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Camelina germination under osmotic stress – Trend lines, time-courses and critical points

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1 CAMELINA GERMINATION UNDER OSMOTIC STRESS - TREND LINES, TIME-
2 COURSES AND CRITICAL POINTS

3
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14
15 Abstract

16 Camelina [*Camelina sativa* (L.) Crantz] has increased cold, heat, and drought tolerance and
17 decreased susceptibility to diseases and pests than oilseed rape (*Brassica napus* L.). Because water
18 deficit at sowing leads to unsatisfactory stand establishment due to irregular seed germination and
19 emergence, the aim of this study was to understand the response of camelina germination under
20 osmotic stress and identify critical soil moisture levels for successful establishment. Two spring
21 cultivars, NS Slatka and NS Zlatka, developed at the Institute of Field and Vegetable Crops Novi
22 Sad, Serbia, were compared under 9 levels of osmotic stress, ranging from 0 MPa to -1.6 MPa.
23 Polyethylene glycol was used to obtain the osmotic potential of the solutions. Results showed that

24 the tested cultivars did not decrease germination under mild and medium osmotic stress levels
25 (down to -0.8 MPa). However, germination significantly decreased in both cultivars under higher
26 levels of osmotic stress, and NS Zlatka was more sensitive. Germination speed significantly
27 increased at -0.4 MPa. The estimated osmotic potentials to stop germination were -1.45 MPa for
28 NS Slatka and - 1.46 MPa for NS Zlatka. Time to 50% germination also showed a significant bi-
29 linear trend in response to osmotic potential, but in the opposite direction than the one observed in
30 germination. Inflection points were recorded at -0.77 MPa for NS Slatka and -0.78 MPa for NS
31 Zlatka, thereafter time to 50% of germination rapidly increased. This study confirmed that camelina
32 can withstand increased levels of drought stress at germination, so it could be considered a more
33 suitable option than oilseed rape on marginal land, or environments with irregular precipitation.

34

35 Keywords: camelina, germination speed, drought stress; establishment; oilseed crops

36

37 1. Introduction

38 Arising awareness on global climate change, emissions of greenhouse gasses and
39 diminishing fossil fuel reserves are shifting the attention of humanity towards alternative
40 sustainable biofuels. A large portion of global biodiesel production currently comes from edible
41 vegetable oils, such as palm (*Elaeis guineensis* Jacq.), soybean (*Glycine max* (L.) Merr.), oilseed
42 rape (*Brassica napus* L.) and sunflower (*Helianthus annuus* L.) (Sainger et al., 2017). To maintain
43 food production and reduce iLUC (indirect land use change), oilseed crops not grown for human
44 consumption should be considered as alternatives for biofuel production; especially those that have

45 satisfactory yields when grown on marginal land and/or sub-optimal conditions (Augustin et al.,
46 2015).

47 Camelina [*Camelina sativa* (L.) Crantz] has increased cold, heat and drought tolerance, and
48 decreased susceptibility to disease and pests than oilseed rape (Guy et al, 2014; Zanetti et al., 2017),
49 and could be competitive with other *Brassicaceae* species regarding yield (Blackshaw et al., 2011;
50 Pavlista et al., 2011). In non-limiting conditions, camelina can produce up to 3000 kg ha⁻¹, with
51 26-43% seed oil content range (Righini et al., 2016; Obour et al., 2017; Sainger et al., 2017).
52 Camelina oil has a unique composition, containing high levels of tocopherols, oleic, linoleic, α -
53 linoleic and eicosenoic acid, as well as low content of erucic acid (Christou et al., 2016; Zanetti et
54 al., 2017, Anderson et al., 2019), making it suitable for food, feed and non-food uses. Camelina oil
55 is used for the production of biodiesel and aviation fuel, but it could also become an important
56 feedstock for the biopolymer and cosmetics industries (Berti et al., 2016; Burnett et al., 2017; Kalita
57 et al., 2018; Zanetti et al., 2021).

58 Water deficit is one of the most important environmental factors limiting the geographical
59 distribution and performance of all staple crops (Shao et al., 2009). Worldwide water resources
60 available for crop production are decreasing, and even the most productive regions are starting to
61 cope with drought periods and uneven precipitation distribution almost every year (Barnabás et al.,
62 2008). All phenological stages of plant development are affected by drought stress, resulting in a
63 significant reduction in yield (Bartels and Sunkar, 2005; Mittler, 2006; Wu et al., 2011). Irregular
64 and delayed seed germination and seedling emergence are induced by water deficit at germination,
65 leading to inadequate stand establishment (Lewandrowski et al., 2017).

66 Different osmotic potentials between dry seeds and the surrounding environment cause
67 water uptake. Camelina has relatively low water requirements and high tolerance to drought at all
68 growth stages, even at germination and early seedling growth (Berti et al., 2016; George et al.,
69 2017a; George et al., 2018). Generally, oilseed rape is the highest yielding Brassicaceous oilseed
70 crop, which has received more attention in research than camelina. However, in dry conditions, or
71 irregular precipitation dispersal, oilseed rape can become unreliable (George et al., 2017b). If
72 seedbed conditions are suboptimal for oilseed rape, George et al. (2017a) suggested that other
73 oilseed species, such as camelina, may yield more reliably, and therefore represent a less risky
74 option.

75 To gain a better understanding on camelina seed germination traits under osmotic stress,
76 controlled environmental studies were conducted using seeds from two camelina genotypes to
77 determine response under differing osmotic potentials, and to identify critical soil moisture levels
78 for successful germination.

