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3 **Agronomic potential of two European pennycress accessions as a winter crop**

4 **under European Mediterranean conditions**

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21   **Abstract**

22   The growing interest in oilseed crops for sustainable biofuel production has promoted the

23   exploration of new plant species with high oil content and quality. One of these species is

24   pennycress (*Thlaspi arvense* L.; Brassicaceae), a winter annual plant that, due to the

25   characteristics of its seed oil, has a great potential as feedstock for advanced biofuels.

26   However, pennycress is not cultivated in Europe and, in contrast to the USA, the research has

27   been very scarce, especially regarding its agronomic behaviour and production. In this work

28   we performed a comparative analysis of the agronomic potential of two pennycress accessions

29   of European origin (French and NASC), with respect to two from USA (Beecher and

30   Elizabeth), to be cultivated under Mediterranean agroclimatic conditions. Stand establishment,

31   growth, and yield data of the four pennycress accessions were collected during two growing

32   seasons (2016-17 and 2017-18) in experimental fields situated in Aragon (NE Spain). The

33   European accessions had less germination success than those from USA (20-50% less).

34   However, the seed yield of the French accession was similar or superior to that of USA origin

35   (730-1390 vs 500-1340 ha^{-1}). This was because French plants were able to compensate for the

36   lower plant density with increased production of tillers and inflorescences. The other

37   European short cycle accession, NASC, requires further research to understand and overcome

38   its erratic germination and low seed yield. In terms of seed oil and erucic acid content, higher

39   variability was found between the growing seasons than among the pennycress accessions,

40   suggesting that weather conditions, especially rainfall distribution, have a pronounced effect

41   on seed, oil and erucic acid yield and must be considered for growing pennycress in Europe.

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44   **Keywords:** *Thlaspi arvense* L., alternative oilseed crop, germination, tillering, seed oil, fatty

45   acids.

46

1. Introduction

Plant seed oils represent an outstanding resource of bioproducts with many applications. Because of their chemical properties, plant seed oils have valuable uses in the food industry, as edible or cooking oils. Other industrial uses that include pharmaceutical, cosmetic and chemical industries, as part of paints, lubricants or plasticizers, are also economically relevant (Dyer et al., 2008). More recently, the need to reduce the dependence on petroleum and the widespread awareness of sustainable development in the context of global climate change and greenhouse gas emissions (GHG), have led to a growing interest in oilseed crops as renewable alternatives to petroleum-derived chemical feedstock (Metzger and Bornscheuer, 2006; Vollmann and Rajcan, 2009; Moser et al., 2009; Moser, 2012). In fact, the use of sustainable aviation fuels (SAF) is perceived as the only renewable liquid fuel option for internal combustion and jet engines by global aviation industry (Black et al., 2011; Trejo-Petch et al., 2019). In the European Union (EU), this perception was reflected in the recommendation of 10.1% increase of biofuel consumption for transport from 2017 to 2018 (EurObserv'ER, 2019). Moreover, the EU relies heavily on imported renewable and non-renewable fuels. Despite this demand, difficulties have emerged to develop strategies for biofuel production based on new feedstock, particularly in the EU, as they might threaten agri-food production because of land-use competition (Harvey and Pilgrim, 2011; Lüdeke-Freund et al., 2012).

The growing demand for oilseed production has promoted not only research and technological development aimed at improving the quantity and quality of seed oil, but also the exploration of new species with uniqueness of their seed oil composition (Moser, 2012; Zanetti et al., 2013). One of these plants is the winter annual Brassicaceae *Thlaspi arvense* L. (pennycress). Although pennycress is native of Eurasia, now it is widely distributed and can be found on all continents except Antarctica (Sedbrook et al., 2014). It is autogamous (Johnston et al., 2005) and its diploid genome has been recently sequenced and mapped (Dorn

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72 et al., 2013, 2015; Chopra et al., 2018). Large mutagenesis studies have been performed in an

73 attempt to improve pennycress breeding techniques (Chopra et al., 2020). Pennycress has been

74 identified as a promising non-food feedstock for bioenergy application related with biofuel

75 obtention (Moser et al., 2009; Moser, 2012). This interest is based on the high oil content in its

76 seeds (>36% dwb) and the high amounts of monounsaturated fatty acids, particularly erucic

77 acid ($22:1^{\Delta 13}$). The erucic content results in high energetic capacity (cetane number of 59.8) as

78 well as outstanding low temperature characteristics (cloud point of -10ºC) (Boateng et al.,

79 2010; Moser, 2012; Fan et al., 2013). Furthermore, as a winter crop, pennycress cultivation

80 might provide ecological and environmental benefits, reducing erosion in soils left bare over

81 the winter and decreasing pest pressure (Fan et al., 2013; Sedbrook et al., 2014).

82   Pennycress seems to be a good candidate for domestication due to its high adaptability to

83 different environmental conditions, short growing season, winter hardiness, and low input

84 requirements (Moser, 2012; Sedbrook et al., 2014; Dose et al., 2017). Approximately, a

85 decade of agronomic research, mostly performed in the USA, has provided relevant

86 information on different aspects of pennycress, such as seed dormancy, behaviour in crop

87 rotation and genetic diversity (Fan et al., 2013; Sedbrook et al., 2014; Altendorf et al., 2019;

88 Cubins et al., 2019). Despite these efforts, the results are variable, especially those related to

89 germination and stand establishment, whose inconsistency can be a limiting factor for its

90 cultivation. Likewise, more research on the most appropriate agronomic management

91 practices is required before integrating pennycress into a generalized production system (Dose

92 et al., 2017; Cubins et al., 2019).

93   The evaluation of pennycress as a winter crop represents an interesting opportunity for

94 Europe. However, unlike the USA, very few studies on its cultivation have been conducted in

95 Europe, although there is a botanical record of its presence throughout the continent. Thus,

96 Groeneveld and Klein (2014, 2015) conducted studies in southern Germany on pollination and

insect visitation in crop rotations that included pennycress. Likewise, the works of Royo-Esnal et al. (2015) and Gesch et al. (2016), carried out in Spain, on concrete aspects of the emergency and seed dormancy of pennycress, have provided valuable information that can help to its cultivation. More recently, several agronomic parameters like sowing date or seeding rate were studied in two different EU locations using USA germplasm (Zanetti et al., 2019). However, as far as we know, no data are available of the agronomic potential of European germplasm of pennycress that could be better adapted to our agroclimatic conditions.

