DOI: /10.5513/JCEA01/20.4.2484

Morphological characteristic of fiber flax seedlings regard to different pH water solution and temperature

Morfološke karakteristike klijanaca predivog lana ovisno o pH vrijednosti vodene otopine i temperature

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ABSTRACT

The aim of this study was to determine the influence of different pH values of water solution (pH 4.5, 5.5, 6.5, 7.5 and 8.5) and temperatures (10, 15 and 20 °C) on fibre flax seed germination and seedlings morphological characteristic (seedling root and stem length and total seedling length). The study was conducted in the controlled conditions by rolled filter paper in 4 replicates for 7 days. The average germination rate of flax seed was 84%. The highest germination rate was found at 20 °C (88%) which was very significant (P<0.01) as compared to germination rate at 10 °C where it was decreased by 9%. The highest share of normal seedlings was determined at pH 5.5 (70%) and the lowest at pH 8.5 (59%). There was no statistically significant influence of different pH on morphological characteristic of fibre flax seedlings (seedling root and stem length and total seedling length). The statistically significant difference (P<0.01) was determined in the length of the seedlings depending on the temperature, whereby after 7 days the largest seedlings were developed at 20 °C (14.4 cm) and the shortest at 10 °C (2.1 cm). In general, the lowest pH value (4.5) as well as the highest pH value (8.5) in this study resulted in a lower share of normal and healthy seedlings (63% and 59% respectively) and compared with seedlings of other pH values they developed a smaller root and stem.

Keywords: fibre flax, seed, temperature, pH, germination

SAŽETAK

Cilj ovog istraživanja bio je utvrditi utjecaj pet vodenih otopina različitih pH vrijednosti (pH 4,5; 5,5; 6,5; 7,5 i 8,5) i tri različite temperature (10, 15 i 20 °C) na klijavost sjemena i morfološka svojstva klijanaca (dužina korjenčića, stabljike i ukupna dužina klijanaca) predivog lana. Naklijavanje sjemena predivog lana provedeno u kontroliranim uvjetima metodom rolanog filter papira u 4 ponavljanja tijekom 7 dana. Ukupna klijavost sjemena lana je iznosila prosječno 84%. Ovisno o temperaturi najveća klijavost je utvrđena pri 20 °C (88%), što je bilo vrlo značajno (P<0,01) u odnosu na klijavost pri 10 °C, koja je bila smanjena za 9%. Najveći udio zdravih i normalno razvijenih klijanaca utvrđen je na pH 5,5 (70%), a najmanji na pH 8,5 (59%). Ovisno o pH, nije utvrđen statistički značajan utjecaj na morfološka svojstva klijanaca lana (dužina klijanca, dužina korijenčića i dužina stabljike). Statistički vrlo značajane razlike (P<0,01) utvrđene su u dužini klijanca ovisno o temperaturi, pri čemu su se nakon 7 dana naklijavanja najveći klijanci razvili na 20 °C (14,4 cm), a najmanji na 10 °C (2,1 cm). Najmanja pH vrijednost (4,5), kao i najviša pH vrijednost (8,5) u ovom istraživanju rezultirale su nižim udjelom normalno razvijenih klijanaca (63%, odnosno 59%) te su u usporedbi s klijancima pri ostalim pH vrijednostima, klijanci razvili manji korijenčić i stabljiku.