79

80 2. Materials and methods

81 This study used two spring camelina genotypes, NS Slatka and NS Zlatka, developed at the
82 Institute for Field and Vegetable Crops (Novi Sad, Serbia) and released by the Ministry of
83 Agriculture, Forestry and Water Management of the Republic of Serbia. Genotype NS Zlatka
84 ~~Slatka~~ was formed by the process of self-fertilisation from the Banat population of camelina, while
85 NS Slatka was formed by the same process from the Ukrainian variety, Stepski-1. These two
86 camelina genotypes are specially selected for the Balkan environment and are characterized by
87 good production traits.

88 Solutions of polyethylene glycol (PEG, molecular weight 6000, Merck KgaA, Darmstadt,
 89 Germany) were used to induce osmotic stress. Nine increasing levels of osmotic stress (0.0, -0.2,
 90 -0.4, -0.8, -1.2, -1.4, -1.6 MPa were applied using the formula from Michel and Kaufmann
 91 (1973). Germination was surveyed in Petri dishes (100 × 15 mm) on double-layer filter paper, in
 92 four replicates of 100 seeds each. Prior to placing in the Petri dishes, the filter paper was submerged
 93 in the prepared solution, with excess left to drain. During the whole experiment, the seeds were
 94 kept at 20°C and 16/8h ~~8/16h~~ light/dark cycle in a growth chamber. To prevent changes in the
 95 osmotic potential during the trial, the filter paper was changed every second day. Seeds were
 96 considered germinated when the radicle was at least 2 mm long. Germination was observed daily,
 97 and final germination was determined when no seeds germinated for three consecutive days (d), or
 98 20 d after incubation.

99 Time to reach 50% germination (T_{50}) was calculated according to the formula given by
 100 Coolbear et al. (1984) modified by Farooq et al. (2005):

$$101 \quad T_{50} = t_i + [(N / 2 - n_i) (t_i - t_j)] / n_i - n_j$$

102 Where N is the final number of germinated seeds and n_i , n_j cumulative number of seeds
 103 germinated by adjacent counts at times t_i and t_j , respectively when $n_i < N/2 < n_j$.

104

105 2.1 Statistical analysis

106 Data were analysed using two-way ANOVA. Mean values ~~Means~~ were compared using
 107 LSD test ($P \leq 0.05$). In order to accomplish the normality and homoscedasticity assumptions of the
 108 ANOVA, percentage data (germination) were first subjected to arc-sine transformation. Values of

109 germination traits were analyzed as a function of osmotic stress. The chosen function for this
110 relationship was a bi-linear regression:

$$111 \quad Y = A + BX \text{ if } X \leq C; \text{ and } Y = A + BC + D(X - C) \text{ if } X > C;$$

112 where X indicates the osmotic stress levels, A is the intercept, B is the first slope, C indicates
113 the level of osmotic stress where the breaking point occurred and D indicates the second slope of
114 the examined trait.

115 3. Results

116 3.1. Osmotic stress effect on camelina germination

117 The results of ANOVA showed that osmotic stress had a significant effect on the
118 germination of camelina seeds, but not the genotype and the interaction between genotype and
119 osmotic stress (Table 1). The lack of interaction between genotype and osmotic stress indicates that
120 both genotypes behaved similarly in response to osmotic stress. On the other hand, both main
121 factors and their interaction had significant effect on time to 50% of germination. For both surveyed
122 traits, the highest percentage of variation was explained by osmotic stress (>97%).

123 Observing the germination averaged by genotype, camelina withstand osmotic stress down
124 up to -0.8 MPa without any significant decrease (Table 2). No differences were found in average
125 germination between the camelina genotypes. However, observing the G x OS interaction, some
126 differences were noticed: NS Slatka was able to withstand osmotic stress levels down to -1.0 MPa
127 without a decrease in germination, while NS Zlatka proved to be more sensitive. At -0.8 MPa, a
128 significant decrease in the germination of NS Zlatka was recorded compared with the control, while
129 NS Slatka showed a more significant decrease in germination only at -1.0 MPa.

130 Time to 50% of germination (T_{50}) is one of the common parameters used to represent
131 germination speed, and it is prolonged with increased osmotic stress. Observing the average, a
132 significant increase in T_{50} was first observed at -0.4 MPa. however, NS Slatka required a longer
133 time to achieve 50% germination than NS Zlatka. Analyzing the G x OS interaction, NS Zlatka
134 showed significantly shorter T_{50} in control (0 MPa) and -0.2 MPa, but when osmotic stress reached
135 below -0.4 MPa, the two genotypes showed only minor differences.

136

137 3.2 Germination trend lines and critical points

138 When decreasing the osmotic potential (i.e., increasing osmotic stress) germination showed
139 a significant bi-linear trend in both genotypes (Fig. 1). In NS Slatka, down to -1.15 MPa,
140 germination was reduced at the rate of 1.25% MPa^{-1} , while after the inflection point, the trend in
141 germination declined rapidly with rate of 315% MPa^{-1} (Fig. 1). The estimated osmotic potential to
142 completely stop germination was at -1.45 MPa, which corresponded to the X-axis intercept.
143 Similarly, in NS Zlatka, lower decrease in germination rate (9.86% MPa^{-1}) was recorded down to
144 -1.18 MPa, followed with rapid decline (307.5% MPa^{-1}) after the inflection point. Osmotic
145 potential to completely block germination was at -1.46 MPa for NS Zlatka.