Our Group has characterized pennycress germplasm of European origin at both the biochemical and molecular levels (Claver et al., 2017) and two accessions, French and NASC have attracted our interest. In this work we performed an agronomic characterization of these Europeans accessions with the following objectives: (i) evaluate the agronomic behaviour and potential of pennycress under the Mediterranean conditions of Europe and (ii) compare two pennycress accessions of European origin with two from USA with accumulated crop cultivation experience in an attempt to estimate differences in germplasm origin in its adaptability for cultivation in Europe.

2. Materials and methods

2.1. Pennycress germplasm collection

Four pennycress accessions were studied and compared in the present study. Two of them, "NASC" and "French", have a European origin (Claver et al., 2017). The NASC seeds (N9661) were obtained from the Nottingham Arabidopsis Stock Centre, NASC (UK), and is a wild accession collected from a field in Wellesbourne (UK). The French seeds were purchased from the B&T World Seeds company (France). The other two pennycress accessions, "Beecher" and "Elizabeth", were developed in the USA and were supplied by Dr. Phippen of

the Western Illinois University (USA). Beecher (Reg. No. PI672505) was collected from a winter fallow corn field at Hanna City (IL) and Elizabeth (Reg. No. PI677360) is an improved variety from the Beecher accession with a reduction in the dormancy of the seeds (Isbell et al., 2017). While the NASC accession acts as a spring annual (vernalization is not required for flowering; Claver et al., 2017), the rest are winter annual accessions.

2.2. Study site

The experimental fields were located at the research farm of the Estación Experimental de Aula Dei (Consejo Superior de Investigaciones Científicas, EEAD-CSIC), in the Aragon region (NE Spain) (41º 43′ N, 0º 48′ W, 230 m alt.). This soil is representative of the soils in semiarid Aragon with a medium texture (loam with 31% sand, 43% silt, 26% clay, 0-40 cm depth), alkaline (pH=8.4; 316 g kg^{-1} of CaCO$_3$) and generally with low organic matter content (9.7 g kg^{-1} of C org.). The climate is semiarid Mediterranean (average annual air temperature of 14.8ºC and annual rainfall of 344 mm).

2.3. Experimental design and cropping management

The four pennycress accessions were compared following a randomized complete block design with threes replicates per treatment. The individual plot size was 24 m x 2.8 m with a separation of 1.5 m between plots.

The study was conducted during two experimental growing seasons (2016-17 and 2017-18). Due to the impossibility of using herbicides, in the second growing season the trial moved a few meters to avoid the effects of a possible regrowth of pennycress coming from the harvest of the first growing season. Even so, the field and the soil were the same in both cases. The field was previously cultivated with barley.

146   The soil preparation consisted of mouldboard ploughing followed by a pass of cultivator

147   and roller for seedbed preparation. In both growing seasons, a N-P-K fertilizer (60-120-40 kg

148   ha^{-1}) was applied at sowing time. Pennycress was sown on 15 November in 2016 and on 17

149   October in 2017 at a rate of 10 kg ha^{-1}. A 6-row plot seeder with 20 cm row spacing (built in

150   the EEAD-CSIC) was used in both growing seasons. Seeds were sown directly on the soil

151   surface (not buried) followed by rolling to ensure a good seed-soil contact. As discussed

152   below, due to the irregular distribution of rainfall in the study area, we decided to install a

153   sprinkler irrigation system after sowing that could be used in case the lack of water could

154   prevent the emergence and establishment of the crop. No herbicide was applied, controlling

155   the weeds by hand and cleaning the corridors and the adjacent areas by rotovator.

156   Pennycress grain was harvested when more than 95% of the silicles were dry with a

157   yellow colour and the colour of the seeds turned dark reddish brown. The harvest was done

158   with a plot combine (Wintersteiger *Elite* model) on 22 May 2017 and on 21 May 2018. Grains

159   were threshed, cleaned and weighed.

160

161   *2.4. Data collection*

162   The development and growth of pennycress was monitored during both experimental

163   growing seasons (2016-17 and 2017-18) through the measurement of different parameters at

164   certain stages of development (the same stages in both seasons). The crop establishment was

165   estimated by counting the number of plants in four areas of 0.25 m^2 in each plot, once full

166   emergence was observed (January-February). The plant height was measured throughout the

167   growing season. The percentage of tillering plants, the number of tillers per plant and the

168   number of inflorescences per plant were also determined during the flowering and the seed

169   maturation stages. These parameters were measured in 10 plants from two different rows of

170   each plot. Seed maturation was assessed qualitatively according to four stages of silicle

171   development based on colour (green, green-yellow, yellow-green and yellow). After harvest, a

172   random sample of 1000 grains was taken from each plot and weighed with a precision balance

173   to determine the mean grain weight. Seed moisture content was determined by oven-drying

174   the seed samples (103ºC for 17±1 hours). The grain yields reported in this study were adjusted

175   to a moisture content of 10% (w/w).

176   Data on precipitation and air temperature during the study period were collected from an

177   automated meteorological station located 50 m from de experimental field in the EEAD-CSIC.

178   This station is part of the network of observatories of the Spanish State Meteorological

179   Agency.

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181   *2.5. Seed oil and fatty acid composition analysis*

182   Total seed oil content was analysed from 10 g of pennycress seeds of each accession and

183   for each experimental growing season. Powdered pennycress seeds were subjected to acid

184   hydrolysis with HCl, following by extraction of the oil fraction with petroleum ether in a

185   semiautomatic Soxhlet extractor (Foss Soxtec® Avanti 2055). After evaporation and

186   desiccation of the extract, the oil content for each fraction was determined gravimetrically.

