PENNYCRESS (*THLASPI ARVENSE*) A NEW NON-FOOD CROP FOR OIL-BASED BIOFUEL PRODUCTION IN EUROPE AND USA

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ABSTRACT: The development of alternative feedstocks for producing oil-based biofuels needs to meet the majority of the following criteria: low cost, high oil content, low agricultural inputs, favorable fatty acid (FA) composition, compatibilitywith existing farm equipment and infrastructure, production in off-season from conventional commodity crops, adaptability to marginal/idle lands, and viable markets for co-products such as seed meal. Recently a "potential weed", pennycress (*Thlaspi arvense* L.) has become one of the most attractive new non-food oil crops. Pennycress is highly tolerant to low temperatures, tolerating temperatures below -15°C after reaching a 4-to-6-leaves rosette stage. The seed contains up to 37% oil (DM) with the major fatty acid as erucic (36%). The fatty acid composition in pennycress has been shown to have physical properties suitable for biofuels like biodiesel and hydro-treated renewable jet fuel (HRJ). In the last decade, pennycress has attracted increasing interest as a potential oilseed for biofuel production in the USA, either biodiesel and/or jet fuel, in Europe very few studies have specifically focused on pennycress. In the present study, we compared the productivity of pennycress in response to environment in two European countries, Italy (Bologna) and Greece (Aliartos), and in two USA states, Illinois (Peoria) and Minnesota (Morris). Seed yield, seed oil content and oil compositions were evaluated in response to growing environment. Representative seed samples from each study location was solvent extracted for total oil recovery and compared across locations.
Keywords: vegetable oil, oil crops, biobased economy, biodiesel, marginal land, erucic acid.

1 INTRODUCTION

The demand for vegetable oils is continuously rising due to population growth and increasing globalization, as well as the share of vegetable oils converted to biobased applications continues to increase[1], while the world plant lipid production is unfortunately remaining quite stable (FAOSTAT 2016). Recently, the increase in vegetable oil requests from the biobased industry [2] is calling for highly efficient solutions in terms of land use and agricultural inputs, with minimal environmental impacts, thus opening the way to the study and development of new non-food oilcrops. Among candidate species, pennycress (*Thlaspi arvense* L.) has attracted considerable attention in the scientific community since it possesses many traits that can easily support its integration in existing rotation systems [3]. Specifically, pennycress is highly cold-tolerant and provides a living crop that covers land over winter, reducing soil erosion and nutrient leaching, and providing habitat for animals and insects [4, 5, 6]. Pennycress is considered a typical non-food oilseed crop [7] since its seeds are characterized by a high content of erucic acid (>30% DM) in the oil and an elevated amount of glucosinolates in the meal, thus making any food/feed use of the seeds unfeasible. Within the last decade, pennycress oil has attracted increasing interest as raw material for biofuel production in the USA, either biodiesel and/or jet fuel [8, 9, 10], whereas in Europe very few studies have been specifically focused on pennycress, but recently the EU project MAGIC has identified pennycress as a suitable crop for marginal land, in relation to its wide environmental adaptability, resistance to low temperatures, short-season and adaptation to marginal lands.

2 MATERIALS AND METHODS

2.1 Field experiments

Four field trials were set up across Europe (Italy and Greece) and USA (Minnesota and Illinois) to evaluate the potential of pennycress as a new oilseed for biofuel production under contrasting environmental conditions (Table I).

Table I: Locations, soil type and main climatic characterization (30-y historical data) for each trial site.

Location (country/state)		Soil type	Cumulative annual precipitation (mm)	Mean annual temp (°C)
Europe	Aliartos (Greece)	Sandy loam	485	16.7
	Bologna (Italy)	Silty clay loam	613	13.4
USA	Morris (Minnesota)	Fine loam	673	5.8
	Peoria (Illinois)	Silty clay loam	1011	10.8