146 T_{50} had significant bi-linear trend with osmotic potential (Fig. 2), but in the opposite
147 direction to the one for germination. In NS Slatka, T_{50} was increased at the rate of 0.23 d MPa^{-1}
148 down to -0.77 MPa, and rate of increase was 8.38 d MPa^{-1} after the inflection point. Down to -
149 0.78 MPa, NS Zlatka had an increase in T_{50} with a rate of 1.59 d MPa^{-1} , while after that point T_{50}
150 was prolonged at a rate of 6.89 d MPa^{-1} .

151

152 3.3. Camelina germination time-course

153 As osmotic potential decreased, the number of days to the start of germination progressively
154 increased, as shown in Fig. 3. Both genotypes showed a similar germination time response.
155 Germination began one day after incubation at 0.0 MPa and -0.2 MPa, with NS Zlatka showing a
156 more rapid response. A germination rate of approximately 90% was observed for both genotypes
157 between 0.0 MPa and -0.4 MPa. Under medium stress (-0.6 MPa) the same germination percentage
158 was surveyed only after 3 d. Germination started after 2 d under more severe stress (-0.8 MPa) and
159 both genotypes reached 90% of germination, but only NS Slatka fulfilled its full germination
160 potential. As osmotic stress increased, the curve of cumulative germination flattened. Germination
161 was delayed for a few days under higher levels of osmotic stress, consequently, maximum
162 germination occurred later. At osmotic stress -1.6 MPa camelina was not able to germinate.

163

164 4. Discussion

165 The seed germination process is undoubtedly impossible not without the presence of
166 enough moisture in the surrounding environment. The absorbed water activates the enzyme
167 hydrolysis which further breaks down the reserve substances into metabolically usable compounds
168 and thus allows the penetration of the radicle through the seed coat. The degree of water absorption
169 (imbibition) depends on the relation between the water potential in the germination medium and
170 the water potential of the seed (Locher and Brouwer, 1965). In conditions of insufficient moisture,
171 the initial water uptake and seed imbibition are hampered, the lag phase is prolonged, and the
172 beginning of the third phase or root protrusion is procrastinated (Kebreab and Murdoch, 1999). In
173 this study, osmotic stress induced by PEG significantly delayed or completely inhibited camelina

174 seed germination, whereby the effect primarily depended on the stress level. Although the initial
175 germination values under full water availability (0.0 MPa) were similar between genotypes, a
176 different mechanism of response to osmotic stress was observed.

177 Final seed germination decrease, due to low water availability, has been confirmed in many
178 plant species: *H. annuus* L. (Kaya et al., 2006), *T. aestivum* L. (Zhang et al., 2010), *G. max*
179 (Wijewardana et al., 2018), *O. sativa* L. (Singh et al., 2017), *B. napus* L. (Channaoui et al., 2019).
180 From the plant production point of view, the soil water potential is regulated by surface forces that
181 attach water in capillaries and by reducing the activity of water caused by dissolved solutes. The
182 amount of water available for plant uptake depends on many factors (such as soil texture, structure,
183 layering, stage of development, etc.) (Tolk, 2003), and depends on its availability at the wilting
184 point, which is estimated to be the water amount in the soil matrix potential of the soil ψ of -1.5
185 MPa (Kirkham, 2014). In this study, the inhibition caused by the osmotic effect was especially
186 noticeable at -1.0 MPa for NS Slatka, and -0.8 MPa for NS Zlatka, or below. The fact that both
187 genotypes were able to adapt to moderate osmotic stress levels down ~~up~~ to -0.8 MPa, with final
188 germination percentages similar to the control indicates their high tolerance to drought stress. The
189 results are in line with Čanak et al. (2020) who examined drought tolerance of different biotypes
190 of camelina genotypes. In some oilseed rape varieties, Channaoui et al. (2017) reported that the
191 osmotic level of -0.9 MPa drastically reduced seed germination and -1.1 MPa completely inhibited
192 it. However, Waraich et al. (2015) observed significant germination decrease of camelina at -0.2
193 MPa. Similarly, Dawadi et al. (2019) evaluated drought tolerance in oilseed species and classified
194 camelina as a drought susceptible one, but the initial percentage of germination (control) of
195 camelina in that study was low (58%), so the conclusions are not highly applicable to other studies.

196 The seed water absorption rate is inversely proportional to the reduction of water potential
197 in the soil, causing water deficiency and thus reducing germination (Asgarpour et al., 2015).
198 However, it is worth mentioning that the causes of low initial germination can be numerous, e.g.
199 low vigorous seed, inadequate storage conditions, etc. Belo et al. (2014) stated that the
200 physiological response of some sunflower genotypes to stress conditions (<-0.3 MPa) may be the
201 accumulation of solutes in the seed, which reduced the osmotic potential and thus allowed the seed
202 to imbibe in conditions of low water availability. The reasons behind camelina drought tolerance
203 at germination might be related to the small seed size, which provides greater total surface contact
204 with the soil, and would require less water for germination (Pereira et al., 2013). Čanak et al. (2020)
205 also indicated that the drought response of camelina depends on seed size, but also on the biotype
206 and the stress level. On the other hand, Oberbauer and Miller (1982) stated the importance of higher
207 soil moisture presence for small seeds due to their insufficient carbohydrate reserves, responsible
208 for rapid germination and growth of radicle necessary for the survival under dry conditions. Also,
209 small seeds of oat (*Avena sativa*) compared with medium and large seeds were significantly more
210 sensitive to high drought levels (-0.75 MPa), while at lower stress levels the difference in seed size
211 was not noticeable (Mut and Akay, 2010). Furthermore, several studies indicate that the seed size
212 of *Brassica campestris* (Pandya et al., 1973) and *Brassica napus* (Pace and Benincasa, 2010) did
213 not affect germination under drought stress.