187   For fatty acid analysis, total lipids were analyzed from pennycress seeds (0.5 g) following

188   the Bligh and Dyer (1959) method as described in Claver et al. (2017). Data from fatty acid

189   analysis were obtained from two independent pools of seeds from the different pennycress

190   accessions in the two experimental growing seasons.

191

192   *2.6. Statistical analysis*

193   The statistical comparisons among pennycress accessions were made using analysis of

194   variance (ANOVA) for a randomized complete block design with three replicates. Treatment

195   means were compared with the Duncan's multiple range test ($P<0.05$). When data showed

196 non-normality (Kolmogorov-Smirnov test), log transformations were made and ANOVA

197 conducted with the transformed data.

198

199 **3. Results**

200 *3.1. Weather conditions*

201   The amount and distribution of precipitation varied considerably between the two

202 experimental growing seasons (Table 1). Growing season precipitation (October-May) in

203 2016-17 was similar to the long-term average (244 mm) and in 2017-18 was 30% above

204 average. In the second season, 90% of the precipitation was concentrated from January to May

205 2018, April and May being exceptionally humid. In contrast, during the October-December

206 2017 period, rainfall was 70% below average and supplemental irrigation was used to enhance

207 seed germination. In the 2016-17 growing season, the precipitation pattern was very different,

208 since 50% of the total rainfall fell in October-November 2016 (126 mm). On the contrary,

209 April and May 2017 were very dry, receiving less than half the normal amount of rainfall for

210 this period. Air temperature was similar between the two crop years and the long-term average

211 year with the exception of January and May 2017, which were almost 2ºC warmer than

212 average (Table 1).

213

214 *3.2. Crop establishment and growth*

215   The germination of pennycress in the 2016-17 growing season was considerably delayed

216 compared with that of the 2017-18 season. Thus, in the first growing season, two months

217 passed after sowing until crop emergence was observed (11 January 2017) while in the second

218 season, it began only after two weeks (31 October 2017). Regarding differences among

219 accessions, it should be noted that, in the second growing season, NASC emergence was

220 delayed approximately two weeks with respect to the rest.

221   Once germination was completed, the initial crop development already revealed the first

222   differences among pennycress accessions. During both growing seasons, Beecher and

223   Elisabeth (USA) had a better establishment than French and NASC (EU). The highest number

224   of plants always corresponded to Beecher and the lowest to NASC. The difference was greater

225   in the second growing season with 10 times less plants from NASC than from Beecher. In

226   fact, the NASC variety had a very low emergence success in this growing season (only 15

227   plants m^{-2}) (Table 2).

228   The height of the pennycress plants followed a parallel dynamic in the four accessions

229   during both growing seasons (Fig. 1). Elisabeth was the tallest plants; once maximum height

230   (late April) was reached, plants were 11-13 cm taller than the European accessions ($P<0.05$;

231   Table 2). Thereafter, with the height declining, Elisabeth tended to lean and lodged if the wind

232   blew. In the second growing season, all plants reached greater height than in the first growing

233   season.

234   The tillering capacity of pennycress was affected by the accession (Table 3). Although in

235   the first growing season the differences among accessions were not statistically significant, a

236   tendency was observed that was confirmed in the second season. Both the percentage of plants

237   with tillers and the number of tillers per plant were higher in the European than in the USA

238   accessions; the latter maintained similar average number of tillers per plant (1-1.4) in the two

239   growing seasons (Table 3). Conversely, the European accessions doubled the number of tillers

240   from the first to the second growing season (1.9-2.5 vs. 4.4-4.7).

241

242   *3.3. Flowering and seed production*

243   During the 2016-17 growing season, French began flowering on 13 March 2017 and one

244   week later the other accessions. In the 2017-18 season, flowering was quite simultaneous in

245   the four accessions, beginning on 7 March 2018. The number of inflorescences per plant

246 varied with the pennycress accession in the same way as the number of tillers (Table 3). Thus,

247 the European plants produced greater number of inflorescences than those from USA,

248 especially during the 2017-18 growing season (more than double). Again, comparing the two

249 growing seasons, the USA accessions maintained similar production of inflorescences (Table

250 3).

251 The different stages of seed maturation were in general quite coincident in the four

252 accessions (Table 4). However, Elizabeth and French were slightly earlier in seed maturity,

253 showing colour change and drying of silicles a little faster when compared with Beecher and

254 NASC. In the second growing season, the appearance of silicles and seed ripening were

255 delayed with respect to the first growing season, possibly due to the abundant rain received in

256 the spring.

257 Seed yields varied significantly among accessions and between growing seasons (Table

258 3). Yield values ranged from 500 to 730 kg ha^{-1} during the 2016-17 growing season and from

259 700 to 1390 kg ha^{-1} during the 2017-18 growing season. With the exception of NASC, the

260 seed production of the first growing season doubled (French and Elizabeth) or, even, tripled

261 (Beecher) in the second growing season. It is worth mentioning that seed yields from the

262 French accession were always similar or significantly higher than those from USA. On the

263 other hand, seed yield from the NASC accession was in a similar range in both growing

264 seasons, although in the second one was only half of that obtained from the other accessions.

265 Pennycress 1000-seed weight ranged from 1.04 to 1.36 g, being highest during the 2017-18

266 growing season (Table 3). These differences among accessions did not correspond to

267 differences observed in seed yield. In fact, French, the highest seed yielding accession,

268 produced seeds with the lowest mass in both growing seasons (4-14% lower).

269

270 *3.4. Seed oil and erucic acid content*

271   Total seed oil content ranged from 34.5% (NASC 2016-17) to 36.7% (Elizabeth 2017-

272   18).   The variation observed between the growing seasons (maximum difference of 1.9

273   percentage point; Table 5) was higher than that among accessions (maximum difference of 0.8

274   percentage point). In fact, while there were no significant differences among accessions within

275   each growing season, all accessions significantly increased the amount of seed oil from the

276   first to the second growing season (increases of 1-2%) (Table 5).

