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MORPHOLOGICAL PARAMETERS OF OIL-PUMPKIN SPROUTS IN DIFFERENT WATER-SOLUTION PH VALUES

IVANA VARGA, D. ILJKIĆ, MIRNA ŠIMUNOVIĆ, D. AGIĆ, G. HERMAN, MANDA ANTUNOVIĆ

Fakultet agrobiotehničkih znanosti Osijek
Sveučilište Josipa Juraja Strossmayera u Osijeku
Faculty of Agrobiotechnical Sciences Osijek,
Josip Juraj Strossmayer University of Osijek

SUMMARY

This study aimed to determine the influence of a water-solution pH value on the germination of Croatian *Bankovci* oil pumpkin (*Cucurbita pepo* L. var. *oleifera*). The study was conducted under controlled conditions. The pumpkin seeds germinated at the different aqueous-solution pH values (2.5, 3.5, 4.5, 5.5, 6.5, 7.5, and 8.5, respectively). Subsequent to germination, a total germination was determined, and the morphological parameters of sprouts were measured: total length, hypocotyl length, and root length (cm). In this research, the average seed germination amounted to 92%. The pH value had a statistically significant influence ($p \leq 0.05$) on all morphological parameters. In this study, the average sprout length amounted to 7.6 cm. The smallest sprout thrived in a very acidic medium at a pH value of 3.5, whereby the average sprout length amounted to only 3.6 cm, whereas the largest sprouts thrived at a pH value of 7.5, whereby the sprout length averagely amounted to 8.4 cm. A sprout development response in the water-solution media having the various pH values can be valuable for the examination of a possibility to grow this genotype in different soil types and for the calculation of a number of seeds for precise sowing.

Keywords: *Cucurbita pepo* var. *oleifera*, Croatian landrace *Bankovci*, germination rate, hypocotyl root length

INTRODUCTION

In the Republic of Croatia, the cultivation of pumpkin (*Cucurbita pepo* var. *oleifera*) has a long tradition. Primarily, common oil pumpkins are cultivated to produce oil for extraction (P1eh et al., 1998). They are usually sown in the areas with a warmer climate, where the conditions for arable farming and soil cultivation are favorable. The cultivation of pumpkins requires a knowledge of sowing time, sow spacing and depth, sufficient irrigation and fertilization, fruit harvesting, and finally processing.

The influence of a pH value on the plant growth is significant, with a low pH value generally having a negative effect. However, some species can tolerate the lower pH values, and the relationship between a pH value and the plant growth is species-specific, with the different plant species requiring different pH values for an optimal growth and germination rate (Martins et al., 2011; Leifert et al., 2004). According to Parađiković (2009), an optimal pH value for the cultivation of pumpkin is neutral—that is, a pH value amounting to 7.

There are several possibilities for the use of pumpkin fruit and seeds. A nutritional value, but also a variability of use, contribute to the promotion and consumption of the product (Delaš, 2010). The application is mostly aimed at the anti-inflammatory and anti-carcinogenic purposes and at the improvement of chronic cardiovascular disease symptoms (Gedi, 2022). The use in households is most often represented by a virgin or a cold-pressed oil, but pumpkins are increasingly used to make the spreads, marmalades, and dishes. Pumpkin seeds contain phytoingredients such as flavonoids, alkaloids, oleic, linoleic, and palmitic acids, which engage in anti-inflammatory, antiulcer, antimicrobial many other activities, and they are used in pharmacology and alternative medicine (Klir et al., 2017; Martinec et al., 2019; Wagner, 2020).

Germination is a significant parameter of seed quality and can be affected by a medium pH value, light, temperature, previous seed preparation, and storage. Therefore, a dependence of plant species' germination on various factors has been known for a long time. Pumpkins are a fairly common species, but an additional study of the most favorable conditions for germination could contribute to a better recommendation for farmers and to a higher yield. So far, no similar or related research has been conducted regarding this pumpkin genotype. Therefore, this study aims to determine the germination of oil pumpkin seeds and morphological parameters of the sprouts depending on an aqueous-solution pH value.