Ključne riječi: predivi lan, sjeme, temperatura, pH, klijavost

INTRODUCTION

Flax (*Linum usitatissimum* L.) is an important industrial plant which is grown widely for its fibers and oils. Fibre flax have the main yield fibre, whereas linseed (oilseed flax) have seeds as the primary yield and straw is the secondary yield. As morphological differences, the fibre flax is 80-150 cm tall and poorly branched, while linseed is 45-80 cm tall and more branched (Couture et al., 2002; Pospišil, 2013; Butorac et al., 2017; Butorac et al., 2018). Nowadays fibre flax, as natural fiber source, is increasingly used in various, innovative sectors of the industry which are today obliged to take into account sustainable development in their production methods. Besides that, many authors accelerate the importance of flax seed in human diet and its medicinal use because containing a mixture of the fatty acids, is rich in two essential fatty acids, alphalinolenic acid (C18:3; ω-3) and linoleic acid (C18:2; ω-6) (Fitzpatrick, 2008; Jhala and Hall, 2010; Bernacchia et al., 2014, Nôžková et al., 2014; Hlavačková et al., 2016; Klir et al., 2017).

From 2012 to 2016 the fiber flax is cultivated on about 200 000 ha with average fiber yield of 3.5 $t*ha^{-1}$ (FAO Stat, 2018). Therefore, the largest harvested area of fiber flax in Europe are in France (72.091 ha), Belarus (48 028 ha), Belgium (12 227 ha), United Kingdom (10 219 ha), and in the world in Russian Federation (46 176 ha), Egypt (9 449 ha) and China (4 216 ha).

Seedlings are the most vulnerable stage in the life cycle of plants and germination determines when and where seedling growth begins. Seedling emergence under field conditions is influenced by several abiotic factors such as temperature, moisture, pH, seedbed preparation, salt stress etc. (Parađiković et al., 2007; Pacanoski et al. 2014; Bukvić et al., 2015; Zebec et al., 2017; Anđelić et al., 2018; Baličević et al., 2018, Bukvić et al., 2018).

Seed germination have different phases which are affected by temperature, moisture, and oxygen. Temperature is important factor that impacts germination and optimal temperature for temperate crops is between 0 °C and 35 °C (Vassilevska-Ivanova and Tcekova, 2002). The min. temperature for flax germination is 2 – 3 °C and the optimum is 16 – 18 °C (Pospišil, 2013). Higher germination temperatures (up to 45 °C) are related to some tropical crops (Vassilevska-Ivanova and Tcekova, 2002).

According to Hall et al. (2016) fibre flax succeeds in a wide range of soil pH, while the optimal pH for fibre flax growth, stand out at pH 6.0. Pospišil (2013) states that the optimum soil pH for its growing is between 6.2 and 7.2, while Jacobs and Merwe (2012) state that optimum pH ranges between 5 and 7.

In Republic of Croatia there are few Family farms which cultivate fibre flax and it is grown on approximately 15 – 30 ha (Pospišil, 2013). This is very small grown area, as compared to the end of 20th century, when fibre flax was grown on approximately 3.000 – 5.000 ha (Pasković, 1966). Butorac et al. (2017) point out that in Croatia there is no own seed production of fibre flax and that for the re-introduction on agricultural surfaces it is important to explore the agronomic, morphological and phenological properties of foreign varieties and the possibility of acclimatization on the agroecological conditions of Croatia.

Thus, the aim of this study was to determine the influence of five different pH values of water solution (pH 4.5, 5.5, 6.5, 7.5 and 8.5) at three temperatures (10, 15 and 20 °C) on seed germination rate and morphological characteristics of seedlings of fiber flax (seedling root and stem length and total seedling length).

MATERIALS AND METHODS

The water solution was made by adding HCl or NaOH to distilled water in order to achieve desired pH value (4.5, 5.5, 6.5, 7.5 and 8.5), which was measured with pH meter (Mettler Toledo, USA). The germination test was done according to the The International Seed Testing Association – ISTA (ISTA, 2006). The seeds were pre-chilled at the temperature 8 °C during 7 days before sowing.

The filter paper (Munktell, 580x580 mm, 80 g/qm) was moistened with 55 ml water solution. Afterwards the 100 seeds (cultivar Lirina, RWA) per replication was sown on each filter paper and then rolled and put into plastic bag. The seeds were sown in 4 replicates. The samples were then put into growth chamber (Memmert, Germany) on three different temperatures: 10, 15 and 20 °C for 7 days. The germination test was done in 24 h dark conditions.

