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

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

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Abstract

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

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

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

1. Introduction

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

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

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

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

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

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

2. Materials and methods

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

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

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

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

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

2.1 Statistical analysis

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

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

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

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

3. Results

3.1. Osmotic stress effect on camelina germination

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

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

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

3.2 Germination trend lines and critical points

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

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

3.3. Camelina germination time-course

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

4. Discussion

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

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

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

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

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

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

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

5. Conclusions

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

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

Author Contributions

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

Declaration of Competing Interest

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

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Table 1. ANOVA results, percentage of variation explained by different sources, calculated for 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

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

Osmotic stress (MPa)	Germination (%)			Time to 50% of germination (days)		
	NS Slatka	NS Zlatka	*Average*	NS Slatka	NS Zlatka	*Average*
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}
Average	*81.72* ^{a}	*81.44* ^{a}	-	*2.95* ^{a}	*2.67* ^{b}	-

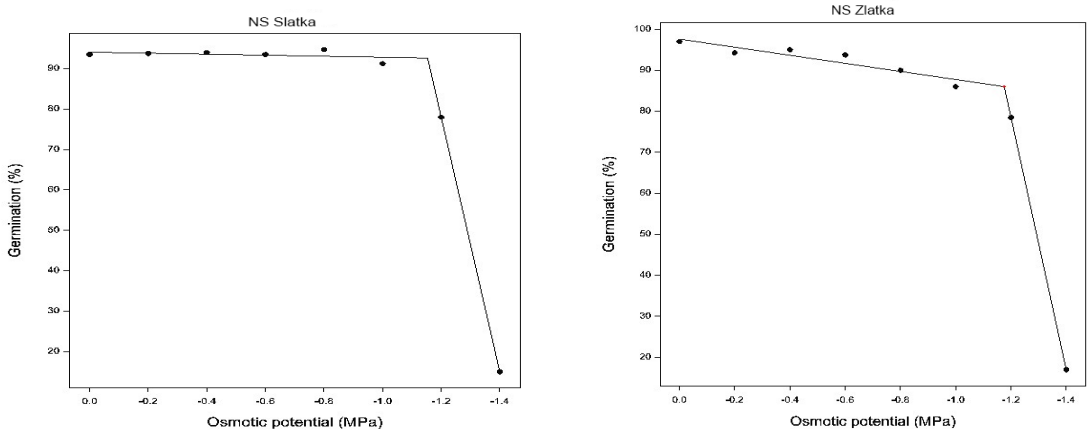

Figure 1. Germination of two camelina genotypes (NS Slatka & NS Zlatka) subjected to different levels of osmotic stress (Control=0.0, -0.2, -0.4, -0.6, -0.8, -1, -1.2, and -1.4 MPa) induced with PEG 6000.

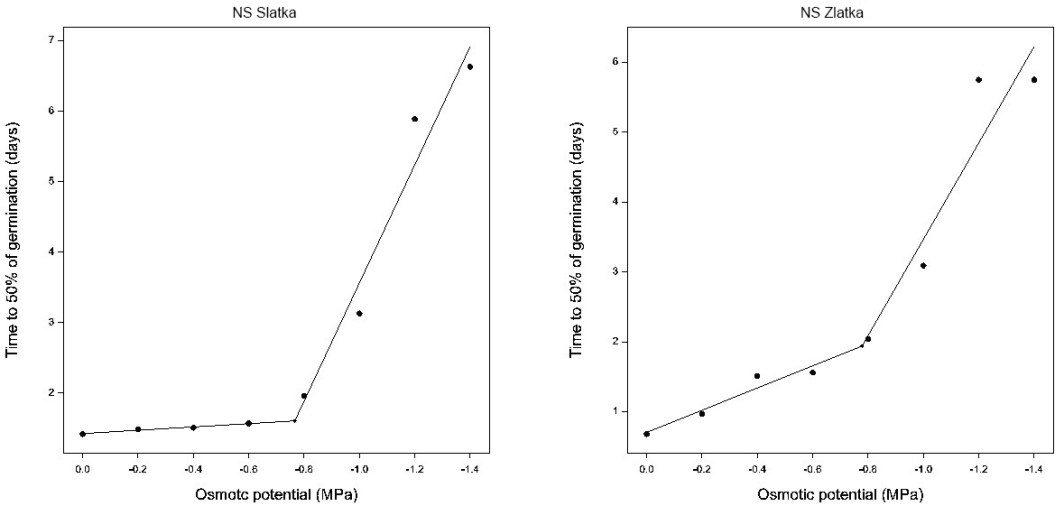

Figure 2. Time to 50% germination of two camelina genotypes (NS Slatka & NS Zlatka) subjected to different levels of osmotic stress (Control=0.0, -0.2, -0.4, -0.6, -0.8, -1, -1.2, and -1.4 MPa) induced with PEG 6000.

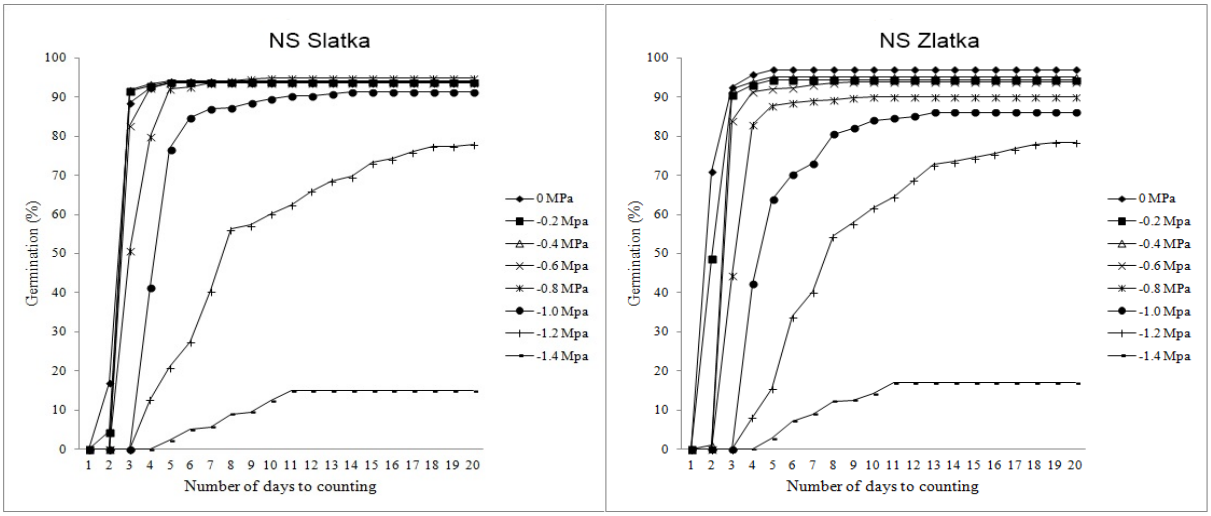

Figure 3. Cumulative seed germination time-courses of two camelina genotypes (NS Slatka & NS Zlatka) under different levels of osmotic stress (Control=0.0, -0.2, -0.4, -0.6, -0.8, -1, -1.2, and -1.4 MPa) induced with PEG 6000.