MATERIAL AND METHODS

A germination test of the oil-pumpkin seeds from the Croatian village of *Bankovc*i, harvested in 2019 and stored at 4°C, was administered in the study. This cultivar is indexed in the CPGRD database (Croatian Plant Genetic Resources Database) under the accession number of IND00084. The experiment was carried out under the controlled conditions in the plant-growth chamber (*Fitoclima 1200 PLH*, Aralab) at the Faculty of Agrobiotechnical Sciences Osijek. The germination test was carried out while following the I S T A protocol by the International Seed Testing Association (2016).

An HCl or a NaOH solution was added to the distilled water to modify the pH value, and a pH meter (Mettler Toledo, USA) was used to measure the pH values. Seven treatments were performed, having the aqueous solution pH values of 2.5, 3.5, 4.5, 5.5, 6.5, 7.5, and 8.5. Twenty-five ml of water solution were added to the 580 mm × 290 mm filter paper (Munktell). Forty seeds per replication were sown on each filter paper

in a total of 4 replications of 40 seeds each (Fig. 1). Each filter paper was rolled up and stored in a PE bag with a corresponding label, and then the bags were placed in the growth chamber, in which a constant temperature was maintained at 20°C.

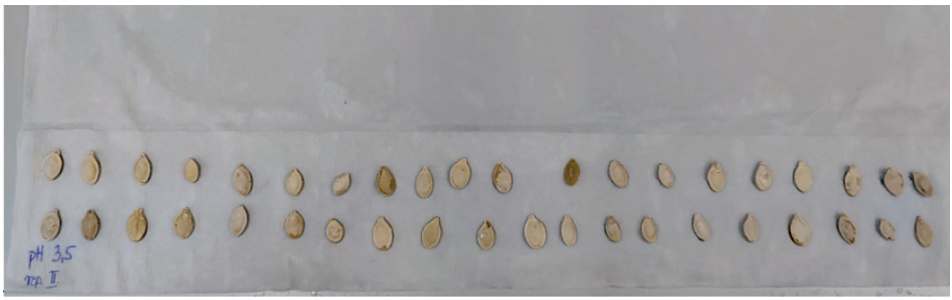

Figure 1 The example of moistened filter paper with the deployed oil pumpkin seeds, a treatment with the pH value amounting to 3.5, 2nd replication
Slika 1. Primjer vlažnoga filter-papira s raspoređenim sjemenkama uljne bundeve, tretman pH 3.5, II. repeticijaž

The sprouts' morphological parameters were determined after eight days. The length (cm) of the sprouts and their parts was measured manually by a ruler (Fig. 2).

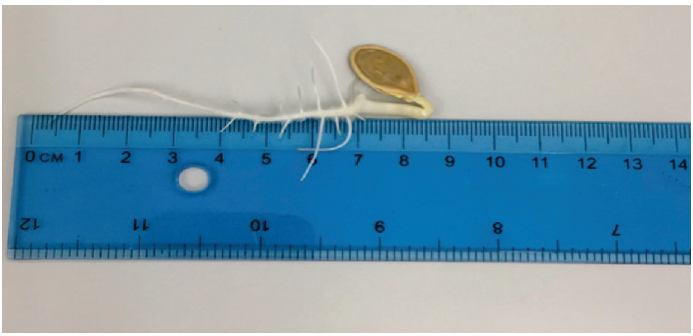

Figure 2 The measurement of oil-pumpkin sprout lengths (cm)
Slika 2. Mjerenje dužine klijanca uljne bundeve (cm)

The data were statistically analyzed by a simple one-way analysis of variance (ANOVA) using the *SAS Enterprise Guide* (7.1) program. Following a significant F-test, the means were compared while administering the LSD test (least significant difference) ($p \leq 0.05$). The columns' vertical error bars in the figures indicate a standard error at 5%.