After 7 days the total germination rate (%), share of normal, abnormal and non-sprouted seeds were determined. Afterwards, the length of root, stem and total length (cm) of every normally developed seedling, were measured. On the seedlings grown at 10 °C there were no clear difference between the root and stem, thus the total length of all healthy and normally developed seedlings was determined, at every pH level.

The ANOVA was performed using SAS 9.4 Software-a (SAS Institute Inc.). LSD test was used to calculate the differences between the means at P<0.05 and P<0.01. Simple linear regression was done to show relationship between length of root and stem of seedlings.

RESULTS

The average germination rate for all the treatments was 84% (Table 1). The different pH of water solution did not have significant influence on germination rate, but the different temperatures and interaction of pH and temperature had significant influence (P<0.01) on germination rate. In average for all the pH values, the lowest germination rate was at 10 °C (79%) and the highest was at 20 °C (88%), with very significant (P<0.01) differences between the means. The interaction between temperature and pH value was very significant (P<0.01) (Table 1). The lowest germination rate was found at pH 6.5 and 10 °C (75%) and the highest at 20 °C and pH 7.5 (94%).

After 7 days there were on average counted 64% healthy and normal seedlings, 17% abnormal seedlings and 19% of non-sprouted seeds (Figure 1). Regard to the pH value of water solution and temperature, the highest share of normal seedlings was observed at pH 5.5 and 20 °C (70% and 68%, respectively), whereas the lowest at pH 8.5 and 15 °C (59% and 62% respectively). The share of abnormal seedlings at different pH value, varied from 10% (pH 5.5) to 21% (pH 8.5) and at different temperatures from 16% (10 °C) to 21% (15 °C). The highest share of non-sprouted seeds was found at pH 5.5 (18%) and at 10°C (20%).

Different pH didn't significantly influence total length and the length of root and stem of seedlings (Table 2). Even though the differences were not significant, at pH 5.5 and 6.5 the total length of seedling was above the average (8.6 cm). On average, the shortest seedlings were at 10 °C (2.1 cm) and the longest at 20 °C (14.4 cm).

Table 1. Fiber flax germination rate (%) at different pH and temperature

Temperature (B)	pH (A)					Average
	4.5	5.5	6.5	7.5	8.5	
10 °C	76	82	75	79	83	79
15 °C	88	84	88	84	80	85
20 °C	84	82	92	94	86	88
Average	83	83	85	86	83	84

$LSD_{0.05}$ (A) = ns; $LSD_{0.01}$ (A) = ns
$LSD_{0.05}$ (B) = 3.0; $LSD_{0.01}$ (B) = 5.5
$LSD_{0.05}$ (A x B) = 6.7; $LSD_{0.01}$ (A x B) = 12.2

Table 2. Total length and the length of root and stem of fibre flax seedlings (cm) at different pH values and temperatures

Total length of fibre flax seedlings (cm)

Temperature (B)	pH (A)					Average
	4.5	5.5	6.5	7.5	8.5	
10 °C	1.8	3.3	1.7	1.8	2.1	2.1
15 °C	9.0	9.8	10.0	9.0	8.2	9.2
20 °C	13.8	14.2	14.9	14.5	14.4	14.4
Average	8.2	9.1	8.9	8.4	8.3	8.6

$LSD_{0.05}$ (A) = ns $LSD_{0.05}$ (B) = 0.97 $LSD_{0.05}$ (A x B) = 2.16
$LSD_{0.01}$ (A) = ns $LSD_{0.01}$ (B) = 1.77 $LSD_{0.01}$ (A x B) = 3.97

Root length of fibre flax seedlings (cm)

Temperature (B)	pH (A)					Average
	4.5	5.5	6.5	7.5	8.5	
15 °C	6.5	6.9	6.9	6.5	5.8	6.5
20 °C	6.8	7.0	8.0	7.3	7.4	7.3
Average	6.6	7.0	7.4	6.9	6.6	6.9