In all locations, pennycress was grown as a winter crop with sowing and harvest dates optimized accordingly to each specific environment. In Italy (Bologna, 44°33'N, 11°23'E) two different pennycress lines (MN-106 and Elizabeth PI677360) [11] were sown in two consecutive dates (SD1: 13/10/2016 and SD2: 25/10/2016) comparing two different seeding rates (5 vs. 2.5 kg ha^{-1} of seeds, HD and LD respectively). Harvest was in mid May 2017 for all the plots. In Greece (Aliartos 8°22'N, 23°6'E) the pennycress line MN-106 was sown adopting a seeding rate of 5 kg ha^{-1} of seeds in two consecutive sowing dates (SD1: 9/10/16 and SD2: 19/11/16) and harvested on 22/05/2017. In Minnesota (Morris 45°35 N, 94°54'W) the

pennycress lines MN-106 and Beecher were compared undertwo different sowing densities (6 vs. 3 kg ha^{-1} of seeds, HD and LD respectively) during the growing season 2014/15. In Illinois (Peoria 40°43'N, 89°36'W) the pennycress line, Elizabeth, was sown in a large strip of about 2500 m^2, without replicates, on 20/09/2016 and harvested 06/06/2017. All the trials were rain fed, neither pesticide application nor chemical weed control were necessary during the growing season, and weeds were controlled manually. At full maturity in all locations pennycress plants were swathed and then threshed to separate seeds. Residual seed moisture content was determined on a representative seed sub-sample from each plot by oven drying at 105°C; upon reaching constant moisture levels, and weighted. Seed yields presented here are based on dry weight.

2.2 Laboratory analyses

All laboratory analyses, carried out on representative pennycress seed samples harvested in selected field trials reported in Table II, were performed at NCAUR laboratories in Peoria (Illinois).

Table II: List of pennycress seed samples chemically analyzed for biodiesel production

Sample name	Location	Line	Sowing date	Seeding rate
A	Peoria	Elizabeth	-	-
B	Aliartos	MN-106	1	-
C	Bologna	MN-106	1	HD
D		MN-106	1	LD
E		Elizabeth	2	HD
F		MN-106	2	HD

The pennycress seeds were ground <0.71 mm using a knife mill (Retsch Mill GM 200, Retsch GmgH, Haan, Germany). The ground samples was loaded into cellulose thimbles (Whatman 60 mm x 180 mm) and then extracted with hexane for at least 3 hours using a Soxhlet extractor. The miscella was filtered through a Whatman #1 filter and the hexane was evaporated using a rotary evaporator. The oils were transferred into capped brown glass bottles and kept at room temperature until use. Moisture of the seed and defatted meal samples were obtained by following AOCS official method Ba 2a-38 (AOCS, 1997). Oil yield was calculated based on the weights of oil extracted and pennycress seeds. Oil recovery was calculated based on the weight of oil extracted and the total amount of oil in the seed determined by its oil content.Fatty acid analyses were conducted on the methyl esters by gas chromatography using the method proposed by Isbell [12]. Total Oil content was determined by pulsed NMR using the method proposed by Isbell [12]. Viscosity measurements were made using a calibrated Cannon-Fenske viscometery tube (Cannon Instrument Co., State College, PA) measuring the kinematic viscosity according to ASTM method D445-97 (ASTM, 1997) at 40°C and 100°C. Viscosity index was calculated using ASTM method D2270-93 (ASTM, 1998). Pour points were measured using ASTM method D97-96a (ASTM, 1996) using 50 mL of sample with an accuracy of ±3°C. Cloud points were measured using ASTM method D2500-99 (ASTM, 1999) using 50 mL of sample with an accuracy of ±1°C. Acid values were determined by AOCS method Cd 3d-63 (1993b) using a Titrino 751 GPD automatic titrator from Metrohm (Riverview, FL). Cold filter plugging points (CFPP, °C) were measured according to ASTM D6371 (ASTM, 2010) utilizing a model FPP 5Gs Automatic CFPP Analyzer provided by PAC, L.P. (Houston, TX) using 45 mL of sample with an accuracy of ±1°C. IP was determined according to EN 15751 using a model 743 Rancimat instrument from Metrohm USA (Riverview, FL). Pennycress biodiesel samples were made using the method of [13] with the modification after base reflux 20.0mL of 1.0 M H_2SO_4/methanol was added then continued reflux for 1 hour. Cooled to room temperature transferred to a separatory funnel, added 20 mL of 0.5 M Na_2PO_4, 20mL of saturated NaCl solution then mixed vigorously, allowed to separate and the bottom aqueous phase removed. Then washed with pH 5 NaH_2PO_4 buffer and removed the bottom aqueous phase. The esters were then transferred to a round bottom flask and dried under vacuum at 90°C for one hour.

3 RESULTS AND DISCUSSION

3.1 Field results

Pennycress survived and grew well in all test locations, but seed yields varied greatly across environmental locations (Figure 1).