214 Another specific feature of camelina seed that may explain its outstanding performance
215 during germination and early seedling growth in dry environments is the presence of mucilage (Cui
216 et al., 2006; Čanak et al., 2020). Mucilage consists of polysaccharides, accumulated in the cell wall,
217 which has a high binding water capacity because they broaden during the imbibition phase,
218 fragmenting the outer cell wall, and surrounding the seed with viscous gel (North et al., 2014). By

219 maintaining seed hydration when water is deficient, the gel affects the process of seed germination
220 and seedling formation during abiotic stress (Tsai et al., 2021). In their study, Čanak et al. (2020)
221 stated that spring genotypes (such as NS Slatka and NS Zlatka) showed higher germination
222 potential under osmotic stress conditions, although the amount of mucilage in spring genotypes
223 was lower compared with winter ones.

224 Observing the value of T_{50} , there were differences between camelina genotypes in the
225 control (0.0 MPa) and under very low water potential (-1.4 MPa), in both cases, NS Zlatka
226 germinated faster than NS Slatka. Based on the average, a significant increase in T_{50} was noticed
227 since -0.4 MPa (1.51 days), while in the study of Waraich et al. (2015) germination of camelina
228 was significantly slower at similar osmotic stress levels (5.08 days). Time to 50% germination, as
229 a parameter of the germination dynamics, indicates the ecology of a plant species (Al-Ansari and
230 Ksiksi, 2016). In other words, the present results are particularly interesting since they could predict
231 the expected germination time under real field conditions at a certain level of soil moisture
232 (Valickova et al., 2017).

233

234 5. Conclusions

235 The present study showed that there is some variability among camelina genotypes in their
236 response to osmotic stress, which is reflected in the endurance of different stress levels during
237 germination. Rapid and uniform germination of NS Slatka and NS Zlatka camelina genotypes can
238 be expected at an osmotic potential above -0.8 MPa, and modestly slower but finish germination
239 can be still achieved from -1.0 MPa to about -1.2 MPa. The estimated osmotic potentials for
240 stopping germination were -1.45 MPa (NS Slatka) and -1.46 MPa (NS Zlatka), which permit to

241 classify them as highly drought tolerant. The osmotic stress levels, defined in this experiment as
242 the most suitable for discriminating drought tolerance of camelina genotypes, might be used in
243 future research to evaluate the responses of other camelina genotypes at different growth stages.
244 Further research should be conducted to determine whether the germination potential under
245 osmotic stress conditions may reflect the response of plants to such stressful conditions at later
246 stages of growth and development.

247

248 Author Contributions

249 P.Č. conceptualization, investigation, methodology, data curation, software, writing—original
250 draft preparation; F.Z. supervision, writing—original draft preparation, writing—review and
251 editing; D.J. writing—original draft preparation, writing—review and editing, visualization; B.V.
252 resources, methodology, data curation; Z.M. resources, methodology, data curation; D.S. software,
253 formal analysis; M.M. software, formal analysis; B.A. writing—review and editing; E.F. writing—
254 review and editing; A.M.J. resources, supervision, writing—review and editing, funding
255 acquisition, project administration. All authors have read and agreed to the published version of
256 the manuscript.

257

258 Declaration of Competing Interest

259 The authors declare that they have no known competing financial interests or personal relationships
260 that could have appeared to influence the work reported in this paper.

261

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271 14/200032 and 451-03-68/2022-14/200032).

272

273 References

274 Al-Ansari, F., Ksiksi, T.S., 2016. A quantitative assessment of germination parameters: the case of
275 *Crotalaria persica* and *Tephrosia apollinea*. Open Ecol. J. 9, 13–21.
276 <https://doi.org/10.2174/1874213001609010013>.

277 Anderson J.V., Wittenberg A., Li H., T. Berti M., 2019. High throughput phenotyping of *Camelina*
278 *sativa* seeds for crude protein, total oil, and fatty acids profile by near infrared spectroscopy,
279 Ind. Crop. Prod. 137, 501-527. <https://doi.org/10.1016/j.indcrop.2019.04.075>.

280 Asgarpour, R., Ghorbani, R., Khajeh-Hosseini, M., Mohammadvand, E., Chauhan, B.S., 2015.
281 Germination of spotted spurge (*Chamaesyce maculata*) seeds in response to different
282 environmental factors. Weed Sci. 63, 502–10. <https://doi.org/10.1614/WS-D-14-00162.1>.

