Effects of Genotype, Seed Age and KNO_3 on Germination of Radicchio (*Cichorium intybus* L.) and Endive (*Cichorium endivia* L.) Seed

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Summary

After purchasing seed it often happens that all the seed is not used during one season, but they are stored for a couple of years. The aim of this study was to examine whether germination of radicchio and endive is still high enough after longer storage. We also wanted to define seed vigor in relation to the year of production and variety, and whether pretreatment with KNO_3 could improve germination of older seed of radicchio and endive.

The seed of three varieties of radicchio ('Pallarossa', 'Verona' and 'Pandizucchero') and three varieties of endive ('Dječja glava', 'Pankalierka' and 'Escariol žuta'), from five seasons of certification (06/07, 07/08 08/09, 09/10 and 10/11) was tested.

The following seed traits were analyzed: 1000 seed weight, moisture, germination rate, standard germination test, seed electrical conductivity, and the amount of absorbed water per gram of seed for 24 hours. Seed was germinated in two soaking treatments: in tap water with cooling pre-treatment, and KNO_3. Based on the results, it was concluded that treatment with KNO_3 significantly increases the standard germination of radicchio, as compared with water, and does not improve the germination of endive seed, but on the contrary, it has a negative effect. In both plant species, conductivity does not increase with seed age, because the oldest seed did not show the highest EC. In order to fully assess the impact of aging on seed vigor in tested plant species it is necessary to conduct further research.

Key words

radicchio, endive, genotype, seed age, seed vigor

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Introduction

During winter time radicchio and endive are for many people the favorite leafy vegetables. Radicchio has a positive effect on the heart, kidney and bladder and has a distinctive bitter taste due to the presence of intibin (Fabek, 2011). Endive contains insulin, which is very suitable for diabetics and also acts as a diuretic (Matotan, 2004). The leaves of both species are high in protein, vitamins A, B and C and minerals, particularly potassium, calcium, phosphorus and magnesium (Parađiković, 2009). In Croatia, vegetables are grown mostly in gardens and used for personal needs (Matotan, 1994). After purchasing seed it is often the case that all of the seed is not used during one season, but they are stored with intent to be used again next year. It is sometimes the case that there is enough seed for the next two, three or more years. However, the question is how long the seed can be stored without losing viability and whether the seed will produce a normal and healthy plant (Jozinović, 2011). Next to the genetic quality of seed, seed viability is the most important aspect of quality (Kolak, 1994). The author points out that in practice most of the quality control of seed focuses on the calculation of germination, assuming that the seed with high percentage of germination have also sufficient germination energy.

The aim of this study was to examine whether germination of radicchio and endive is still high enough after longer storage, and whether KNO_3 treatment is recommendable for improving the germination of seed with lower vigor. We also wanted to define seed with lower vigor in relation to the season of production and variety, and whether pretreatment with KNO_3 could improve germination of older seed of radicchio and endive.

Material and methods

Analysis were carried out at the Seed testing laboratory of the Institute for Seed and Seedlings, Croatian Centre for Agriculture, Food and Rural Affairs, using seed of three varieties of radicchio ('Pallarossa', 'Verona' and 'Pandizucchero') and three varieties of endive ('Dječja glava', 'Pankalierka' and 'Escariol žuta'). Seed was used from five seasons of certification 06/07, 07/08 08/09, 09/10 and 10/11. Each laboratory sample was tested as follows: 1000 seed weight, determination of moisture content, germination energy, standard germination test, electrical conductivity of seed and the amount of water per gram of seed adsorbed in 24 hours of soaking. For the seed germination test, two soaking treatments were applied: tap water with cooling pre-treatment, and KNO_3 solution. 1000 seed weight was determined instrumentally, running fractions of pure seed through a seed counter (Contador PfeufferSeed Counter).

Moisture content of seed was determined using an oven method, according to ISTA Rules (ISTA, 2011) and Ordinance on methods for seed sampling and seed quality testing (OG 99/08). Seed samples were weighed between 4-5 g, and dried in an oven at a temperature of 130-133°C during one hour. This parameter was determined in two replications, and the result is shown as the average of repetitions.