$LSD_{0.05}$ (A) = ns $LSD_{0.05}$ (B) = 0.60 $LSD_{0.05}$ (A x B) = ns
$LSD_{0.01}$ (A) = ns $LSD_{0.01}$ (B) = ns $LSD_{0.01}$ (A x B) = ns

Stem length of fibre flax seedlings (cm)

Temperature (B)	pH (A)					Average
	4.5	5.5	6.5	7.5	8.5	
15 °C	2.5	2.9	3.1	2.5	2.4	2.7
20 °C	7.1	7.2	6.9	7.2	7.0	7.1
Average	3.8	4.4	3.9	3.8	3.8	4.0

$LSD_{0.05}$ (A) = ns $LSD_{0.05}$ (B) = 0.52 $LSD_{0.05}$ (A x B) = 1.15
$LSD_{0.01}$ (A) = ns $LSD_{0.01}$ (B) = 0.95 $LSD_{0.01}$ (A x B) = 2.12

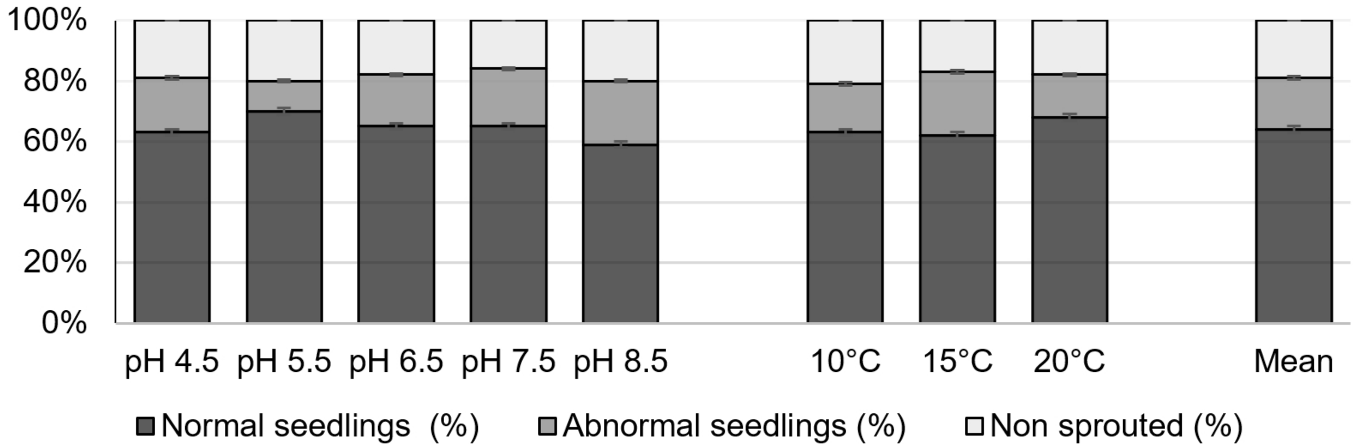

Figure 1. The share (%) of health and normal, abnormal seedlings and non-sprouted seeds at different pH and temperature