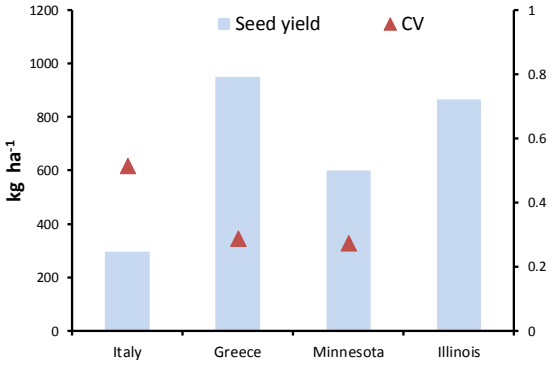

Figure 1: Mean seed yield of pennycress (grand mean for each test environment) in response to different growing sites. Red triangles represent the coefficient of variation (CV) of seed yield within each environment.

In particular, pennycress productivity (lower CV) was greatest when growing season precipitation was adequate, as in the case of Greece, which received exceptionally high rainfall that is uncharacteristic of South Mediterranean climate. Otherwise, the growing season in Italy was exceptionally dry (-50% of usual precipitation) and this could partially explain the low productive potential demonstrated by the tested pennycress lines in such environment (*e.g.*, Elizabeth yielded 867 kg ha^{-1} in Peoria but only one third in Bologna). When comparing sowing dates (Italy and Greece, Figure 2), SD1 lead to significant higher seed yields (950 vs. 361 kg ha^{-1}, $P \leq 0.05$) in both Mediterranean environments, presumably in relation to high water availability and increased competition against weeds. These results are in strong agreement with those of Dose et al 2017 [14] under a northern USA climate (Minnesota), which reached higher seed yields when pennycress was sown in early autumn (beginning of September). Interestingly, the sowing date did not influence the final harvest date, and pennycress confirmed the extreme shortness of its growth cycle (almost 2 weeks shorter than camelina, another new oilseed species, under the same environmental conditions).

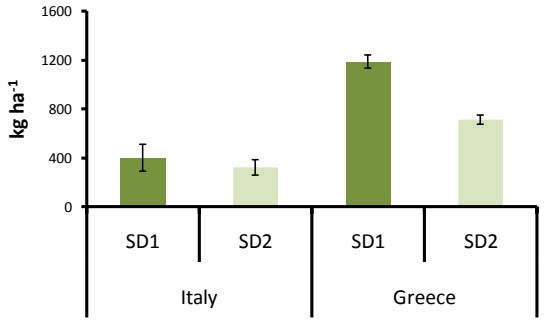

Figure 2: Seed yield of pennycress (line MN-106) in response to different sowing dates in Italy and Greece. Vertical bars: standard error.

When different seeding rates were compared (Italy vs. Minnesota, Figure 3) pennycress resulted in higher seed yields if sown at higher density (550 vs. 379 kg ha^{-1} for HD vs LD, respectively, $P \leq 0.05$), and this guaranteed also a more stable production across different growing conditions, presumably in relation to an improved stand establishment since pennycress still has an issue linked to seed dormancy.

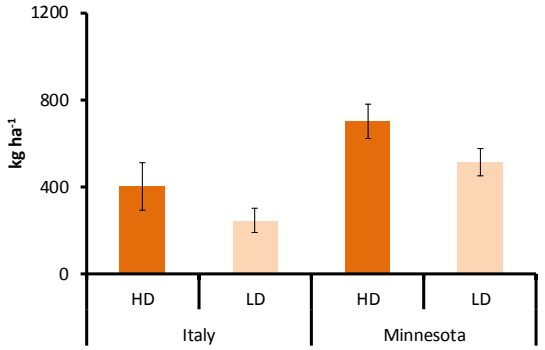

Figure 3: Seed yield pennycress (line MN-106) in response to different seeding rates in Italy and Minnesota. Vertical bars: standard error.

Further studiesto determine an optimized seeding rate for pennycress are for sure needed before the establishment of this new oil crop at commercial scale.

3.2 Analytical results

Hexane extraction recovered 94 to 99% of the oil in ground pennycress seed samples (Table II). In terms of starting seed weights, the oil yield varied from 28 to 31% (Table III). The crude oils had <0.5% FFA (free fatty acids) content, similar to screw pressed pennycress oil [15]. Typical FFA contents of crude soybean and rapeseed oils are 0.5-1.5 and 0.75%, respectively [16, 17]. The oil color was the same for each sample at 9 Gardner, which is lighter than what was observed (11-12 Gardner) for full-pressed oil [18].