RESULTS AND DISCUSSION

The average oil-pumpkin seeds' germination rate was high, amounting to 92% (Fig. 1). In this study, there were no statistically significant differences detected with regard to the total germination. The highest germination rate amounted to 94% at the highest water-solution pH value, and the lowest one amounted to 90%, at a pH value of 6.5. Three aqueous solutions with the pH values of 2.5, 4.5, and 7.5 achieved the same germination rate of 93%.

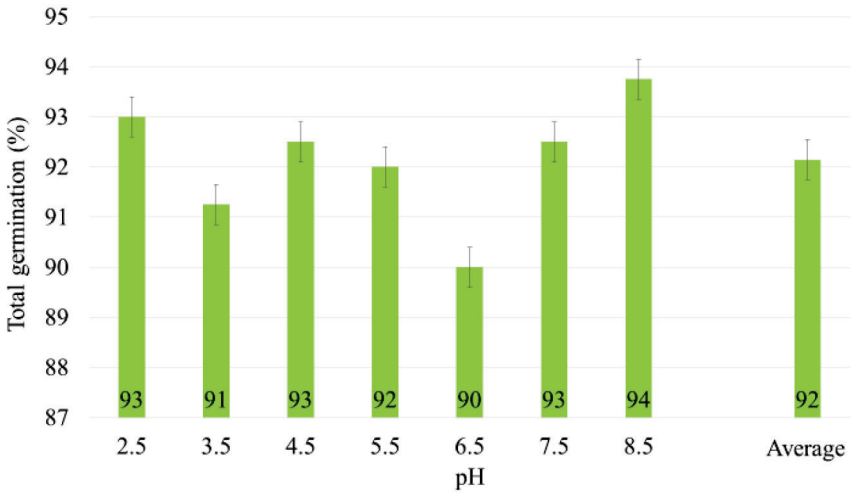

Figure 1 The effect of water-solution pH value on the total germination rate of the oil-pumpkin seeds, landrace *Bankovci*. (Different letters indicate significant differences between the mean values at p≤0.05)

Grafikon 1. Utjecaj pH vrijednosti vodene otopine na ukupnu klijavost sjemena uljne bundeve populacija Bankovci. (Različita slova označuju značajne razlike između srednjih vrijednosti pri p≤0,05)

According to the results of the one-way ANOVA, a significant F value was determined in case of the all analyzed morphological parameters (total sprout length, root length, and hypocotyl length) depending on a water-solution pH value.

The average length of the sprouts amounted to 7.6 cm (Fig. 2). The longest sprouts thrived at the pH value of 7.5, whereby the length mounted to 8.4 cm, while the smallest sprouts were found in an extremely acidic environment having a pH value of 3.5, whereby the length was 6.8 cm. However, for the length of sprouts at the pH values of 2.5, 3.5, 6.5, and 8.5, no statistically significant differences were found.

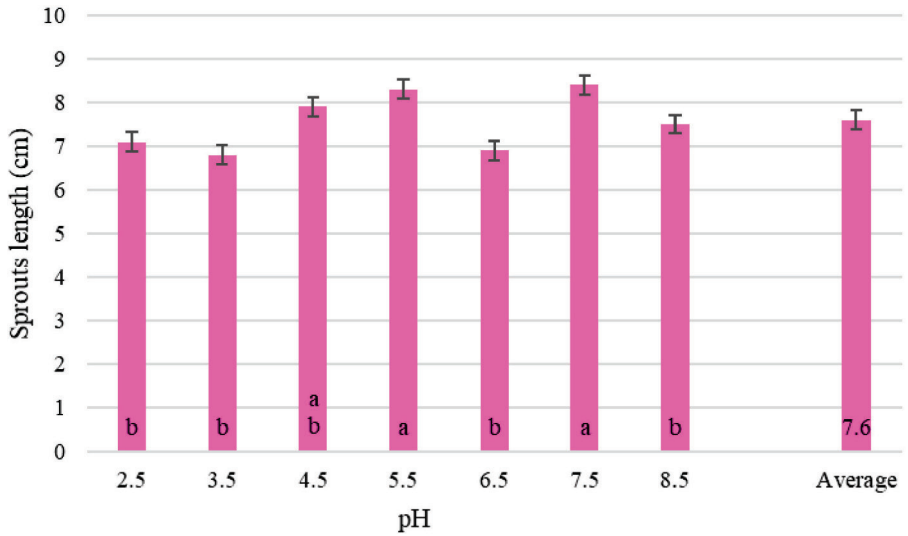

Figure 2 The effect of a water-solution pH value on the length of the oil-pumpkin sprouts, landrace *Bankovci.* (Different letters indicate significant differences between the mean values at p≤0.05)