277   Fatty acid composition and erucic acid (C22:1) were also monitored in the four accessions

278   and both experimental growing seasons. In all cases, erucic acid was the most abundant fatty

279   acid present in the pennycress seed oil, with average values of 34.4-38.0%, depending on the

280   accession and the year (Fig. 2). Differences in erucic acid among the pennycress accessions

281   were not statistically significant in either growing season although, averaging the two seasons,

282   the USA accessions had slightly higher percentages than the European accessions (differences

283   of 0.8-1.4 percentage points). As occurred with the total oil content, the highest content of

284   erucic acid corresponded to the 2017-18 season (36.6-38.0% vs 34.4-36.6% in 2016-17),

285   contributing to this increase all four accessions in a lesser or greater degree. In fact, the higher

286   percentage of the erucic acid in the second than in the first season was statically significant in

287   all pennycress accessions with the exception for the French one (Fig. 2).

288

289   **4. Discussion**

290   *4.1. First experience of pennycress cultivation in Spain*

291   This study evaluated the suitability of two pennycress accessions of European origin

292   (French and NASC) to be cultivated under semiarid Mediterranean conditions. Two USA

293   accessions (Beecher and Elizabeth) were also cultivated for the purpose of serving as

294   reference since, practically, all the available information on pennycress cultivation comes

295   from studies and trials carried out in the USA (Cubins et al., 2019) or in the UE (Zanetti et al.,

296 2019) with USA accessions. In this work, we have compared the agronomical potential of two

297 European accessions that could be better adapted to our agroclimatic conditions.

298 Pennycress seed yields obtained in our study (500-1400 kg ha^{-1}) are within the range

299 reported in the USA. Although values between 1100 and 2250 kg ha^{-1} are considered as

300 expected yields for pennycress grown in USA (see reviews by Moser, 2012 and Sedbrook et

301 al., 2014), much lower productions have been also recorded due to many influence factors

302 such as cropping system, planting date or weather conditions (Johnson et al., 2015; Dose et

303 al., 2017; Cubins et al., 2019). For example, the yields obtained from the Beecher variety

304 grown in Minnesota (USA) during three growing seasons varied from 99 to 1109 kg ha^{-1},

305 depending on the sowing date (Dose et al., 2017).

306 During the experimental period, differences in seed yield were higher between growing

307 seasons than among pennycress accessions. With the exception of NASC, which maintained

308 closer seed yields in both growing seasons, the production obtained in the 2016-17 growing

309 season doubled in 2017-18 for the French and the Elizabeth accessions and almost tripled for

310 Beecher. The highest yields in the second growing season are attributed to the most favourable

311 weather conditions. Rainfall was 30% higher in the 2017-18 growing season than in that of

312 2016-17, with April and May (flowering and grain formation) being exceptionally wet.

313 The influence of weather conditions was already evident during the emergence phase of

314 pennycress. In the first growing season, the excess of soil moisture during the fall of 2016,

315 prevented the seeder from entering the field until middle November. In addition, once sowing

316 done, the dense and continuous fog in December 2016 probably contributed to the long delay

317 in the beginning of crop emergence (2 months from sowing). Evidence of the stimulating

318 effect of light on the germination of pennycress seeds was provided long ago (Courtney, 1967;

319 Hazebroek and Metzger, 1990a). In this sense, the monthly mean solar radiation from

320 November-December 2016 was 62 W m^{-2} compared with 174 W m^{-2} from September-October

321   2016. Likewise, the delayed sowing in 2016 implied a reduction in both the mean air

322   temperature (from 20/16ºC in September/October to 9ºC in November; see Table 1) and the

323   daily thermal amplitude (from average of 16/12ºC to 10ºC). As reported in USA studies

324   (Phippen et al., 2010a; Johnson et al., 2015; Dose et al., 2017), the decrease in soil

325   temperature as the seeding date is delayed throughout the fall, not only has a detrimental

326   effect on pennycress germination and establishment, but can also significantly reduce seed and

327   oil yields.

328   Regardless of the weather conditions, in both growing seasons the USA accessions had

329   higher germination success than the European ones. However, in the case of French, its lower

330   stand establishment did not ultimately result in reduced seed production. In fact, its yield was

331   similar or even higher than that of the USA accessions. The French plants were able to

332   compensate for the lower plant density with higher production of tillers and inflorescences,

333   especially in the second growing season since 2018 was exceptionally wet. The compensation

334   strategy is confirmed with the negative and significant correlation coefficient found between

335   the values of plant density and the number of inflorescences per plant (Fig. 3). By grouping

336   the data by variety and year, we can see that the values corresponding to the European

337   accessions vary with the growing season (Fig. 3). On the contrary, the values from Beecher

338   and Elizabeth are grouped to the right of the graph, which indicates that they are more

339   conservative, maintaining a similar production of tillers and inflorescences in the two growing

340   seasons. Anyway, their emergence values did not vary much between growing seasons. We

341   must bear in mind that USA pennycress accessions used in this study have been cultivated for

342   several years, contributing to fix some these traits. In addition, Elizabeth is a line with a

343   reduction in the dormancy of the seeds obtained from the wild Beecher by consecutive cycles

344   of seed propagation (Isbell et al., 2017).

345   There is not much information about the ability of pennycress to compensate for low

346   stand establishment with an increase in yield components. Matthies (1990) reported a

347   considerable plasticity of the reproductive components for this species. According to this

348   author, pennycress reduced its reproductive output (number of flower buds per plant, seed

349   weight, etc.) in response to the increase in plant density and resource limitation. In an

350   experiment with different seeding rates of pennycress, Phippen et al. (2010b) found a higher

351   number of branches per plant at low seeding rates due to less competition for resources. Data

352   of the present study indicate that both European pennycress accessions showed a

353   compensatory strategy based in branching ability that was not found in the USA accessions in

354   our study. This branching ability is typical of many Brassicaceae species and has been

355   recently reported for Camelina grown in the USA (Gesch et al., 2018). It is worth mentioning

356   that Zanetti et al. (2019) reported the absence of branching in the pennycress USA accessions

357   cultivated in Italy. Our data might suggest that this branching ability might be specific of these

358   European accessions.

359   Although the European NASC accession used the same compensatory strategy as the

360   French, this was not enough to overcome the disastrous establishment of plants in the second

361   growing season. We do not know the reason for this bad response. It may be related to the fact

362   that NASC is a spring line. Seed dormancy is also a possible cause since the degree of

363   dormancy differs among pennycress accessions (Hazebroek and Metzger, 1990b; Sedbrook et

364   al., 2014; Royo-Esnal et al., 2015). In fact, seed dormancy is a negative trait of this and many

365   other oilseed species, especially those undomesticated or partially domesticated (see reviews

366   by Zanetti et al., 2013 and Sedbrook et al., 2014). The NASC behaviour requires further

367   research with the objective to reduce its erratic germination and low seed yield. However, the

368   differences in plant emergence and establishment among the four pennycress accessions

369 studied here, offer the possibility of choosing the most suitable for cultivation under our

370 agroclimatic conditions.

