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COUNTER AGRONOMIC SYSTEMS AND MAIZE SEED VIGOUR

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SUMMARY

The quality and provenance of seed are of exceptional importance for the production and yield of cultivated plants. There are numerous tests for determination of seed vigour. The seed accelerated ageing test is one of the most important tests that provide determination of the degree of germination preservation and the seed longevity in storages. The aim of this study was to observe the effect of different production methods – organic and conventional – on seed vigour of maize (variety Rumenka), using the seed accelerated ageing test. Maize seeds were exposed to double stress conditions (temperature of 45 °C, air humidity of 100%) for 72h. Maize seed germination was determined by the standard germination test and was expressed as a percentage. The results of vigour of maize seeds organically produced indicated that the application of the seed accelerated ageing test increased the first count (78.5%) in relation to the standard germination test (70.75%), and a significantly higher percentage of nongerminated seeds (10.25%) were recorded. Furthermore, the radicle length decreased (120.75 mm), while the length of the seedling above-ground part (117.13 mm) and fresh weight (4.56 g) increased. In maize seeds conventionally produced, the first count and germination were higher (41.5% and 46.25%, respectively) after the application of the seed accelerated ageing test, while the percentage of nongerminated seeds were higher (38.75%). In addition, the length (105.88 mm) and fresh weight (4.43 g) of the seedling above-ground part decreased, while the values of the radicle length (137.5 mm), fresh weight (2.39 mm) and dry weight (0.28 mm) as well as dry weight of above-ground parts of seedlings (0.31 g) were higher than after the application of the standard laboratory method (127.88 mm, 1.89 g, 0.17 mm and 0.31 g, respectively).

Key words:
vigour,
organic production,
conventional production,
maize seed

INTRODUCTION

Maize (*Zea mays* L., Poaceae, Maydeae tribe) is evolutionarily the most developed and most domesticated plant species in the world (Babić et al., 2012). Globally, maize, wheat and rice account for 87% of the total cereal production, while on the other hand they supply significant 43% of total calories in food (Radosavljević, 2010). The essential economic importance of maize results from its diverse utilisation in human and animal nutrition, as well as in industrial processing – the application of technological procedures to maize results in over 1500 various industrial and food products and commodities, such as starch, sweeteners, oil, beverages, glue, industrial alcohol, ethanol, etc. (Ranum et al., 2014). Such a wide range of maize-based products is obtained owing to its chemical composition, which encompasses approximately 71% starch, 10% proteins, 4.7% oil and 2.5% cellulose, giving 365 Kcal/100 g

(Ranum et al., 2014). In Serbia, maize is the most widely distributed field plant species, necessary to provide for the domestic needs, and it is a strategic product of the country intended for export. Maize was grown on the area of 906,753 ha and 962,083 ha in 2017 and 2019, respectively, and the total production amounted to 7,344,542 tonnes or 7.6 t ha^{-1} (Statistical Office of the Republic of Serbia, 2021). Seed vigour is its most important biological trait, which in the broadest sense includes several interrelated traits, such as germination, vitality, seed strength, energy of growing, viability, power of growing, capacity of being viable, germination capacity (Lekić, 2009). Seed vigour shows not only the percentage of viable seeds in a particular sample, but also the seed ability to successfully form normal seedlings under unfavourable field conditions (ISTA Rules, 2016). It is known that genetic factors strongly affect vigour, particularly the seed chemical composition, hardseededness and resistance to pathogens (AOSA, 2002). As the entire seed production is based on the maintenance and improvement of seed vigour, in order to ensure high and stable yields and high-quality seeds, it is necessary to provide rapid and uniform emergence of plants under field conditions (Jovičić, 2014). Seed vigour tests are used in the selection of seeds for sowing because, in the relation to results of the standard germination test, they are the best parameters of the behaviour that seeds will have during germination and emergence under field conditions. They are also used in plant species selection and breeding and in the assessment of seed store capacity (Marcos-Filho, 2015; Finch-Savage & Bassel, 2016).