Germination energy and standard germination test were determined using the paper towels on the alternate temperature 20-30°C for period of 14 days, according to ISTA rules (ISTA, 2011) and the Ordinance on methods for seed sampling and seed quality testing (OG 99/08). In the first test the paper towels were soaked with tap water in a quantity of 80 mL per sample, and in the second test paper towels are soaked with 0.2% solution of KNO_3, also using the amount of 80 mL per sample. On the prepared paper towels 100 seeds were sown in four repetitions. Containers with samples from the first test were placed in the cooling chamber at 7°C for five days, and then moved into the cooling chamber at 20-30°C. Containers with samples from the second test were immediately placed at the temperature of 20-30°C. Five days after germination under controlled conditions of 20-30°C, germination energy was determined. Samples were then returned to the chamber, and after a total of 14 days normal and abnormal seedlings and dead seed were recorded.

To determine the electric conductivity of seed, from all seed samples 1 g of seed was taken. Then the seed was placed in Falcon tubes and 50 ml of demineralized water was added. Tubes with samples were kept in a thermostat at 20°C for 24 h. The solution above the imbibed seed was used for measuring conductivity using a standard conductometer. Prior to each use, a conductometer was calibrated using 0.1% and 0.01% KCl solution. The final result is expressed in $\mu Scm^{-1}g^{-1}$ of seed.

For determining the amount of absorbed water per gram of seed for 24 hours of soaking, the same seed was used as for testing conductivity. After the conductivity test, seed was dried on paper towels and weighed. The result of this measurement was the difference between initial mass and mass after 24-hour of seed imbibition.

The results of all tests carried out in four replicates were analyzed using analysis of variance through the SAS software package, using the F test and LSD test for testing the significance of differences between treatments and their impact on measured parameters.

Results

Indicators of seed germination and seed vigor of radicchio

Variety Pallarosa had the highest germination energy and variety Pandizucchero the lowest (Table 1a). The oldest seed (06/07) had the lowest energy of germination, and the highest had seed produced in the season 09/10. The seed germination energy of radicchio was significantly affected by variety, season and their interaction, and interactions of season and treatment, as well as the interaction of all three factors (Table 1b).

The final germination (SG) was significantly influenced ($P \leq 0.01$) by variety, season, their interaction and interaction of all three factors. The treatment of seed was significant at level of $P \leq 0.05$. Treatment with KNO_3 significantly increased ($P \leq 0.05$) the standard germination of radicchio, as compared to the control (1.23%).

The number of abnormal seedlings was significantly ($P \leq 0.01$) dependent on the variety, season and their interaction, as well as on the interaction between season and treatment. The most abnormal seedlings were found in seed of variety Pandizucchero from season 06/07.

Table 1a. The significance of differences between the indicators of germination of radicchio according to LSD test

		GE %	SG %	Abnormal seedlings	Dead seed
Season	06/07	70.50d	72.50e	10.38a	17.13a
	07/08	80.13b	81.17c	4.75c	14.08bc
	08/09	75.83c	78.04d	6.00b	16.04ab
	09/10	88.63a	89.46a	3.04d	7.50d
	10/11	81.67b	83.50b	3.46d	13.04c
Variety	Pallarossa	82.25a	84.70a	4.55b	10.75c
	Verona	80.50b	81.58b	4.33b	14.15b
	Pandizucchero	75.30c	76.53c	7.70a	15.78a
Seed treatment	Water	79.65a	80.32b	5.62a	14.10a
	KNO_3	79.05a	81.55a	5.43a	13.02a

GE-germination energy; SG-standard germination; a,b,c,d,e –means marked by the same letter are not significantly different according to the LSD test; $P \leq 0.05$

Table 1b. Significance (P) of influence of variety, season, seed treatment and the interaction according to the F test on the parameters of germination of radicchio