371 The values of seed oil content obtained for the four pennycress accessions (34-37%) are

372 consistent with previous data for both NASC and French accessions (Claver et al., 2017), and

373 for the USA accessions (Sedbrook et al., 2014; Altendorf et al., 2019; Zanetti et al., 2019). In

374 the recent analyses of 41 pennycress accessions grown at three Minnesota locations (USA),

375 Altendorf et al. (2019) obtained oil contents ranging from 27.5 to 35.6%. In the previous

376 characterization study of 114 accessions with different origin, Sedbrook et al. (2014) showed a

377 higher variation with oil contents moving from 13.5 to 38.7%. Comparatively, the narrow

378 range of our values can be explained by the fact that all four pennycress accessions were

379 grown under the same agronomic, edaphic, and climatic conditions. The relatively small

380 variation found is attributed to the different weather conditions of the two growing seasons,

381 especially in the rainfall distribution, rather to differences among the pennycress accessions.

382 The higher seed oil content produced in 2017-18 with respect to 2016-17 (differences of 1-2

383 percentage points) coincided with the higher precipitation received, particularly during April-

384 May 2018. This is in agreement with the study from Dose et al. (2017) showing that higher

385 precipitation, particularly in spring, favoured higher seed oil contents in this species. Although

386 all four accessions increased the seed oil content in response to better rainfall conditions,

387 Elizabeth was the line most favourably affected (increase of nearly 2 percentage points).

388 Erucic acid (C22:1) was the most abundant fatty acid in pennycress oil with values

389 ranging between 34.4 and 38.0%. These percentages fall within the range of previous studies

390 for either European or USA accessions [27.5–39.0% (Sedbrook et al., 2014), 35.1-39.7%

391 (Claver et al., 2017) and 29.4-39.6% (Altendorf et al., 2019)]. Although differences among

392 pennycress accessions were small (below 2.1 percentage points) and not statistically

393 significant, there was a trend of lower percentages in the European accessions than in those

16

394   from the USA. Likewise, the four pennycress accessions showed an increase of erucic acid in

395   the 2017-18 growing season with respect to the 2016-17 one. Although it is difficult to

396   separate the genetic influence from the effect of environmental conditions (Gesch et al., 2016;

397   Claver et al., 2017; Altendorf et al., 2019), precipitation seems to favour the preponderance of

398   erucic acid in the seed oil of pennycress under the agroclimatic conditions of this study. Data

399   from additional growing seasons will be necessary to stablish a potential relation between

400   weather conditions and seed oil properties.

401

402   *4.2. Strategies of pennycress cultivation in Aragon (NE Spain)*

403   As previously indicated, pennycress is a plant species, mostly considered a weed,

404   extensively distributed throughout the world (Sedbrook et al., 2014). Due to its high

405   adaptability to a wide variety of environmental conditions, hardiness and low input

406   requirements, pennycress can be considered a good candidate for cultivation in many areas of

407   Europe, including the Mediterranean South region (Zanetti et al., 2013). Our experience in

408   Aragon (NE Spain), in these first two experimental growing seasons, has been successful in

409   three of the four pennycress accessions considered, confirming the high growth potential in

410   our region.

411   A possible pennycress cultivation strategy in our region would be in rotation with corn

412   under irrigation conditions. For this, short-season lines of corn would be used to avoid

413   overlapping the two crops both at the time of planting the corn and at the time of the

414   pennycress harvest. However, rainfed agriculture in Aragon is very important. According to

415   the most recent statistics (MAPA, 2019), rainfed arable land in Aragon extends over 1.38

416   million ha (77% of total agricultural land). Within this surface, about 729,000 ha are

417   cultivated with herbaceous crops (mainly wheat and barley) and 487,000 ha are fallowed

418   every year. In these areas, the most common cropping system is the traditional cereal-fallow

419   rotation (one crop in 2 years) with a long-fallow period of 16-18 months. During fallowing,

420   the risk of soil erosion increases due to insufficient crop residue cover and highly pulverized

421   soils by repeated tillage (López et al., 2001, 2005). Pennycress cultivation during the fallow

422   period, as shown in Figure 4, would be a sustainable management alternative, which would

423   provide environmental and economic benefits due to the energy and industrial applications of

424   the oil in its seeds. However, in Central Aragon, the precipitation is low and highly variable

425   (mean annual rainfall <400 mm), and, in contrast with other semiarid regions, there is not a

426   well-defined rainy season. This is a limitation for the pennycress production in this area since

427   soil moisture, together with temperature amplitude and exposition to light, is crucial for seed

428   germination (Hazebroek and Metzger, 1990a; Royo-Esnal et al., 2015). On the contrary, the

429   subhumid drylands of the sub-Pyrenean zone of Aragon, with 500-700 mm of average annual

430   rainfall, or even the semiarid agricultural fields of the highlands of the Iberian System and the

431   Ebro Valley, with rainfall of 400-500 mm, may be optimal areas for the rainfed cropping

432   system proposed here (Fig. 4).