Table III: Oil extraction results

Sample	Oil%	Ext%	Recv%	Resid%	FFA
A	30.6	27.9	97.8	2.4	0.47
B	32.7	27.7	94.1	2.9	0.40
C	34.0	30.8	99.1	2.9	0.33
D	34.0	30.6	97.9	1.3	0.43
E	34.2	28.4	90.7	3.0	0.29
F	33.5	29.1	94.5	2.8	0.43

Oil% is Dry weight basis pulsed NMR measured on whole seed. Ext% is the percentage oil extracted by mass from oil seed. Recv% is the observed mass percentage of oil extracted from what is theoretically possible. Resid% is the percentage of oil left in the meal after extraction. FFA is the free fatty acid content of the oil.

Oil samples wereconverted to methyl esters using a catalytic sodium methoxide in methanol method [13] and biodiesel yields were greater than 96% for all samples. The major methyl esters for each trial are reported in Table IVThe pennycress grown in Greece of MN-106 line (sample B, Table IV) had the largest erucic acid content of 39.9% whereas the Elizabeth germplasm grown at Peoria(sample A, Table IV) had the lowest at 37.5%. When both accessions (MN-106 and Elizabeth) were grown at the same location under the same plant densities (samples E and F, Table IV) the erucic acid content varied only slightly with the Elizabeth germplasm producing 38.9% compared to 38.2%. forMN-106 germplasm The variability in the erucic acid content showed an inverse correlation to the oleic acid content within each accession and location.

Table IV: Major fatty acids relative content (%)

Sample	18:1	18:2	18:3	20:1	22:1
A	11.9	19.5	12.0	10.4	37.5
B	11.1	18.8	12.0	10.0	39.9
C	10.0	19.6	13.6	10.1	38.6
D	9.9	19.4	13.5	10.0	38.0
E	9.4	19.4	13.0	9.9	38.9
F	11.1	19.2	13.3	10.3	38.2

Physical properties for the biodiesel samples were examined and reported in Table V. The Gardner color for all biodiesel samples was 8 which was slightly less color than the starting oils of 9. Pour point values for each accession were not significantly different with values of -15˚C to -18˚C where the error of measurement is ±3˚C. The cloud point values of the samples ranged from -12˚C for Elizabeth grown at Italy to -18˚C for the MN-106 line grown in Italy (samples E and D, Table V), where the type of accession had the largest impact on cloud point compared to thelocation the seed was grown. The cold filter plugging point (Table VI) provided good results for a biodiesel with the location in Italy (Bologna) giving the lowest CFPP regardless of accession. The viscosity of the samples were all similar and not significantly different. Lastly, the oxidative stability (Table VI) of the samples were all low and fall outside of both the U.S. and European standard for a biodiesel and would need and oxidative stability package to meet the standard. Previous biodiesel synthesized from pennycress grown at NCAUR just met theinduction period standard and the poor oxidative stability of the samples reported here most likely reflects a change from a citric acid wash in the previous study by Moser [9] where these samples did not receive that treatment.

Table V: Physical properties of biodiesels.

Sample	FFA	PP	CP	V40ºC	V100ºC
A	0.25	-15	-16	5.432	2.059
B	0.24	-18	-17	5.445	2.068
C	0.25	-15	-15	5.328	2.037
D	0.25	-18	-18	5.341	2.039
E	0.25	-15	-12	5.360	2.049
F	0.24	-18	-15	5.318	2.042

FFA is free fatty acid as percent. PP is pour point in ˚C. CP is cloud point in ˚C. V40˚C is the 40˚C viscosity in cSt. V100˚C is the 100˚C viscosity in cSt.

Table VI: Cold filter plug points and oxidative stability

Sample	CFPP	IP
A	-16.0	1.38
B	-16.1	1.26
C	-19.7	1.44
D	-19.8	1.61
E	-17.4	1.36
F	-17.7	1.85

CFPP is the cold filter plug point ˚C. IP is the induction period (oxidative stability) in hours.

4 CONCLUSIONS

Pennycress demonstrates a good potential for new oilseed crop with promising potential for biofuel production in Europe and USA. Still some agronomic constraints need to be overcome before a large adoption of pennycress is observed (i.e. high variability across growing conditions, high susceptibility to drought, difficult stand establishment) but the shortness of its growth cycle and the increased frost tolerance, compared for example to more productive and diffused oilseed rape, represents a valuable trait for farmers who are looking for alternative crops to include in their rotations.

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6 ACKNOWLEDGEMENTS

The MAGIC project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 727698.Authors want to thank Angela Vecchi for the technical supervision of field trials in Italy.

7 MAGIC PROJECT LOGO