- 283 Augustin, J.M., Higashi, Y., Feng, X., Kutchan, T.M., 2015. Production of mono-and
284 sesquiterpenes in *Camelina sativa* oilseed. *Planta* 242, 693–708.
285 <https://doi.org/10.1007/s00425-015-2367-4>.
- 286 Barnabás, B., Jäger, K., Fehér, A., 2008. The effect of drought and heat stress on reproductive
287 processes in cereals. *Plant Cell Environ.* 31, 11–38. [https://doi.org/10.1111/j.1365-](https://doi.org/10.1111/j.1365-3040.2007.01727.x)
288 [3040.2007.01727.x](https://doi.org/10.1111/j.1365-3040.2007.01727.x).
- 289 Bartels, D., Sunkar, R., 2005. Drought and salt tolerance in plants. *Crit. Rev. Plant Sci.* 24, 23–58.
290 <https://doi.org/10.1080/07352680590910410>.
- 291 Belo, R.G., Tognetti, J., Benech-Arnold R., Izquierdo, N.G., 2014. Germination responses to
292 temperature and water potential as affected by seed oil composition in sunflower. *Ind. Crop.*
293 *Prod.* 62, 537–544. <https://doi.org/10.1016/j.indcrop.2014.09.029>.
- 294 Berti, M., Gesch, R., Eynck, C., Anderson, J., Cermak, S., 2016. *Camelina* uses, genetics,
295 genomics, production, and management. *Ind. Crop. Prod.* 94, 690–710.
296 <http://dx.doi.org/10.1016/j.indcrop.2016.09.034>.
- 297 Blackshaw, R., Johnson, E., Gan, Y., May, W., McAndrew, D., Barthet, V., McDonald, T.,
298 Wispinski, D., 2011. Alternative oilseed crops for biodiesel feedstock on the Canadian
299 prairies. *Can. J. Plant Sci.* 91, 889–896. <http://dx.doi.org/10.4141/cjps2011-002>.
- 300 Burnett, C.L., Fiume, M.M., Bergfeld, W.F., Belsito, D.V., Hill, R.A., Klaassen, C.D., Liebler, D.,
301 Marks, J.G., Shank, R.C., Slaga, T.J., Snyder, P.W., Andersen, F.A., 2017. Safety
302 assessment of plant-derived fatty acid oils. *Int. J. Toxicol.* 36, 51S–129S.
303 <https://doi.org/10.1177/1091581817740569>.
- 304 Cui, J.W., Qun, S., Baoqi, S., 2006. Drought resistance of *Camelina Sativa* (L.) Crantz's seeds in
305 germination. *Chin. Agric. Sci. Bull.* 10, 203–205.

- 306 Čanak, P., Jeromela, A.M., Vujošević, B., Kiproovski, B., Mitrović, B., Alberghini, B., Facciolla,
307 E., Monti, A., Zanetti, F. 2020. Is drought stress tolerance affected by biotypes and seed
308 size in the emerging oilseed crop camelina? *Agron.* 10, 1856.
309 <https://doi.org/10.3390/agronomy10121856>.
- 310 Channaoui, S., El Kahkahi, R., Charafi, J., Mazouz, H., El Fechtali, M., Nabloussi, A., 2017.
311 Germination and seedling growth of a set of rapeseed (*Brassica napus*) varieties under
312 drought stress conditions. *Int. J. Envir. Agric. Biotechnol.* 2, 487–494.
313 <http://dx.doi.org/10.22161/ijeab/2.1.61>.
- 314 Channaoui, S., Saghour El Idrissi, I., Mazouz, H., Nabloussi, A., 2019. Reaction of some rapeseed
315 (*Brassica napus* L.) genotypes to different drought stress levels during germination and
316 seedling growth stages. *OCL.* 26, 23. <https://doi.org/10.1051/ocl/2019020>.
- 317 Christou, M., Alexopoulou, E., Di Girolamo, G., Righini, D., Monti, A., Stolarski, M., Krzyzaniak,
318 M., Van Loo, E.N., Eynck, C., Gruschow, J., 2016. Camelina & crambe: underutilized oil
319 crops with new perspectives for Europe. 24th European Biomass Conference and
320 Exhibition, Amsterdam, Netherlands. 147–150.
- 321 Coolbear, P., Francis, A., Grierson, D., 1984. The effect of low temperature pre-sowing treatment
322 under the germination performance and membrane integrity of artificially aged tomato
323 seeds. *J. Exp. Bot.* 35, 1609–1617. doi:10.1093/jxb/35.11.1609.
- 324 Dawadi, D., Seepaul, R., George, S., Groot, J., Wright, D., 2019. Drought tolerance classification
325 of common oilseed species using seed germination assay. *J. Oilseed Brassica.* 10, 97–105.
- 326 George, N., Levers, L., Thompson, S., Hollingsworth, J., Kaffka, S., 2017a. Modeling identifies
327 optimal fall planting times and irrigation requirements for oilseed rape and camelina at
328 locations across California. *Calif. Agr.* 71, 214–220. <https://doi.org/10.3733/ca.2017a00>.

- 329 George, N., Hollingsworth, J., Yang, W., Kaffka, S., 2017b. Canola and camelina as new crop
330 options for cool-season production in California. *Crop Sci.* 57, 693–712.
331 <https://doi.org/10.2135/cropsci2016.04.0208>.
- 332 George, N., Thompson, S.E., Hollingsworth, J., Orloff, S., Kaffka, S., 2018. Measurement and
333 simulation of water-use by canola and camelina under cool-season conditions in California.
334 *Agr. Water Manage.* 196, 15–23. <https://doi.org/10.1016/j.agwat.2017.09.015>.
- 335 Guy, S.A., Wysocki, D.J., Schillinger, W.F., Chastain, T.G., Karow, R.S., Garland-Campbell, K.,
336 Burke, I.C., 2014. Camelina: Adaptation and performance of genotypes. *Field Crop. Res.*
337 155, 224–232. <https://doi.org/10.1016/j.fcr.2013.09.002>.
- 338 Kalita, D.J., Tarnavchyk, I., Sibi, M., Moser, B.R., Webster, D.C., Chisholm, B.J., 2018. Biobased
339 poly(vinyl ether)s derived from soybean oil, linseed oil, and camelina oil: Synthesis,
340 characterization, and properties of crosslinked networks and surface coatings. *Prog. Org.*
341 *Coat.* 125, 453–462. <https://doi.org/10.1016/j.porgcoat.2018.09.033>.
- 342 Kaya, M.D., Okcu, G., Atak, M., Cıkkı, Y., Kolsarıcı, O., 2006. Seed treatments to overcome salt
343 and drought stress during germination in sunflower (*Helianthus annuus* L.). *Eur. J. Agron.*
344 24, 291–295. <https://doi.org/10.1016/j.eja.2005.08.001>.
- 345 Kebreab, E., Murdoch, A.J., 1999. Modelling the effects of water stress and temperature on
346 germination rate of *Orobanche aegyptiaca* seeds. *J. Exp. Bot.* 50, 655–664.
347 <https://doi.org/10.1093/jxb/50.334.655>.
- 348 Kirkham, M.B., 2014. Field capacity, wilting point, available water, and the nonlimiting water
349 range, in: Kirkham M.B. (Ed.), *Principles of Soil and Plant Water Relations*. Academic
350 Press, pp. 153–170.