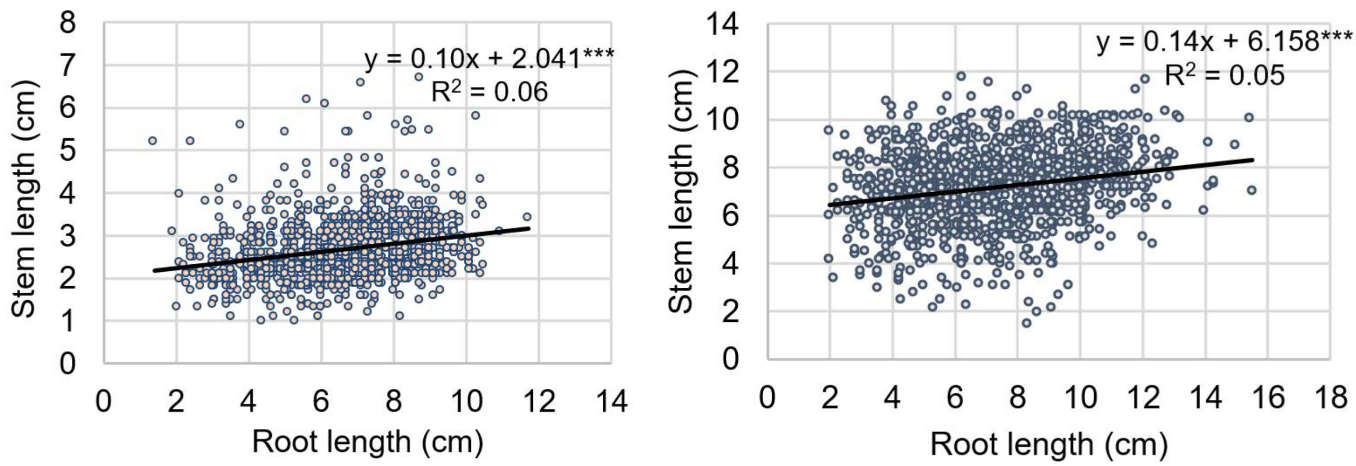

Figure 2. Scatterplot diagram of stem and root length at 15 °C (n = 1252; on the left) and 20 °C (n = 1354; on the right)

Interaction between pH and temperature significantly influenced the examined parameters (P<0.01) (Table 2). Therefore, at 20 °C and pH 6.5 seedlings were the longest (on average 14.9 cm), whereas at the same pH (6.5) at 10 °C resulted with the shortest seedlings (1.7 cm).

Temperature significantly influenced the root lenght (P<0.05), where at the 20 °C the root was on average 7.3 cm, which was for 0.8 cm higher as compared to root length at 15 °C (Table 2). The more expressed diference in elongation was found for stem length, where the average length at 15 °C was 2.7 cm, while at 20 °C seedlings were 4.4 cm long what is 61% longer as compared to the treatment mentioned before.

Single linear regression showed extremely significant relationship between stem and root length (Figure 2). Even though the R^2 is small, it can be seen that according to simple regression analysis for every centimeter increment of root length, the stem length increase for 0.10 cm at 15 °C and for 0.14 cm at 20 °C.

DISCUSSION

In this study, seed germination was under the significant influence of temperature. Thus, the highest germination rate was observed at 20 °C (94%) with the highest average root, stem and seedling length (7.3 cm, 7.1 cm and 14.4 cm, respectively). On the contraty, the lowest germination rate was at 10 °C (79%) with smallest seedlings (average total length 2.1 cm). In experiment set up in glass Petri dishes at room temperature, Jain (2013) stated that germination rate of 10 flax cultivars (TLP-1, RLC-29, LC-54, LC-185, T-397, Kiran, Nagarkot, Neelum, Shubra and Gaurav) after seven days averaged 89.75%, with an average root length of 4.1 cm, stem 7.1 cm and a total seedlings length 11.2 cm. Veerhoff (1940) stated that for two oil-flax cultivars germination was markedly reduced at 38.5 °C, and no germination occurred at 43 °C. Kurt and Bozkurt (2006) found that lower temperatures have a dramatic effect on the flax seedlings emergence rate. Authors found the highest percent seedling emergence (86.9%) at 30 °C and the lowest percent seedlings emergence (33.9%) at 10 °C. The fibre flax in Croatia is usually sown in early spring when the soil temperature is around 8 – 12 °C. Saeidi (2008) states that earlier sowing of flax has many advantages such as avoiding high temperatures at flowering time, grain filling, early harvest, but also, avoiding disease and pests. But disadvantage of earlier sowing may be in the early stage of growth when low soil temperatures can negatively affect growth and development of young seedlings. In our study, the highest share of non-sprouted seeds was found at 10 °C (20%). Thus, it is recommended to use higher seed rate in sowing time to get the optimum plant density at harvest.