433

434   **5. Conclusions**

435   Agronomic data obtained during the first two field growing seasons in Aragon (NE Spain)

436   indicate that pennycress has the potential to be cultivated in Mediterranean Europe. However,

437   differences among the four pennycress accessions studied here (two European and two USA

438   accessions) offer the possibility of choosing the most suitable for cultivation under

439   Mediterranean agroclimatic conditions. The French accession was able to compensate for the

440   lower germination rate with higher production of tillers and inflorescences, reaching similar or

441   higher seed yields than those from the USA accessions. This European accession is a

442   promising option with which to continue working. Future work will be conducted to evaluate

443   cropping strategies for the Mediterranean region with pennycress as a winter annual crop in

444   rotation with corn under irrigation or during fallowing in the traditional cereal-fallow rotation

445   in rainfed conditions.

446

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Table 1. Monthly total precipitation (P) and mean air temperature (T) recorded at the experimental site during the study period (2016-2018) and the 27-yr average (1990-2017).

	2016-17		2017-18		1990-2017	
	Mean T (°C)	P (mm)	Mean T (°C)	P (mm)	Mean T (°C)	P (mm)
October	15.4	43	16.5	10	15.6	39
November	9.0	83	8.9	7	9.8	33
December	5.5	6	5.5	11	6.1	22
January	5.4	3	7.9	49	6.0	22
February	8.6	28	6.2	36	7.6	19
March	11.6	49	9.6	40	10.9	28
April	14.0	12	13.6	100	13.4	41
May	19.4	20	16.9	62	17.7	44
June	24.3	87	22.0	16	22.1	28
July	24.9	6	26.0	15	24.5	16
August	24.2	9	24.9	50	24.3	17
September	21.4	8	18.7	29	20.0	36
Mean/Total	15.3	354	14.7	424	14.8	344

Table 2. Plant density and maximum height of four accessions of *Thlaspi arvense* (French, NASC, Beecher and Elizabeth) in the 2016-17 and 2017-18 growing seasons.

Growing season	Accessions	Plant density (plants m^2)	Plant height (cm)
2016-17	French	114	67.5
	NASC	84	66.5
	Beecher	162	73.4
	Elizabeth	140	79.5
	LSD (0.05)[a]	32	7.7
2017-18	French	78	90.7
	NASC	15	91.0
	Beecher	153	97.1
	Elizabeth	147	101.8
	LSD (0.05)	55	8.9

[a] LSD, least significant difference (*P*<0.05).

Table 3. Percentage of plants with tillers, number of tillers and inflorescences per plant, seed yield, and weight of 1000 seeds of four lines of *Thlaspi arvense* (French, NASC, Beecher and Elizabeth) during the 2016-17 and 2017-18 growing seasons.

Growing season	Accession	Plants with tillers (%)	Mean number (and range) of tillers per plant	Mean number (and range) of inflorescence per plant	Seed yield (kg ha^{-1})	Seed weight (g 1000 seeds^{-1})
2016-17	French	50	2.5 (0-8)	8.8 (1-36)	732	1.036
	NASC	40	1.9 (0-7)	6.3 (1-15)	555	1.137
	Beecher	48	1.4 (0-8)	4.6 (1-15)	502	1.081
	Elizabeth	43	1.1 (0-5)	4.3 (1-13)	551	1.120
	LSD (0.05)[a]	ns	ns	ns	191	0.096
2017-18	French	65	4.7 (0-23)	10.9 (1-60)	1394	1.168
	NASC	67	4.4 (0-14)	13.7 (1-57)	704	1.281
	Beecher	38	1.3 (0-8)	4.6 (1-17)	1339	1.228
	Elizabeth	27	1.0 (0-8)	3.4 (1-16)	1301	1.356
	LSD (0.05)	26	2.1	3.7	362	0.066

[a] LSD, least significant difference ($P<0.05$). ns, not significant.

Table 4. Seed stage development based on the silicle colour (S, appearance of some silicles; G, green silicles; GY, green-yellow; YG, yellow-green; Y, yellow) and percentage of dry silicles (seeds with a dark reddish brown colour) in four lines of *Thlaspi arvense* (French, NASC, Beecher and Elizabeth) during the 2016-17 and 2017-18 growing seasons.

| Growing season	Accession	Silicle colour					Dry silicles (%)		
		29 March	3 April	10 April	25 April	2 May	9 May	15 May	22 May
2016-17	French	S	G	G	YG	Y	58	95	100
	NASC	S	G	G	GY	Y	52	93	100
	Beecher	-	G^a	G	GY	Y	43	94	100
	Elizabeth	S^a	G	G	GY	Y	58	94	100
		4 April	18 April	26 April	3 May	10 May		16 May	21 May
2017-18	French	S	G	G	YG	Y	nd	87	100
	NASC	S^a	G	G	YG	Y	nd	47	100
	Beecher	S	G	G	YG	Y	nd	58	100
	Elizabeth	S	G	G	YG	Y	nd	73	100

[a] Still in the initial phase. nd, not determined.