- 351 Leishman, M.R., Wright, I.J., Moles, A.T., Westoby., M., 2000. The evolutionary ecology of seed
352 size. In *Seeds: The Ecology of Regeneration in Plant Communities*; Fenner, M., Ed.; CAB
353 International: Wallingford, UK, pp. 31–57.
- 354 Lewandrowski, W., Erickson, T.E., Dixon, K.W., Stevens, J.C., 2017. Increasing the germination
355 envelope under water stress improves seedling emergence in two dominant grass species
356 across different pulse rainfall events. *J. Appl. Ecol.* 54, 997–1007.
357 <https://doi.org/10.1111/1365-2664.12816>.
- 358 Locher, J.T., Brouwer, R., 1965, Influence of different root temperature on transpiration and
359 exudation of young maize plants. Wageningen: Jaarb. I.B.S. pp. 57–65.
- 360 Michel, B.E., Kaufmann, M.R., 1973. The osmotic potential of polyethylene glycol 6000. *Plant*
361 *Physiol.* 51, 914–916. <https://doi.org/10.1104/pp.51.5.914>.
- 362 Mittler, R., 2006. Abiotic stress, the field environment and stress combination. *Trends Plant Sci.*
363 11, 15–19. <https://doi.org/10.1016/j.tplants.2005.11.002>.
- 364 Mut, Z., Akay, H., 2010. Effect of seed size and drought stress on germination and seedling growth
365 of naked oat (*Avena sativa* L.). *Bul. J. Agr. Sci.* 16, 459–467.
- 366 North, H.M., Berger, A., Saez-Aguayo. S., Ralet, M.C., 2014. Understanding polysaccharide
367 production and properties using seed coat mutants: future perspectives for the exploitation
368 of natural variants. *Ann. Bot.* 114, 1251–63. <https://doi.org/10.1093/aob/mcu011>.
- 369 Oberbauer, S., Miller, P.C., 1982. Effect of water potential on seed germination. *Holarctic Ecol.* 5,
370 218–220. <http://www.jstor.org/stable/3682461>.
- 371 Obour, A.K., Obeng, E., Mohammed, Y.A., Ciampitti, I.A., Durrett, T.P., Aznar-Moreno, J.A.,
372 Chengci, C., 2017. Camelina seed yield and fatty acids as influenced by genotype and
373 environment. *Agron. J.* 109, 947–956. <https://doi.org/10.2134/agronj2016.05.0256>.

- 374 Pace, R., Benincasa P., 2010. Effect of salinity and low osmotic potential on the germination and
375 seedling growth of rapeseed cultivars with different stress tolerance. *Ital. J. Agron.*, 5, 69–
376 77. <https://doi.org/10.4081/ija.2010.69>.
- 377 Pandya, R.B., Khan, M.I., Gupta, S.H., Dhindsa, K.S., 1973. Effect of seed size upon germination,
378 moisture uptake, seedling growth, dry weight changes and soluble sugars under
379 polyethylene glycol (Peg) induced stress. *Biochem. Physiol. Pfl.*, 164, 80–87.
380 [https://doi.org/10.1016/S0015-3796\(17\)30663-7](https://doi.org/10.1016/S0015-3796(17)30663-7).
- 381 Pavlista, A., Isbell, T., Baltensperger, D., Hergert, G., 2011. Planting date and development of
382 spring-seeded irrigated oilseed rape: brown mustard and camelina. *Ind. Crops Prod.* 33,
383 451–456. <https://doi.org/10.1016/j.indcrop.2010.10.029>.
- 384 Pereira, W.A., Pereira, S.M.A., dos Santos Dias, D.C.F., 2013. Influence of seed size and water
385 restriction on germination of soybean seeds and on early development of seedlings. *J. Seed*
386 *Sci.* 35, 316–322. <https://doi.org/10.1590/S2317-15372013000300007>.
- 387 Righini, D., Zanetti, F., Monti, A., 2016. The bio-based economy can serve as the springboard for
388 camelina and crambe to quit the limbo. *OCL*, 23, D504. <https://doi.org/10.1051/ocl/201602>.
- 389 Sainger, M., Jaiwal, A., Sainger, P.A., Chaudhary, D., Jaiwal, R., Jaiwal, P.K., 2017. Advances in
390 genetic improvement of *Camelina sativa* for biofuel and industrial bio-products. *Renew.*
391 *Sust. Energ. Rev.*, 68, 623–637. <https://doi.org/10.1016/j.rser.2016.10.023>.
- 392 Shao, H.-B., Chu, L.-Y., Abdul Jaleel, C., Manivannan, P., Panneerselvam, R., Shao, M.A., 2009.
393 Understanding water deficit stress-induced changes in the basic metabolism of higher plants
394 – biotechnologically and sustainably improving agriculture and the eco-environment in arid
395 regions of the globe. *Crit. Rev. Biotechn.* 29, 131–151.
396 <https://doi.org/10.1080/07388550902869792>.