	GE	SG	Abnormal seedlings	Dead seed
Variety (A)	<0.0001	<0.0001	<0.0001	<0.0001
Season (B)	<0.0001	<0.0001	<0.0001	<0.0001
Seed treatment (C)	0.3000	0.0349	0.6204	0.0999
A x B	<0.0001	<0.0001	<0.0001	<0.0001
A x C	0.7277	0.2680	0.1660	0.8936
B x C	0.0227	0.1169	0.0181	0.5460
A x B x C	0.0009	0.0023	0.0826	0.0277

GE-germination energy; SG-standard germination

Table 2a. The significance of differences in moisture content, 1000 seed weight, electrical conductivity of seed (EC) and the quantity of absorbed water in radicchio seed during imbibition according to LSD test

		Seed moisture %	1000 seed weight (g)	EC ($\mu Scm^{-1}g^{-1}$)	Absorbed water (g/g seed)
Season	06/07	6.60a	1.53a	146.93d	0.55b
	07/08	6.30e	1.44d	168.40b	0.51c
	08/09	6.40d	1.47c	182.63a	0.58a
	09/10	6.43c	1.52b	142.69e	0.52c
	10/11	6.50b	1.52b	159.81c	0.55b
Variety	Pallarossa	6.64a	1.43c	160.19a	0.53b
	Verona	6.40b	1.49b	159.64a	0.54b
	Pandizucchero	6.30c	1.56a	160.45a	0.56a

a,b,c,d,e – means marked by the same letter are not significanlty different according to the LSD test; $P \leq 0.05$

Table 2b. Significance (P) of influence of variety, season, seed treatment and the interaction according to the F test on the moisture content, 1000 seed weight, electrical conductivity (EC) and amount of absorbed water in radicchio seed

	Moisture	1000 seed weight	EC	Absorbed water
Variety (A)	<0.0001	<0.0001	0.6710	<0.0001
Season (B)	<0.0001	<0.0001	<0.0001	<0.0001
A x B	<0.0001	<0.0001	<0.0001	<0.0001

In the case of a dead seed, besides the very significant impact ($P \leq 0.01$) of variety, season and their interaction, there was a significant interaction ($P \leq 0.05$) between all three factors as well. As with other indicators of germination, dead seed were mostly found in samples from the season 06/07 and the least in seed from the season 09/10. Considering variety, the lowest amount of dead seed had a variety Pallarossa.

Regarding the moisture of radicchio seed, significant effects ($P \leq 0.05$) of variety and season were established, as well as their interaction. The highest moisture content of seed had the variety Pallarosa, and the lowest Pandizuccero. Seed from the season 06/07 had the highest moisture content and seed from 07/08 the lowest.

In the case of 1000 seed weight, F test showed the same results as with moisture. The lowest seed weight had the variety Pallarosa, and the highest variety Pandizucchero. Seed produced in 07/08 had the lowest weight and seed produced 06/07 the highest. 1000 seed weight was not significantly different for seed produced in seasons 09/10 and 10/11. Season of production of radicchio seed had a very significant impact ($P \leq 0.01$) on the electrical conductivity of seed, as well as the interaction of variety x season. Variety was not a significant factor in the seed electrical conductivity. The seed with the highest conductivity was from 08/09, and the lowest was from seed produced in the season 09/10.

The amount of absorbed water per gram of seed for 24 h was highly significantly influenced ($P \leq 0.01$) by both factors and their interaction. The highest water uptake was found in variety Pandizuccero while 'Verona' and 'Pallarossa' were not significantly different by the quantity of absorbed water. The highest water uptake was found in seed produced in 08/09. There was significantly lower water uptake from seed produced in the seasons 10/11 and 06/07, and the lowest was from seed produced in seasons 09/10 and 07/08.

Indicators of seed germination and seed vigor of endive

The highest germination energy was in endive seed from 09/10 and the lowest from 07/08 (Table 3a). Germination energy of seed from 10/11 did not differ in relation to seed from 09/10 and 06 /07. The highest germination energy was from the variety Pankalierka and the lowest from the variety Dječja glava. Seed treatment with KNO_3 significantly ($P \leq 0.05$) reduced seed germination energy of endive in relation to seed germinated in water.