The aim of this study was to observe the effect of organic and conventional production on seed vigour of maize using the seed accelerated ageing test.

MATERIAL AND METHODS

The tested seeds of organically and conventionally produced maize variety ZP Rumenka were sown on the 30-are experimental plot of the Maize Research Institute, Zemun Polje in 2017. Prior to primary tillage of calcareous chernozem, NPK fertilisation was performed at the 120:80:60 kg ha^{-1} rate for the conventional production and 500 kg $ha-^{1}$ of organic fertiliser DIX 10N for the organic production. Herbicides based on nicosufuron and mesotrione (for the conventional production) and mechanical weed suppression by manual hoeing and inter-row cultivation (for the organic production) were used as a part of cropping measures.

Maize seed vigour was tested by the seed accelerated ageing test. Seeds were exposed to double stress conditions of high temperature (45 °C) and high air relative humidity (100%) for 72 hours. Afterwards, seeds were sown in sand to determine their germination. Seed germination was determined by the standard germination test with 100 seeds in four replications and was expressed as a percentage (ISTA Rules, 2016). Four days later, the first count was determined, while germination was recorded on the seventh day (ISTA Rules, 2016).

The percentage of seed germination was determined by the evaluation of a typical seedling. A normal or typical seedling is a seedling with well developed primary root, a straight and undamaged shoot, healthy cotyledons or an undamaged coleoptile, as well as rudiments of the first true leaves. At the end of the incubation period, the following growth parameters were determined: the length of the above-ground parts and roots (mm), fresh and dry weight of the above-ground parts and roots (g). The length of the above-ground parts and roots was determined by measuring the mean value of 10 seedlings of each replication using a ruler. Furthermore, the fresh weight of the above-ground part and the root was determined by measuring the mean value of 10 seedlings of each replication and then they were dried in the thermostat at the temperature of 80 °C for 24 h and their dry weight was measured.

To examine the influence of the production methods and tests, as well as their interactions on the tested characteristics, a two-factor analysis of variance with a balanced design was used. For post-hoc comparisons, the least significant difference test (LSD) was used. A significance level of 5% was used in all tests, software Statistica, version 12.0 (StatSoft Inc., USA). The correlation analysis between the observed parameters in the accelerated ageing test and standard germination test was performed by calculating the Pearson's correlation coefficient (r), ($p < 0.05$).

RESULTS AND DISCUSSION

To observe effects of the production methods on maize seed vigour with the use of two types of tests – standard germination test and accelerated ageing test – on the monitored parameters, a two-factor analysis of variance according to complete randomised design was used. The *Tuckey*'s test was used for subsequent pairwise comparisons (significance level 5%). The results are presented in Table 1.

Table 1. Mean values of investigated traits

Parameter	Production Method				Two way ANOVA results		
	Organic Test		Conventional Test		F rations		
	Germination standard test	Accelerated ageing test	Germination standard test	Accelerated ageing test	Pr. method	Test	Pr. method×Test
First count (%)	70.75±8.06 a*A*	78.50±5.57 b*A*	34.25±6.95 a*B*	41.50±6.14 b*B*	118.84**	4.95*	0.01^{ns}
Germination (%)	88.25±3.95 a*A*	84.25±2.75 a*A*	43.25±2.99 a*B*	46.25±6.18 a*B*	391.79**	0.06^{ns}	2.79^{ns}
Off-type seedlings (%)	5.75±2.87 a*A*	5.5±1.73 a*A*	25.00±2.16 *aB*	15.00±2.16 *bB*	160.63**	20.42**	18.47**
Non-germinated seeds (%)	6.00±1,.83 *aA*	10.25±2.5 *b*A	31.75±4.35 a*B*	38.75±6.7 b*B*	160.35**	6.9*	0.41^{ns}
Length of seedling above-ground part (mm)	107.75±4.27 *a*A	117.13±4.23 *bA*	111.38±11.24 aA	105.88±4.01 a*B*	1.3^{ns}	0.34^{ns}	4.96*
Root length (mm)	132.63±22.44 aA	120.75±21.71 aA	127.88±12.99 aA	137.5±7.76 aA	0.48^{ns}	0.02^{ns}	1.54^{ns}
Fresh weight of seedling above-ground part (g)	3.88±0.54 aA	4.56±0.51 aA	4.61±0.46 aA	4.43±0.17 aA	1.81^{ns}	1.25^{ns}	3.78^{ns}
Root fresh weight (g)	1.99±0.29 aA	2.04±0.52 aA	1.89±0.31 aA	2.39±0.31 aA	0.43^{ns}	2.24^{ns}	1.49^{ns}
Dry weight of seedling above-ground part (g)	0.33±0.06 aA	0.36±0.03 a*A*	0.27±0.05 a*B*	0.31±0.02 a*B*	7.35*	2.87^{ns}	0.001^{ns}
Root dry weight (g)	0.23±0.03 a*A*	0.26±0.05 bA	0.17±0.03 a*A*	0.28±0.08 *b*A	0.72^{ns}	7.68*	2.4^{ns}