Grafikon 2. Utjecaj pH vrijednosti vodene otopine na duljinu klijanaca uljne bundeve populacije Bankovci. (Različita slova označuju značajne razlike između srednjih vrijednosti pri p≤0,05)

The average root length of sprouts was 6.2 cm (Fig. 3). The root length varied from 5.5 (pH 6.5) up to 7.2 cm (pH 7.5). There was no significant difference between the root length of 4.5 cm and the pH value of 5.5 (with an average length amounting to 6.7 cm). Furthermore, no significant difference was found between the mean pumpkin sprouts' root-length values of at a low pH amounting to 2.5 and 3.5, respectively, and for a neutral pH value of 6.5 (5.8, 5.6, and 5.5 cm, respectively).

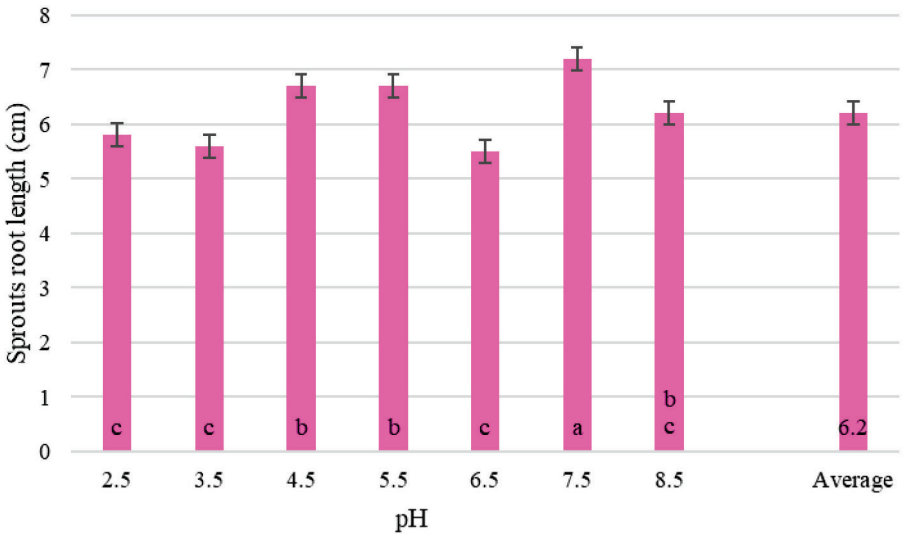

Figure 3 The effect of a water-solution pH value on the oil-pumpkin sprouts' root length, landrace *Bankovci.* (Different letters indicate significant differences between the mean values at p≤0.05)

Grafikon 3. Utjecaj pH vrijednosti vodene otopine na duljinu korijena klijanaca uljne bundeve populacije Bankovci. (Različita slova označuju značajne razlike između srednjih vrijednosti pri p≤0,05)

The average sprouts' hypocotyl length amounted to 1.3 cm (Fig. 4). In general, the hypocotyl was smaller than the sprout roots. Moreover, the sprouts at the lower pH values developed a shorter hypocotyl, in comparison with the other pH values. The highest hypocotyl was observed concerning the sprouts at the pH value of 5.5 (1.6 cm) and the lowest hypocotyl was in an acid medium, with a pH value of 2.5 to pH 4.5, in which the length of the hypocotyl amounted to 1.2 cm.