According to the F test, the germination energy was very significantly influenced ($P \leq 0.01$) by age, variety and treatment, by the interaction variety x season, season x treatment, and variety x treatment as well as by the interaction of all three factors (Table 3b).

Table 3a. The significance of differences between the indicators of germination of endive according to LSD test

		GE %	SG %	Abnormal seedlings	Dead seed
Season	06/07	82.38b	83.29b	2.13b	14.58b
	07/08	71.71d	73.25d	2.71ab	24.04a
	08/09	76.88c	80.00c	2.83ab	17.17b
	09/10	86.13a	87.25a	2.67ab	10.08c
	10/11	84.54ab	85.79ab	2.88a	11.33c
Variety	Dječja glava	69.65c	72.30c	2.93a	24.78a
	Pankalierka	88.73b	89.13a	2.78ab	8.10c
	Escariol žuta	82.60b	84.33b	2.23b	13.45b
Seed treatment	Water	86.87a	87.18a	2.48a	10.33b
	KNO_3	73.78b	76.65b	2.80a	20.55a

GE-germination energy; SG-standard germination; a,b,c,d,e –means marked by the same letter are not significantly different according to the LSD test; P≤0.05

Table 3b. Significance (P) of influence of variety, season, seed treatment and the interaction according to the F test on the parameters of germination of endive

	GE	SG	Abnormal seedlings	Dead seed
Variety (A)	<0.0001	<0.0001	0.0407	<0.0001
Season (B)	<0.0001	<0.0001	0.2420	<0.0001
Seed treatment (C)	<0.0001	<0.0001	0.1670	<0.0001
A x B	<0.0001	<0.0001	0.5186	<0.0001
A x C	<0.0001	<0.0001	0.6570	<0.0001
B x C	<0.0001	<0.0001	0.4999	<0.0001
A x B x C	<0.0001	<0.0001	0.0495	<0.0001

GE-germination energy; SG-standard germination

Table 4a. The significance of differences in moisture content, 1000 seed weight, electrical conductivity of seed (EC) and the quantity of absorbed water in endive seed during imbibition according to LSD test

		Seed moisture %	1000 seed weight (g)	EC ($\mu Scm^{-1}g^{-1}$)	Absorbed water (g/g seed)
Season	06/07	6.47b	1.62a	152.78d	0.60b
	07/08	6.23d	1.39d	210.47b	0.60b
	08/09	6.17e	1.31e	259.33a	0.64a
	09/10	6.33c	1.53b	196.71c	0.59b
	10/11	6.50a	1.46c	205.51b	0.57c
Variety	Dječja glava	6.44b	1.55a	174.30c	0.59b
	Pankalierka	6.10c	1.34c	233.31a	0.60a
	Escariol žuta	6.48a	1.50b	207.28b	0.61a

a,b,c,d,e –means marked by the same letter are not significantly different according to the LSD test; *P*≤0.05

Table 4b. Significance (P) of influence of: variety, season, seed treatment and the interaction according to the F test on: the moisture content, 1000 seed weight, electrical conductivity (EC) and amount of absorbed water of endive seed

	Moisture	1000 seed weight	EC	Absorbed water
Variety (A)	<0.0001	<0.0001	<0.0001	0.0003
Season (B)	<0.0001	<0.0001	<0.0001	<0.0001
A x B	<0.0001	<0.0001	<0.0001	<0.0001

Standard germination was also affected by all tested factors and their interactions. According to the LSD test the differences in standard germination, depending on the seed age, were the same for germination energy as well as differences among varieties (Table 3a). Effect of seed treatment with KNO_3 was similar as for germination energy (Table 3a).