Legend: lowercase letters (a, b) - indicate the statistical significance between the standard germination test and the accelerated ageing test (n=4, mean ± SD); capital letters (A, B) - indicate the statistical significance between organic and conventional production methods (n=4, mean ± SD); * - significant at 5 probability level; ** - significant at 1 probability level; ns – non significant

The results of this study indicate that the application of the accelerated ageing test in organically produced seeds led to increase in the first account (78.50%) compared to the standard germination test (70.75%). Germination was lower (84.25%) than in the standard germination test (88.25%), but even this difference was not statistically significant. However, a statistically significant difference was recorded after the accelerated ageing test in a higher percentage of non-germinated seeds (10.25%). Germination of samples of organically produced maize seeds, both in the standard germination test and the accelerated ageing test, was above the limit value (80%) between highly vigorous and medium vigorous seed lots. When testing vigour of conventionally produced maize seeds, it was determined that the first count (41.50%), germination (46.25%; but without statistical significance) and the percentage of non-germinated seeds (38.75%) were higher after the seed accelerated ageing test. In contrast to organically produced maize seeds in which stress conditions during testing led to the reduced seed germination (84.00%) compared to the standard germination test (88.25%), higher germination was recorded in conventionally produced seeds in the accelerated ageing test (46.25%). However, both values of seed germination were below 80%, which was considered low vigorous seed lots. From the aspect of differences in the standard germination test between organically and conventionally produced seeds, a statistical significance was recorded in the first count (two-fold higher in organically produced seeds – 70.75%) and germination (88.25% – two-fold higher in organically produced seeds). From the aspect of differences in the seed accelerated ageing test between organically and conventionally produced seeds, a statistical significance was recorded in the first count (almost two-fold higher in organically produced seeds – 78.50%) and germination (84.25% – higher in organically produced seeds). High relative humidity during the accelerated ageing test caused high-vigour seeds to germinate faster, but the high temperature still affected some biochemical compounds, which resulted in a lower germination percentage after this test. In contrast, Kavitha et al. (2017) found that accelerated seed ageing affects the reduction of germination, shoot and root length, dry matter production vigour index. They also found that these conditions reduce the activity of ἀ-amylase, catalase and peroxidase. According to the results obtained by Radić & Milošević (2004), after the application of the standard germination test, maize seed germination ranged from 88% to 96% and from 91% to 95%, in the first and the second year of investigation, respectively. These values are significantly higher than germination values obtained in organically and conventionally produced seeds in our study. By testing seed quality of maize inbred lines of different maturity groups and the genetic background, Milivojević (2016) came to the conclusion that the average values of the total germination in the accelerated ageing test were 87.87% and 63.07% for the seeds produced in 2014 and 2011, respectively. We can conclude that the germination percentage of 84.25% in organically produced seeds in our study is similar to the germination value determined in 2014, and significantly higher than values recorded in 2011. However, the germination in conventionally produced seeds in our study (46.25%) is drastically lower than the value obtained by Milivojević (2016) in both years of investigation. Maize seed vigour affects crop development, whereby low-vigour seeds inhibit crop development during the growing period (Vaz Mondo et al., 2015), which is in accordance with the report made by Vanzolini & Carvalho (2002). In order to establish the effects of seed vigour on the crop cultivation cycle, Vaz Mondo et al. (2015) sowed four maize seed lots of various vigour and evaluated the daily growth for each phenophase. Their results indicate that all lots of tested seeds had similar germination (95%-99%), which points out to high quality in terms of seed viability. Seedling emergence varied from 67% to 98%, and accelerated ageing from 5% to 91%. The authors concluded that seed vigour affected the initial plant phenophases up to the 12-leaf stage. According to Santipracha et al. (1996), the application of the accelerated ageing test in three-way cross and double cross hybrids resulted in similar values of germination as when the standard germination test was applied, while there was a statistically significant difference in seed vigour in single cross hybrids between these two tests. Hussein et al. (2012) showed that accelerated ageing resulted in the progressive loss of seed viability and vigour: the initial germination of maize seeds was 99%, but it then progressively declined until it reached no germination after 14 days of ageing. Hussein et al. (2012) stated that the reduction in germination might be due to the degradation of the mitochondrial membrane leading to the reduction in energy supply necessary for germination. Although it is still not entirely clear which compound has the greatest effect on seed deterioration and on high vigour, storage components such as proteins, lipids and carbohydrates are known to participate in the germination process by providing carbon and nitrogen and are directly associated with seed quality (Wu et al., 2017). Andrade et al. (2019) found that seed strength is associated with effective reserve utilisation and mobilisation. The reduction in seed viability, germination rate and vigour after seed ageing is related to biochemical changes such as decrease in soluble proteins and the sugar content (Rastegar et al., 2011). Since seed vigour is a complex trait that is formed during different growing stages of the parent plant and is under great influence of the prevailing environment (Finch-Savage & Bassel, 2016), the obtained differences between different production methods are expected.