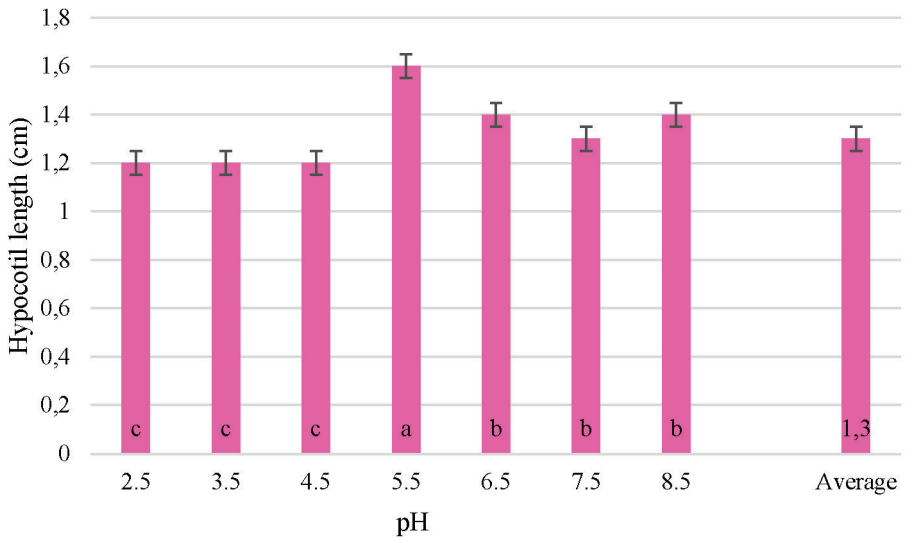

Figure 4 Hypocotyl length (cm) in the water solutions of oil-pumpkin sprouts, landrace *Bankovci*, having different pH values

Grafikon 4. Duljina stabljika (cm) klijanaca bundeve (genotip Bankovci) pri različitim pH vrijednostima. (Različita slova označuju značajne razlike između srednjih vrijednosti pri p≤0,05)

Asadi Aghbolagh i et al. (2022) researched the effect of different concentrations of humic acid on the germination fraction of pumpkin seeds under cadmium stress conditions. They stated that the different pH values could have a great influence on a heavy metal availability and that using humic acid could reduce the stress effect on pumpkins. The authors indicated that the application of humic acid (400 mg L^{-1}) increased a total germination rate of pumpkin, and that the highest percentage of germination was about 80%, which was by 25% higher than the control group without humic acid. Horak et al. (1994) investigated the effects of scarification, temperature, osmotic potential, and a pH value on the germination rate of buffalo gourd (*Cucurbita foetidissima*). The study showed that the germination rate increased from 15% at a pH value of 2.2 to 90% at a pH value of 8, while mechanical and chemical scarification did not increase the germination rate if compared to a non-scarified seed.

Guirra et al. (2020) evaluated the effect of phytohormones as abiotic stresses attenuators during the germination and initial growth of *Cucurbita moschata* seedlings under different types of water, with the pH values of 7.9, 8.0, 8.1, and 8.4. The authors found that the seedlings' physiological system in stressful environments will be focused on a root growth that needs to be established quickly, so that the plant could prioritize the root cell elongation. Furthermore, the authors found the highest root dry mass under the pH value of 8.4.

There are several studies available about the effect of a water-solution pH value on the red clover's (Bukvić et al., 2009a; 2010) alfalfa's (Bukvić et al., 2009b), soybean's (Grljušić et al., 2007), pea's (Bukvić et al., 2007), white clover's (Bukvić et al., 2008a), and cereal seeds' (Kraljičak, 2009; Ervačić, 2023) germination and growth. Bukvić et al. (2009) found that the length of alfalfa-sprout roots was significantly different at various water-solution pH values and that the sprout root length was greater at the pH value of 4 (2.75 cm) than at pH value of 6 (2.13 cm), regardless of a cultivar and temperature. In another study by Bukvić et al. (2008a), the authors found that the lowest white clover's sprout length was the one at the pH value of 4 and in the neutral media at the pH value of 6. In our study, pumpkins developed shorter roots in the neutral media at the pH value of 6.5. Fiber-flax seedlings were tested by Buranji et al. (2019) at different water-solution pH values. The authors found that the low (4.5) and the high water-solution pH values (8.5) reflected on fibre flay seedling due to a higher share of abnormal and deformed seedlings. Shoemaker and Carlson (1990) studied different pH values (from 4.5 to 7.5) of some flowers species using a filter paper and stated that they did not found any germinated seeds at pH 4.5, for Petunia x hybrida, Salvia splendens, Begonia x sempervirens, and Lobularia maritima species. In our study, oilseed pumpkin was not so sensitive to the low or high pH values, and germination energy was even higher in case of extremely acid or alkalic water solutions. This might well be a sprouts' response in the early growth stages, whereas a pH value should fluctuate between 6.0 and 7.5 (Pospišil, 2013) with regard to the field cultivation.