The number of abnormal seedlings was significantly influenced ($P \leq 0.05$) only by the variety and interaction of all three factors. Depending on the season of seed production, a significant ($P \leq 0.05$) difference in the number of abnormal seedlings was determined in seed produced in 10/11 and 06/07 (Table 3a). Significant differences ($P \leq 0.05$) in the number of abnormal seedlings were also found between variety Dječja glava, which had the highest, and variety Escariol žuta, in which there was the lowest number of abnormal seedlings. Number of abnormal seedlings was higher in seed treated with KNO_3, but the difference compared to control (water) was not significant.

Percentage of dead seed significantly ($P \leq 0.05$) depended on all the investigated factors and their interactions (Table 3b). The lowest number of dead seed was found in seed from 09/10 and 10/11 and the highest in seed from 07/08 (Table 3a). Variety Dječja glava had the highest amount of dead seed and 'Pankalierka' the lowest. Significantly ($P \leq 0.05$) more dead seed was found in treatment with KNO_3 as compared to control (10.22%).

Endive seed produced in season 10/11 had the highest moisture content and seed from 08/09 the lowest (Table 4a). The highest 1000 seed weight and also the lowest EC had seed from 06/07, while the seed from 08/09 had the lowest 1000 seed weight and the highest EC, and absorbed significantly more water during imbibition.

Variety, seed age and their interactions had a highly significant ($P \leq 0.01$) influence on % of moisture, 1000 seed weight and quantity of absorbed water in endive seed during imbibition (Table 4b).

Discussion

The aim of standard germination test is to estimate potential of seed germination for a seed lot. On this basis it is possible to compare the quality of two seed lots and also to estimate the required seed quantity for sowing (ISTA, 1999). The standard germination test is usually used to assess the seed quality and it provides results which can predict seed germination in field when conditions are good (Kolasinska at al., 2000). Only high quality seed is capable of developing strong plants in the field and to give crops with full yield potential of a particular variety. Damaged seed commonly shows reduced germination in the field, which is especially expressed in adverse conditions for germination (Haramija, 2007).

Yousif (2010) was studying the effect of seed age, size and moisture content on seed quality of sorghum (*Sorghum bicolor* L. Moench) and came to the conclusion that a new, large seeds and seeds with moderate moisture content always show characteristic of higher vigor as compared to the old and the small seed with either higher or lower moisture content. Ujević (1988) argues that the germination energy and germination of seed are generally higher in seed with higher 1000 seed weight, and at the same seed mass is the result of biological activity, but also external factors, such as nutrition, water, temperature and others.

In the case of the endive in this study, 1000 seed weight ranged from 1.116 g in variety Pankalierka from 08/09, to 1.780 g in variety Dječja glava from 06/07. These values are slightly less than 2 g as stated by Parađiković (2009). In case of radicchio, 1000 seed weight was between 1.399 g in variety Pallarossa from 06/07 and 1.736 g for variety Pandizucchero of the same season, and the value of 1000 seed was slightly more than 1 - 1.5 g as claimed by Parađiković (2009). Values for seed moisture for chicory ranged from 6.1% in variety Pandizucchero from 06/07 to 6.8% in variety Pallarossa of the same season, and for the endive from 5.6% in variety Pankalierka from 08/09 to 6.8% in the variety Dječja glava from 06/07 and Escariol žuta from 10/11.

Pimpini et al. (2002) stated that the quality of radicchio seed is dependent on genetic traits and methods of seed selection, but also depends on the method of harvesting, seed manipulation during processing as well as storage conditions. Corbineau and Come (1990) conducted a study on endive that showed that there were significant differences in germination, depending on the color of seed, and that during storage seed becomes more sensitive to low temperature of germination.

The standard germination test and germination energy as the main indicators of physiological seed quality are often limited by the agrochemical properties of the substrates used for seed germination. Firstly the pH and EC of the substrate are what directly affect the number of normal seedlings, and the normal development of the root system, hypocotyl, and seedlings mass (Palenkić et al., 2011). The same author has set up an experiment in which the goal was to determine the effect of agrochemical properties of three different substrates (Green vital, acidic substrate and Potgrond H) on the germination characteristics of radicchio. The authors concluded that the proper choice of substrate for cultivation of radicchio seedlings plays an important role in seed germination, especially in developing roots.