Table 5. Seed oil content (%, dwb) of four accessions of *Thlaspi arvense* (French, NASC, Beecher and Elizabeth) in the 2016-17 and 2017-18 growing seasons.
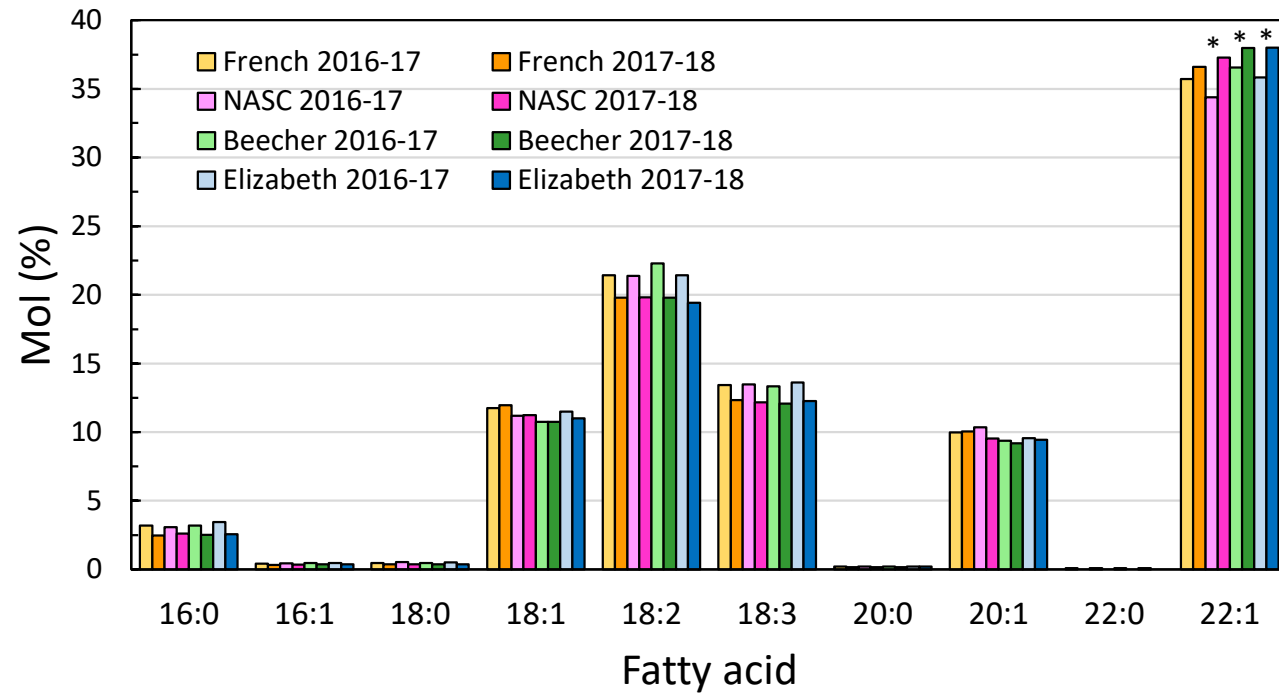
Accession	Growing season		
	2016-17	2017-18	LSD (0.05)[b]
French	34.85	36.18	0.34
NASC	34.46	35.94	0.91
Beecher	34.81	35.90	0.48
Elizabeth	34.80	36.70	1.89
LSD (0.05)[a]	ns	ns	

[a] LSD, least significant difference (P<0.05) when comparing accessions. ns, not significant.

[b] LSD, least significant difference (P<0.05) when comparing growing seasons.
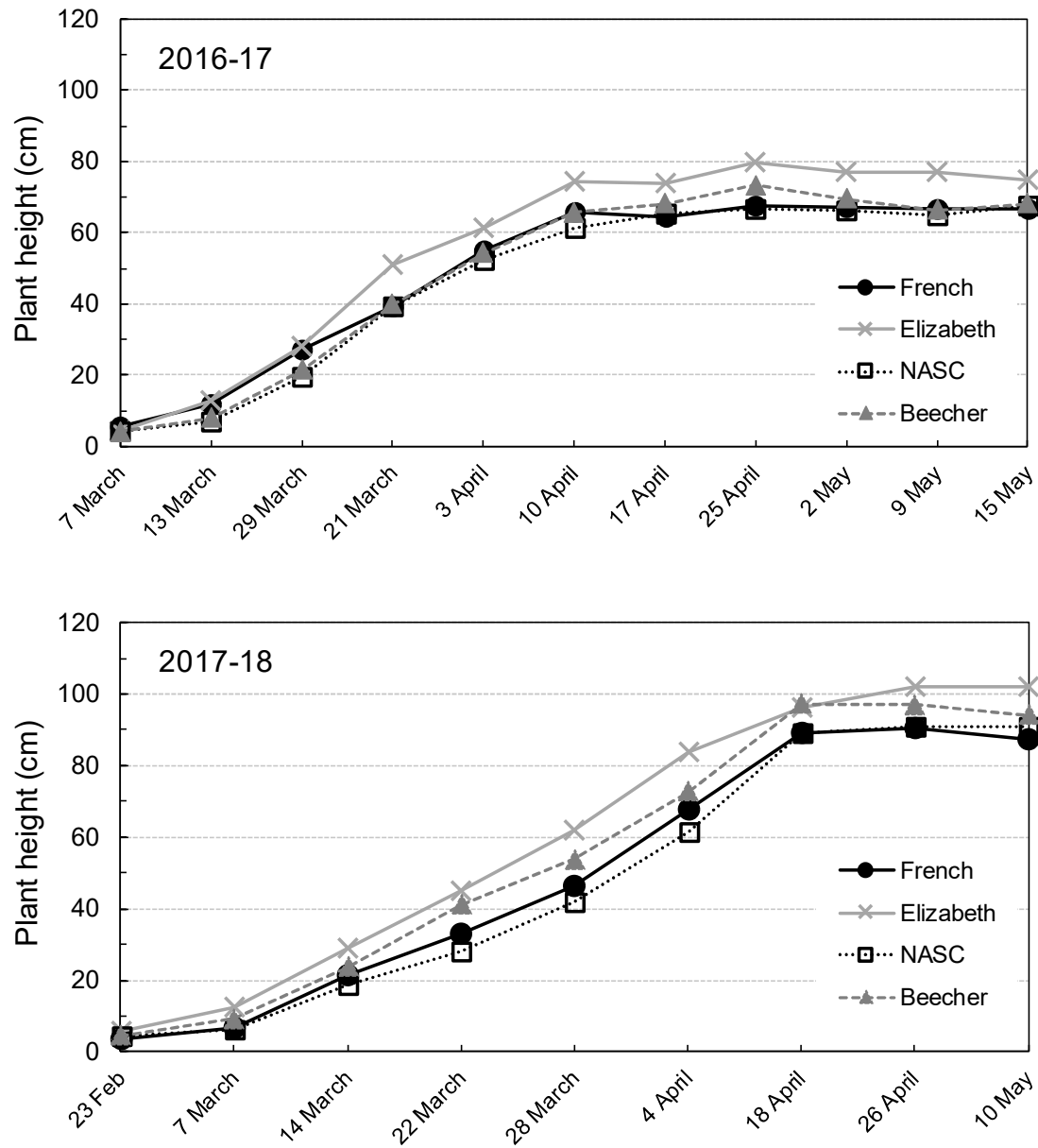
Fig. 1. Evolution of plant height of four accessions of *Thlaspi arvense* (French, NASC, Beecher and Elizabeth) during the 2016-17 and 2017-18 growing seasons.