CONCLUSION

This study was set up at a constant temperature amounting to 20°C but with the different applications of water-solution pH. There was no significant difference in the total germination rate, and it averaged 92%. Different water-solution pH values exerted a statistically significant ($p \leq 0.05$) influence on all morphological pumpkin-sprout parameters (root and hypocotyl length and total sprout length). Root development was greater than that of the hypocotyl. The lowest hypocotyl was formed at the pH value amounting to 2.5 to 4.5 (1.2 cm on average), and the shortest route length was developed in an extremely acid (pH amounting to 2.5 and 3.5, respectively) and neutral medium (a pH of 6.5), and it averaged 5.6 cm. In the future, in would be valuable to grow this domestic genotype on the soil having various pH values to examine germination, having it initially grown on the acid and alkaline soils.

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MORFOLOŠKI PARAMETRI KLIJANACA OBIČNE ULJNE BUNDEVE PRI RAZLIČITIM PH VRIJEDNOSTIMA VODENE OTOPINE

SAŽETAK

Cilj ovoga rada bio je utvrditi utjecaj pH vodene otopine na klijavost obične bundeve (*Cucurbita pepo* var. *oleifera*) primke *Bankovci*. Istraživanje je provedeno u kontroliranim uvjetima. Sjeme bundeve naklijano je na različitim pH vrijednostima vodene otopine (2,5, 3,5, 4,5, 5,5, 6,5, 7,5 i 8,5). Nakon naklijavanja utvrđena je ukupna klijavost te su izmjereni morfološki pokazatelji klijanaca: ukupna dužina, dužina hipokotila i dužina korijena (cm). Prosječna klijavost sjemena u ovome istraživanju iznosila je 92 %, dok je pH vrijednost imala statistički značajan utjecaj ($p<0,05$) na sve morfološke pokazatelje. Prosječna dužina klijanaca u ovome istraživanju iznosila je 7,6 cm. Najmanji klijanci razvili su se u vrlo kiseloj sredini, na pH 3,5, gdje je prosječna dužina klijanaca iznosila svega 3,6 cm, a najveći klijanci razvili su se na pH 7,5, gdje je dužina klijanaca bila prosječno 8,4 cm. Razvoj klijanaca u vodenim otopinama s različitim pH vrijednostima može biti važan za ispitivanje mogućnosti uzgoja ovoga genotipa na različitim tipovima tala, posebno pri izračunu broja sjemenaka za preciznu sjetvu.

Ključne riječi: *Cucurbita pepo* var. *oleifera*, sjeme *Bankovci*, klijavost, duljina korijena, duljina hipokotila

Adresa autora - Authors' address
Doc. dr. sc. Ivana Varga
e-mail: ivana.varga@fazos.hr
Izv. prof. dr. sc. Dario Iljkić
Mirna Šimunović, mag. ing. agr.
Izv. prof. dr. sc. Dejan Agić
Goran Herman, mag. ing. agr.
Prof. dr. sc. Manda Antunović

Sveučilište Josipa Juraja Strossmayera u Osijeku,
Fakultet agrobiotehničkih znanosti Osijek,
Vladimira Preloga 1, 31000 Osijek, Hrvatska

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