Bekendan et al. (1987) studied the germination behavior of 17 lots of different varieties of endive in different combinations of constant and alternating temperatures, in light and darkness. They came to the conclusion that the moistening of substrate with KNO_3 gave better results in all tested combinations. Tzortzakis (2009) found that the percentage of germination of endive and radicchio has increased when pre-treatment with KNO_3 or GA_3. In this study, treatment with KNO_3 significantly increased the standard germination of radicchio, as compared to the control (1.23%). According to the Ordinance on methods for seed sampling and seed quality testing (OG 99/08), KNO_3 is recommended for breaking of seed dormancy in endive. However in this research, treatment with KNO_3 in applied concentration did not improve seed germination of endive, on the contrary it had a negative effect, because in this variant of the experiment there was significantly more of dead seed observed, as well as significantly lower EC and SG recorded in relation to the control, for all varieties and seed age.

Vieira et al. (2004) considered the test of seed electric conductivity as fast and convenient procedure that allows obtaining objective information, it can be easily performed in most laboratories for the seed analysis, while does not require expensive equipment or specialized staff. Seed of all agricultural plant species (rice, soybean, maize, wheat, sunflower, peas, lupines, cotton, alfalfa, barley, tomato, onion, sugar beet, paprika, bean, seed of flower and forest plant species) can be tested using the test of seed electric conductivity (ISTA, 1995, quoted by Salinas et al., 2010). The authors considered EC as good indicator of physiological quality of seed, which is the result of interaction of environmental conditions and genotype.

Powell and Matthews (1978) concluded that the longer storage reduces seed vigor due to leakage of electrolyte from seed. Membrane damage due to deterioration of seed, which leads to poor selectivity and increased leaching of substances from the seed into the environment, is one of the main causes of reduction in physiological quality of seed. As a result, the electrical conductivity test is considered as an important indicator of the quality of seed vigor, as it indirectly determines the degree of degradation of the membrane by measuring the amount of electrolyte in solution in which the seed is submerged (Panobianco et al., 2007). Despite the statistically significant effects of season of seed production, in both plant species, in this study it has not been determined an increase of conductivity with the age of seed, because the oldest seed did not show the largest EC. It can be assumed that the production conditions essential for the initial quality of seed produced in 08/09, contributed to the largest value of EC of seed in this study. However, these seeds in both plant species did not show the lowest germination.

In this study, the seed of endive variety Dječja glava had the highest 1000 seed weight on average, the lowest EC and absorbed the least amount of water. Contrary to the expectations, this variety had the lowest germination, the most abnormal seedlings and dead seed. Pankalierka variety had the highest EC, the lowest 1000 seed weight, the highest germination and lowest amount of dead seed. In order to fully assess the impact of seed aging on EC, and more precisely determine the applicability of this indicator in the evaluation of seed quality of radicchio and endive, it would be necessary to monitor changes of EC during storage of several years, but using seed produced under identical agroecological conditions.

Conclusion

Based on the results of the conducted research with the seed of radicchio and endive, we can conclude the following:

1. Treatment with KNO_3 significantly increases ($P \leq 0.05$) the standard germination of radicchio, as compared to water and cooling pre-treatment.
2. Treatment with KNO_3 does not improve the germination of endive seed, but had a negative effect. That was demonstrated by significantly higher level of dead seed as well significantly lower EC and SG in comparison with control and for all varieties and age of seed in average.

3. In both plant species, conductivity does not increase with seed age, because the oldest seed did not show the largest EC.
4. The seed of endive, variety Dječja glava, on average had the highest 1000 seed weight, the lowest EC and absorbed the least water. This variety had the lowest germination, the largest amount of abnormal seedlings and dead seed. Variety Pankalierka had the highest EC, the lowest 1000 seed weight, the highest germination and lowest number of a dead seed.
5. In order to fully assess the impact of aging on seed EC, and to more precisely determine the applicability of this indicator in assessing the quality of endive and radicchio seed, it is necessary to conduct further research.

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