. Camelina variety trial in low N soil in 2022

 Table 2. Camelina variety trial in high N soil in 2022

Variety	Planting date	Harvest date	Height (cm)	Biomass (kg/ha)	Yield (kg/ha)	HI	Oil (%)	Oil Yield (kg/ha)
MT144	5/16/2022	8/5/2022	73.3	7131.0	1809.0	0.3	40.1	726.8
MT229	5/16/2022	8/5/2022	77.5	7869.0	2103.7	0.3	41.1	864.9
Suneson	5/16/2022	8/1/2022	68.8	6703.0	1932.9	0.3	36.7	711.8
Mean			73.5	7234.3	1948.5	0.3	39.3	767.8
P-value			0.1596	0.6117	0.5122	0.8169	< 0.0001	0.2388
CV (%)			7.9	22.6	17.9	22.6	0.9	16.9
LSD								
(0.05)			NS	NS	NS	NS	0.6	NS

Variety	Stand Count (plants/m ²)	Height (cm)	Biomass (kg/ha)	Yield (kg/ha)	НІ	Oil (%)	Oil Yield (kg/ha)	Bolting Days	Flowering Days	Maturity Days	Downy Mildew (Scale 1-10)
144	127.5	72.9	4881.3	1837.3	0.4	42.9	787.1	39.3	45.0	87.0	0.1
229	91.5	74.0	6325.3	1970.8	0.3	41.3	814.1	43.8	45.5	84.0	0.1
Suneson	119.0	64.3	4926.3	1818.8	0.4	40.2	729.3	34.5	39.3	81.0	0.9
Mean	112.7	70.4	5377.6	1875.7	0.4	41.5	776.8	39.2	43.3	84.0	0.4
P-value	0.1933	0.0025	0.0052	0.4913	0.0211	0.0650	0.2681	<0.0001	<0.0001	0.0071	0.0029
CV (%)	23.7	4.3	9.7	10.1	8.4	3.4	9.0	3.9	2.4	2.4	66.7
LSD (0.05)	NS	4.8	831.0	NS	0.05	NS	NS	2.4	1.7	3.2	0.4

 Table 3. camelina variety trial in low N soil in 2023 (planted on 05/04/2023)

 Table 4. camelina variety trial in High N soil in 2023 (planted on 05/04/2023)

Variety	Stand Count (plants/m ²)	Height (cm)	Biomass (kg/ha)	Yield (kg/ha)	н	Oil (%)	Oil Yield (kg/ha)	Bolting Days	Flowering Days	Maturity Days	Downy Mildew (Scale 1-10)
144	124.5	74.7	6716.1	2264.6	0.3	41.1	930.7	37.8	45.5	87.0	0.1
229	124.0	78.3	7245.1	2322.5	0.3	41.4	961.0	42.5	46.0	87.0	0.0
Suneson	140.5	68.3	6371.4	2145.5	0.3	39.0	837.3	34.8	39.0	79.5	1.1
Mean	129.7	73.7	6777.5	2244.2	0.3	40.5	909.7	38.3	43.5	84.5	0.4
P-value	0.7035	0.0059	0.5404	0.5275	0.8867	0.0113	0.1618	0.0009	0.0003	0.0002	0.0052
CV (%)	23.9	4.4	16.0	9.7	16.6	2.3	9.5	5.0	3.7	2.0	93.8
LSD (0.05)	NS	5.2	NS	NS	NS	1.5	NS	3.0	2.6	2.8	0.6

Variety	Stand Count (plants/m ²)	Height (cm)	Yield (kg/ha)	Oil (%)	Oil Yield (kg/ha)	Bolting Days	Flowering Days	Maturity Days	Downy Mildew (Scale 1-10)	Downy Mildew (% Infected)
144	273.0	61.0	1739.8	40.6	706.9	42.5	46.3	83.0	2.3	0.3
229	217.5	56.3	2225.1	40.5	901.4	43.5	46.0	83.0	1.5	0.2
Suneson	277.0	57.1	1820.6	38.8	705.8	41.0	45.0	81.8	2.3	0.3
Mean	255.8	58.1	1928.5	40.0	771.3	42.3	45.8	82.6	2.0	0.3
P-value	0.0190	0.7368	0.0250	0.0479	0.0156	0.1292	0.2200	0.6007	0.7479	0.7083
CV (%)	10.3	15.5	11.3	2.5	11.2	3.7	2.2	2.4	79.1	78.9
LSD (0.05)	42.2	NS	348.0	1.6	137.7	NS	NS	NS	NS	NS

Table 5. Camelina variety trial in low N soil in 2024 (planted on 05/02/2024)

 Table 6. Camelina variety trial in high N soil in 2024 (planted on 05/02/2024)

Variaty	Stand Count (plants/m ²)	Height (cm)	Yield (kg/ha)	Oil (%)	Oil Yield (kg/ha)	Bolting Days	Flowering Days	Maturity Days	Downy Mildew (Scale 1-10)	Downy Mildew (% Infected)
144	244.5	59.2	2069.5	39.6	820.3	41.8	44.3	82.5	2.0	0.2
229	258.5	59.8	2605.2	39.6	1030.7	41.0	46.0	82.0	2.3	0.2
Suneson	258.5	58.6	2303.2	37.3	859.6	40.5	44.0	82.5	1.3	0.1
Mean	253.8	59.2	2325.9	38.8	903.5	41.1	44.8	82.3	1.8	0.2
P-value	0.9102	0.9757	0.0002	<0.0001	0.0001	0.5425	0.4747	0.9346	0.6724	0.4053
CV (%)	20.7	13.5	4.7	1.0	4.4	3.8	5.4	2.7	88.1	26.6
LSD (0.05)	NS	NS	173.6	0.6	63.3	NS	NS	NS	NS	NS

	Se	ed Yield (k	g/ha)	Oil C	Concentratio	on (%)	Oil Yield (kg/ha)			
	MT									
	144	MT 229	Suneson	MT 144	MT 229	Suneson	MT 144	MT 229	Suneson	
2022 LN	1565.0	1554.1	1193.3	40.1	40.3	37.6	627.6	627.1	448.9	
2022 HN	1809.0	2103.7	1932.9	40.1	41.1	36.7	726.8	864.9	711.8	
2023 LN	1837.3	1970.8	1818.8	42.9	41.3	40.2	787.1	814.1	729.3	
2023 HN	2264.6	2322.5	2145.5	41.1	41.4	39.0	930.7	961.0	837.3	
2024 HN	2069.5	2605.2	2303.2	39.6	39.6	37.3	820.3	1030.7	859.6	
2024 LN	1739.8	2225.1	1820.6	40.6	40.5	38.8	706.9	901.4	705.8	
Mean	1880.9	2130.2	1869.0	40.7	40.7	38.3	766.6	866.5	715.5	
% of CK	100.6	114.0		106.4	106.4		107.1	121.1		

Table 7. Seed yield, oil concentration, and oil yield averaged over six environments.