- 397 Singh, B., Reddy, K.R., Redoña, E., Walker, T., 2017. Developing a screening tool for osmotic
398 stress tolerance classification of rice cultivars based on in vitro seed germination. *Crop Sci.*
399 57, 387–394. <https://doi.org/10.2135/cropsci2016.03.0196>.
- 400 Tolk, J.A., 2003. Soils, permanent wilting points. *Encyclopedia of Water Science*, Marcel Dekker,
401 New York, N.Y., pp. 927–929.
- 402 Tsai, A.Y.L., McGee, R., Dean, G.H., Haughn, G., Sawa, S., 2021. Seed Mucilage: Biological
403 functions and potential applications in biotechnology. *Plant Cell Physiol.*
404 pcab099. <https://doi.org/10.1093/pcp/pcab099>.
- 405 Valičková, V., Hamouzová, K., Kolářová, M., Soukup, J., 2017. Germination responses to water
406 potential in *Bromus sterilis* L. under different temperatures and light regimes. *Plant Soil*
407 *Environ.* 63, 368–374. <https://doi.org/10.17221/406/2017-PSE>.
- 408 Wijewardana, C., Alsajri, F.A., Reddy, K.R., 2018. Soybean seed germination response to in vitro
409 osmotic stress. *Seed Technol.* 39, 143–154. <http://www.jstor.org/stable/45135884>.
- 410 Wu, C., Wang, Q., Xie, B., Wang, Z., Cui, J., Hu, T., 2011. Effects of drought and salt stress on
411 seed germination of three leguminous species. *Afr. J. Biotechnol.* 10, 17954–17961.
412 <https://doi.org/10.5897/AJB11.2018>.
- 413 Zanetti, F., Eynck, C., Christou, M., Krzyżaniak, M., Righini, D., Alexopoulou, E., Stolarski, M.J.,
414 Van Loo, E.N., Puttick, D., Monti, A., 2017. Agronomic performance and seed quality
415 attributes of camelina (*Camelina sativa* L. Crantz) in multi-environment trials across
416 Europe and Canada. *Ind. Crop. Prod.* 107, 602–608.
417 <https://doi.org/10.1016/j.indcrop.2017.06.022>.
- 418 Zanetti, F., Alberghini, B., Marjanović Jeromela, A., Grahovac, N., Rajković, D., Kiproviski, B.,
419 Monti, A., 2021. Camelina, an ancient oilseed crop actively contributing to the rural

420 renaissance in Europe. A review. *Agron. Sustain. Dev.* 41, 118.

421 <https://doi.org/10.1007/s13593-020-00663-y>.

422 Zhang, X., Chen, S., Sun, H., Wang, Y., Shao, L., 2010. Water use efficiency and associated traits

423 in winter wheat cultivars in the North China Plain. *Agric. Water Manag.* 97, 1117–1125.

424 <https://doi.org/10.1016/j.agwat.2009.06.003>.

425

426 Table 1. ANOVA results, percentage of variation explained by different sources, calculated for
427 sum of squares.

Source of variation	d.f.	Germination	Time to 50% germination
Genotype (G)	1	<0.01	0.52**
Osmotic stress (OS)	7	98.26**	97.81**
G x OS	7	0.32	0.81**
Residual	48	1.42	0.87

428

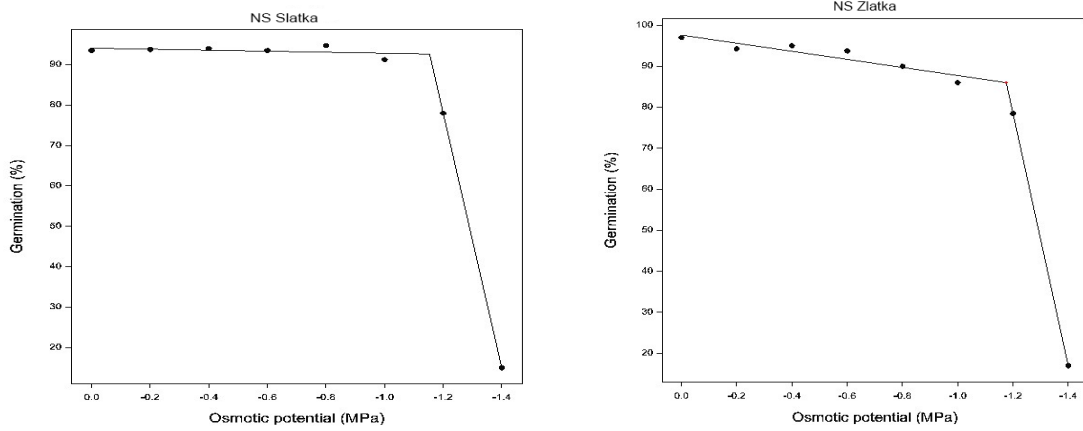
429

430 Table 2. Camelina seed germination and time to 50% germination under different levels of
 431 osmotic stresses, from 0 to -1.4 MPa in the controlled environment experiment comparing NS
 432 Slatka and NS Zlatka genotypes. Means with different letters are significantly different for $P \leq$
 433 0.05 (LSD test).