Fig. 2. Fatty acid composition of four *Thlaspi arvense* accessions (French, NASC, Beecher and Elizabeth) during two growing seasons (2016-17 and 2017-18). Data represent means of two independent pools of seeds obtained from the different pennycress accessions. Only in the case of the 22:1 acid (erucic acid), the asterisk indicates differences statistically significant ($P<0.05$) between the two growing seasons for the same pennycress accession.
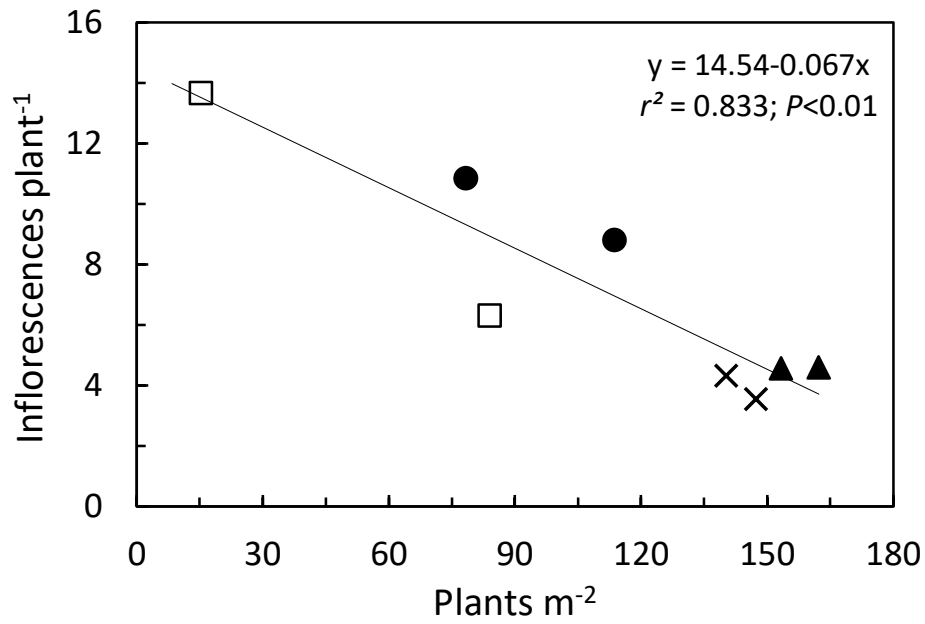
Fig. 3. Relationship between the number of plants per m^2 and the number of inflorescences per plant of *Thlaspi arvense*. Each point corresponds to the mean value obtained for each of the four varieties studied (● French, □ NASC, ▲ Beecher, ✕ Elizabeth) and for the two experimental growing seasons (2016-17 and 2017-18).
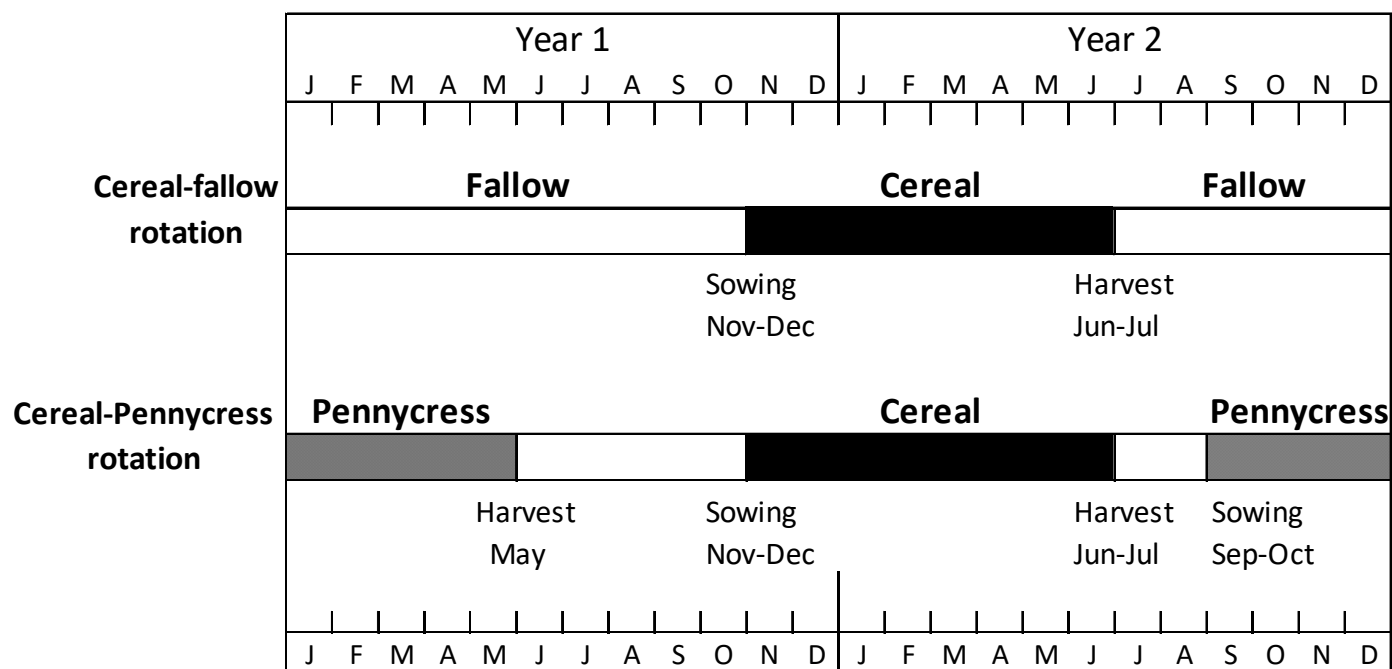
Fig. 4. Scheme of the traditional cereal-fallow rotation in rainfed Aragon (NE Spain) and the alternative cereal-pennycress rotation.