The percentage of off-type seedlings was not statistically significant between the standard germination test and the accelerated ageing test (5.75% vs. 5.50%). There was a significant difference in off-type seedlings in conventionally produced maize seeds (25% vs. 15%) between the two tests (standard germination test and seed accelerated ageing test). The latter test showed lower values of off-type seedlings than the former one. From the aspect of differences in the standard germination test between organically and conventionally produced seeds, a statistical significance was recorded in off-type seedlings (four-fold higher percentage in the conventionally produced seeds – 25.00%) and non-germinated seeds (five-fold higher percentage in the conventionally produced seeds – 31.75%). From the aspect of differences in the seed accelerated ageing test between organically and conventionally produced seeds, a statistical significance was determined for off-type seedlings (almost three-fold higher percentage in the conventionally produced seeds – 15.00%) and non-germinated seeds (three-fold higher percentage in the conventionally produced seeds – 38.75%). Vujošević et al. (2018) tested five seed lots of the maize hybrid NS 640 with different proportions of off-type seedlings to observe parameters of the germination rate, number of plants in the reproductive phase and yields. The percentage of off-type seedlings amounted to 1%, 3%, 38%, 57% and 64% in Lot5, Lot4, Lot3, Lot2 and Lot1, respectively. The percentage of off-type seedlings in organically produced seeds determined in our study (5.75%; 5.50%) was the closest to the Lot4 with 3% of off-type seedlings. Results gained by Vujošević et al. (2018) showed that, under favourable conditions, a large number of off-type seedlings emerged, reached the reproductive phase and participated in the formation of yields, while the emergence of seeds from lots with a high number of off-type seedlings was delayed. According to Milivojević (2016), in the first evaluation, four days after germination, the percentage of normal seedlings amounted to 79.67% and 31.87% for the seeds produced in 2014 and 2011, respectively.