Osmotic stress (MPa)	Germination (%)			Time to 50% of germination (days)		
	NS Slatka	NS Zlatka	<i>Average</i>	NS Slatka	NS Zlatka	<i>Average</i>
0.0	93.50 ^{bc}	97.00 ^a	95.25 ^a	1.42 ^e	0.68 ^f	1.05 ^f
-0.2	93.75 ^{abc}	94.25 ^{ab}	94.00 ^a	1.49 ^e	0.97 ^f	1.23 ^f
-0.4	94.00 ^{abc}	95.00 ^{ab}	94.50 ^a	1.51 ^e	1.51 ^e	1.51 ^e
-0.6	93.50 ^{abc}	93.75 ^{abc}	93.63 ^a	1.57 ^e	1.56 ^e	1.56 ^e
-0.8	94.75 ^{ab}	90.00 ^{cd}	92.38 ^a	1.96 ^d	2.04 ^d	2.00 ^d
-1	91.25 ^{bc}	86.00 ^d	88.63 ^b	3.13 ^c	3.09 ^c	3.11 ^c
-1.2	78.00 ^e	78.50 ^e	78.25 ^c	5.89 ^b	5.75 ^b	5.82 ^b
-1.4	15.00 ^f	17.00 ^f	16.00 ^d	6.63 ^a	5.74 ^b	6.18 ^a
<i>Average</i>	81.72 ^a	81.44 ^a	-	2.95 ^a	2.67 ^b	-

434

435



436

437 Figure 1. Germination of two camelina genotypes (NS Slatka & NS Zlatka) subjected to different
438 levels of osmotic stress (Control=0.0, -0.2, -0.4, -0.6, -0.8, -1, -1.2, and -1.4 MPa) induced with
439 PEG 6000.

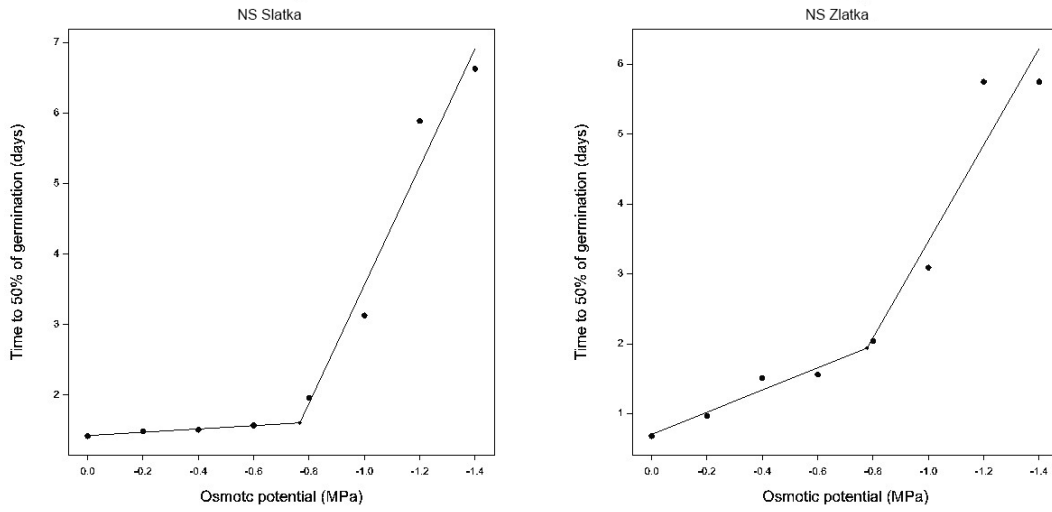
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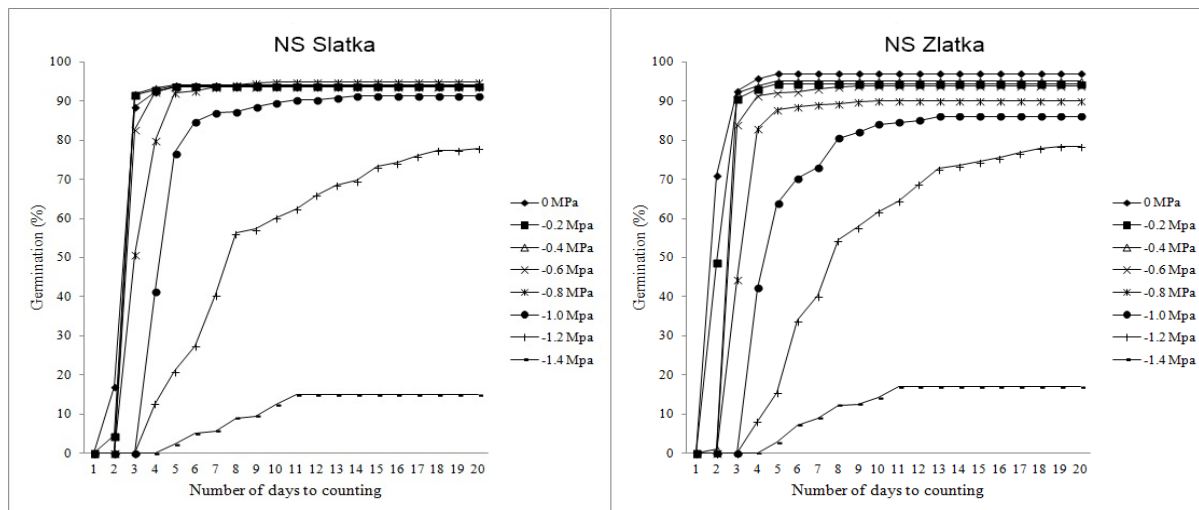
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445

446 Figure 2. Time to 50% germination of two camelina genotypes (NS Slatka & NS Zlatka) subjected
447 to different levels of osmotic stress (Control=0.0, -0.2, -0.4, -0.6, -0.8, -1, -1.2, and -1.4 MPa)
448 induced with PEG 6000.

449



450

451 Figure 3. Cumulative seed germination time-courses of two camelina genotypes (NS Slatka & NS
452 Zlatka) under different levels of osmotic stress (Control=0.0, -0.2, -0.4, -0.6, -0.8, -1, -1.2, and -
453 1.4 MPa) induced with PEG 6000.