The one of the advantage of fibre flax cultivation is that it could be cultivated at the soils which are not suitable for crops which are sensitive to the pH of the soil, like legumes (Ritchey and Carter, 1993; Bukvić et al., 2008a; Bukvić et al. 2008b; Bukvić et al., 2010). Even though for fibre flax production it is assumed that about 60% of sown seeds will developed the whole plant until the harvest, the extreme soil pH as 8.5 could result with lower germination what consequently with lower yield. This may be due to higher concentration of Na^+ ions. It is well known that salinity can affect germination of seeds either by creating osmotic potential which prevent water uptake, or by toxic effects of ions on embryo viability (Lisjak et al., 2015; Wu et al., 2015; Henschke, 2016). This is also partly confirmed in our study, whereas the highest percentage of abnormal seedlings (21%) was found at pH 8.5. In experiment of unfavorable pH and Zn with two flax cultivars (Mogilevsky and Norlin), Dimitrev et al. (2019) found that for flax, Zn deficiency had less negative effects on plants, while the increase in pH level (7.5 or higher) results in crucial damages of plants that indicates greater sensitivity of flax to non-optimal soil acidity.

There are several similar studies of forage crops. For example, Bukvić et al. (2008a) found very significant influence of temperatures (10 and 20 °C) and pH values of water solution (4, 5, 6 and 7) on germination and seedlings length of three cultivars of white clover (Regal, Jura and Rivendel), whereas the highest seedling length was observed at pH 5. On the contrary, in experiment with red clover (cultivar Rajah and Viola), Bukvić et al. (2010), using the solutions with the same pH values, the highest length of seedlings was found at pH 4 and 6, while the lowest at pH 5. Mandić et al. (2012) found that germination media of pH 6 significantly increased alfalfa root length (3.17 cm) and seedling length (8.90 cm) as compared to the germination media at pH 5 (3.03 cm and 8.53 cm, respectively).

Other stressed environment conditions also have great influence on flax seed germination. Heavy metal toxicity is one of the main current environmental health problems, and potentially harmful because of bioaccumulation through the food chain and plant products for human consumption (Ivezić et al., 2015; Novoselec et al., 2018; Japundžić-Palenkić et al., 2019; Kovačević et al., 2019). There are several studies of flax germination in heavy metals contaminated soils (Soudek et al., 2010; Kavuličová et al., 2012; El-khawaga, 2014). Even though flax seed germination and the early seedling growth are more sensitive to metal stress because some of the defence mechanisms have not developed and it is also strongly related to the seed coat permeability to metal ions, the fibre flax could be cultivated on contaminated soils, because its stem is the raw material for fibre production, not for human consumption.

According to our results, it was confirmed that flax seed can germinate in slightly acidic to neutral media or even those that are a little alkaline. Even though the soil is complex system and the pH value is not the only factor which influence seed germination, this kind of study can be a guideline for selection of soil type in fibre flax cultivation.

CONCLUSIONS

In this study the influence of pH values of water solution and temperatures on fibre flax seed (cultivar Lirina) germination and morphological characteristic of seedlings were analyzed in the controlled conditions. Different pH of water solution did not have significant influence on germination rate and morphological characteristic of seedlings. Different temperature has very significant influence on germination rate, total seedlings length and stem length and significant influence on root length. After 7 days the average germination rate was 84%. The seedlings were the longest at 20 °C (14.4 cm) and the shortest at 10 °C (2.1 cm). In this study it was shown that fibre flax cv. Lirina can germinate in wide range of pH, which can be advantage of uses the acid or alkaline soils, which may not suitable for some other crops.

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