The accelerated ageing test applied to organically produced seeds showed that double stress conditions led to decrease in the radicle length, increase in the fresh and dry weight of both roots and above-ground parts, but the observed differences were not statistically significant. However, the accelerated ageing test led to significant increase in the above-ground part length (117.13 mm). There was a significant difference in root dry weights in conventionally produced seeds (0.17 g vs. 0.28 g) between the two tests (standard germination test and seed accelerated ageing test). When testing vigour of conventionally produced maize seeds, a statistically significant difference was established in a greater root dry weight (0.28 g) after the accelerated ageing test, while differences in remaining parameters, such as reduction in the length (105.88 mm) and the fresh weight of the seedling above-ground parts (4.43 g), and increase in the length (137.5 mm) and fresh weight (2.39 g) of roots and dry weight of seedling above-ground parts (0.31 g) were not statistically significant. From the aspect of differences in the standard germination test between organically and conventionally produced seeds, a statistical significance was recorded in the dry weight of seedling above-ground parts (greater in organically produced seeds – 0.33 g). From the aspect of differences in the accelerated ageing test between organically and conventionally produced seeds, a statistical significance was determined in the length of the seedling above-ground parts (greater in organically produced seeds – 117.13 mm) and the dry weight of the seedling above parts (greater in organically produced seeds – 0.36 g). Milivojević (2016) stated that the primary root length established in the standard germination test was significantly lower in seeds produced in 2011 than in seeds produced in 2014. As the seed aged, the primary stem length was not significantly changed. A genotype and seed ageing significantly affected the seedling length in the accelerated ageing test. Seeds of maize inbred lines stored at 5 °C for a year compared to seeds stored at 18 °C for four years had a longer primary root (5.56 cm vs. 4.95 cm) after four-day germination in the standard germination test. The longest primary root (8.5 cm) was recorded in the 2014 seeds of the inbred ZPL-217/415D-3. According to the same author, a longer maize radicle was observed in the cold test than in the standard germination test and the accelerated ageing test. Namely, the longer maize radicles obtained in vigour tests of one-year old seed samples were a consequence of seed imbibition during the stressful storage period. Such seeds, after being placed under optimal growing conditions have an advantage over dry seeds in the standard germination test. Compared to non-stressed seeds, ageing was demonstrated by the decreased first count (48.9%), total number of seedlings (40.8%) and seedling growth (70.0% for root length, 44.0% and 10.5% for root and shoot fresh weight, respectively, 5.0% for seed rest fresh weight, 12.0% and 11.5% for root and shoot dry weight, respectively and 15.3% for seed rest dry weight), as well as by the increased number of off-type seedlings (22.0%) (Kravić et al., 2021). Hussein et al. (2012) stated that accelerated ageing decreased seedling dry and fresh weights, seedling length, seedling vigour index, germination speed index and the seedling shoot and root length. The probable explanation of this reduction can be the reduction of biochemical activities in seeds. Seed ageing adversely affects enzymes vital for conversion of reserve food in the embryo into the usable form and eventually production of normal seedling (Iqbal et al., 2002). The decrease in the shoot length, root length and seedling vigour index can be attributable to DNA degradation with ageing, which results in impaired transcription causing incomplete or faulty enzyme synthesis indispensable for earlier stages of germination (Kapoor et al., 2010). Kravić et al. (2021) showed that maize genotypes with higher antioxidant contents (phenols, flavonoids and anthocyanins, total antioxidant capacity) were less exposed to changes in seedling growth parameters under artificial ageing conditions, indicating a positive effect of these antioxidants on improving seed vigour.

Table 2. presents the values of the coefficients of correlation among the measured quantities. To determine the coefficient of correlation, the mean values calculated from four replications and both production methods, both tests and both types of seeds were used. The highest positive correlation was established between the first count and germination ($r = 0.958$), and then between off-type seedlings and non-germinated seeds ($r = 0.730$), as well as between the root fresh and dry weight ($r = 0.669$). The highest negative correlations were determined between germination and non-germinated seeds ($r = - 0.964$), followed by off-type seedlings and the first count ($r = - 0.914$) as well as between germination and off-type seedlings ($r = - 0.886$). In the standard germination test, Radić and Milošević (2004) stated that there was a positive correlation between the maize root length and the maize seedling above-ground part length, as well as between the root fresh weight and the root length, while a negative correlation existed between the seedling above-ground part length and the seedling above-ground part fresh weight. However, these correlations were not statistically significant. According to the same authors, the application of the accelerated ageing test showed statistically significant differences in correlations between the seedling above-ground part length and the seedling above-ground part fresh weight.

When performing the accelerated ageing test, the temperatures to which seeds are exposed as well as the duration of the test, vary depending on researchers. By testing vigour of hybrid maize seed samples, TeKrony (1996) applied the following temperatures in the accelerated ageing test: 1) 41 °C for 96 h and 2) 45 °C for 72 h. At these temperatures, the results of germination differed: significantly lower germination was recorded in 23 seed lots (34 in total) at the temperature of 45 °C, and the author instructed laboratory researchers to apply the combination of these temperatures in order to obtain the most reliable results.

Table 2. Pearson's correlation coefficients and probability level of their significance

Variables	First count (%)	Germination (%)	Off-type seedling (%)	Non-germinated seed (%)	Length of seedling above-ground part (mm)	Root length (mm)	Fresh weight of seedling above-ground part (g)	Root fresh weight (g)	Dry weight of seedling above-ground part (g)	Root dry weight (g)
First count (%)	**1**	**0.958****	**-0.914****	**-0.886****	0.280	-0.147	-0.179	0.030	**0.665****	0.331
Germination (%)		**1**	**-0.886****	**-0.964****	0.241	-0.160	-0.280	-0.066	**0.606***	0.244
Off-type seedling (%)			**1**	**0.730****	-0.084	0.075	0.338	-0.145	**-0.620***	-0.486
Non-germinated seed (%)				**1**	-0.306	0.193	0.218	0.181	**-0.537***	-0.080
Length of seedling above-ground part (mm)					**1**	-0.211	**0.561***	-0.087	0.470	-0.025
Root length (mm)						**1**	-0.400	0.096	-0.390	0.251
Fresh weight of seedling above-ground part (g)							**1**	0.310	0.376	-0.002
Root fresh weight (g)								**1**	0.273	**0.669****
Dry weight of seedling above-ground part (g)									**1**	0.307

Legend: * - significant at 5 probability level; ** - significant at 1 probability level

CONCLUSION

The determination of vigour of organically produced maize seeds by the application of the accelerated ageing test and standard germination test, showed that there was a statistically significant difference in a higher percentage of the first count (78.50%), a higher percentage of nongerminated seeds (10.25%), longer seedling above-ground parts (117.13 mm) and dry weight of seedling above-ground parts (0.26 g) after the accelerated seed ageing test.

The same tests were used to determine vigour of conventionally produced maize seeds and a statistically significant difference was recorded in a higher percentage of the first count (41.50%), a lower number of off-type seedlings (15.00%), a higher percentage of non-germinated seeds (38.75%) and a greater radicle dry weight (0.28 g) after the application of the accelerated ageing test.

From the aspect of differences in the standard germination test between organically and conventionally produced seeds, a statistically significant difference in organically produced seeds was recorded in the first count (70.75%), germination (88.25%) and the root dry weight (0.23 g), while statistically significant differences in conventionally produced seeds were observed in the higher percentage of off-type seedlings (25.00%) and non-germinated seeds (31.75%).

From the aspect of differences in the accelerated ageing test between organically and conventionally produced seeds, a statistical significance was recorded in the first count (78.50%), germination (84.25%), seedling above-ground part length (117.13 mm) and above-ground part dry weight (0.36 g), while a statistically significant difference in conventionally produced seeds was observed in the higher percentage of off-type seedlings (15.00%) and non-germinated seeds (38.75%).

The obtained results show that the accelerated ageing test did not provide enough information on physiological quality of seeds and that due to the particularity of the production conditions, additional information on seed vigour is needed.

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