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ORIGINAL SCIENTIFIC ARTICLE

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EFFECTS OF PRODUCTION METHODS ON SEED VIGOUR OF SOYBEAN SEEDS

ABSTRACT: The seed accelerated ageing test is one of the most important tests for testing seed vigour, which provides the determination of the degree of preservation for germination and the determination of the duration of the seed storage. The aim of this study was to observe effects of two different production methods (organic and conventional) on seed vigour of soybean cultivar *Kaća*, by the application of the seed accelerated ageing test. The seeds were exposed to stress conditions for 72 h (temperature of 45 ºC and air humidity of 100%). After the test was applied, the number of non-germinated seeds of organically produced soybean increased, which resulted in the reduction of the germination percentage. Compared to the standard laboratory method, after the seed accelerated ageing test was applied, the length of the seedling above-ground part (121.63 mm), fresh weight of the seedling above-ground part (8.9 g) and dry weight of the seedling above-ground part (1,05 g) were higher. Moreover, the length (100.25 mm), fresh (1.26 g) and dry weight (0.1 g) of the root were also higher. After the test was applied, the percentage of the off-type seedlings (10.75%) and non-germinated seeds (26%) was higher in conventionally produced soybean seeds, while the length of the seedling above-ground part (100.63 mm), root length (106.75 mm) and root fresh weight (1.39 g) were lower.

KEYWORDS: accelerate ageing test, conventional production, organic production, soybean

INTRODUCTION

Soybean (*Glycine max* (L.) Merr.) belongs to the family of legumes and is one the oldest cultivated species in the world. The mature soybean grain

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typically contains about 40% proteins, 20% oil, 17% cellulose and hemicellulose, 7% sugar, 5% solid fibres and approximately 6% dry weight-based ash (Ciabotti et al., 2016). Such a favourable grain chemical composition makes soybean one of the most important sources of vegetable oils and proteins in the world, due to which it ranks highly in the proper diet (Hoffman and Falvo, 2004). The soybean production in Serbia has been growing. Since 2008, its favourable price has been contributing to the increased production. Moreover, the demand for soybean is high both in international and national markets. According to Kalentić et al. (2014), organic protein crops, primarily soybean, used as feedstuff of organically raised livestock, are highly imported in Germany and thus can be a significant export item of Serbian producers. The analysis of the agricultural production index indicates that a cyclical phenomenon of extreme weather conditions during the last decade strongly influenced the plant production, and these changes were especially obvious in the areas cultivated with soybean (Živanović and Popović, 2016). The production and processing of organically produced soybean have been increasing. Organic farming maintains and improves soil biodiversity, controls and increases soil fertility, protects the environment and applies the highest standards for the protection of plant and animal health (Ugrenović and Filipović, 2012).

The seed accelerating ageing test is characterised by the seed exposure to two changeable environmental factors, high temperature and high relative humidity, in a short period, which affect seed deterioration. High-vigour seeds endure these stress conditions and deteriorate more slowly with maintained high germination even after ageing in contrast to low-vigour seeds. Several already performed studies have shown that this test provided a very precise assessment of seed vigour in soybean, maize and some other crops. Quite a few studies have confirmed that the results of this test provided better predictability of field seed germination under stress conditions than standard germination tests (TeKrony, 2005). During the seed accelerating ageing test, seeds absorb water from the wet environment; hence there is a moisture increase in seeds, which together with a high temperature to which seeds are exposed, results in the process of accelerated ageing and deterioration of seeds (ISTA, 2014).

MATERIALS AND METHODS

Samples

Seeds of organically and conventionally grown soybean cultivar NS *Kaća* originated from the experimental plot of the Institute of Field and Vegetable Crops in Novi Sad (Bački Petrovac). A sown plot size amounted to three hectares. Prior to primary tillage, fertilisation with 120 kg NPK ha^{-1} at the ratio of 16:16:16 was applied in the conventional production, while fertilisers were not applied in the organic production. Appropriate herbicides based on imazamox and quizalofop-P-tefuryl were applied in the conventional production, while mechanical weed control, by manual hoeing was applied in the organic production.

Accelerated ageing test

Soybean seed vigour was determined by the accelerated ageing test. Seeds were exposed to double stress conditions – high temperature (41 °C) and high relative air humidity (100%) for 72 hours. After that, seeds were sown in the sand to determine seed germination.

The standard laboratory test, with four replications of 100 seeds, was applied to determine soybean seed germination and it was expressed as percentages (ISTA Rules, 2016). The germination first count was done on the fifth day, while germination was read on the eighth day (ISTA Rules, 2016). The seed germination percentage was determined by calculating the proportion of normal seedlings in the working sample. When seed incubation was completed, the following growth parameters were determined: the length of above-ground parts of seedlings and roots (mm), fresh and dry weights of the seedling above-ground parts and the root fresh and dry weights (g). The length of above-ground parts of seedlings and roots was determined by measuring the mean values of 10 seedlings from each replication, using a ruler. The fresh weights of the above-ground parts and roots were established by measuring mean values of 10 seedlings in four replications, in which, after drying in a thermostat at the temperature of 80 °C for 24 hours, the seedling dry weight was measured.

Statistical analysis

Results of all analyses were expressed as the mean of three measurements ± standard deviation (SD), while the significance of differences between the means ($p < 0.05$) was determined by using Tukey's test, software Statistica, version 12.0 (StatSoft Inc., USA). The correlation analysis between observed parameters in the accelerated ageing test and the standard germination test was performed by calculating the Pearson's correlation coefficient (r) ($p < 0.05$).

RESULTS AND DISCUSSION

The results of the vigour test of organically produced soybean seeds are presented in Table 1.

After the application of the accelerated ageing test, a statistically significant decrease in the first count and germination (26.75% and 29.25%, respectively) was recorded compared to the standard germination test (52.00% and 54.25%, respectively). Moreover, the percentage of non-germinated seeds statistically significantly increased to 61.75%. The percent of off-type seedlings did not statistically significantly differ between these two tests. Such results indicate that double stress conditions of the high temperature and high relative air humidity in the accelerated ageing test increased the number of non-germinated organic soybean seeds and thus lowered the germination percentage, which affected seed deterioration. The length of the seedling above-ground

part (121.63 mm), fresh (8.93 g) and dry weights (1.05 g) of the seedling above-ground part were higher after the accelerated ageing test, while the root length (100.25 mm), and root fresh (1.25 g) and dry (0.12 g) weights were greater in the standard germination test. However, these differences were not statistically significant.

Table 1. Results of soybean seed accelerated ageing test

Parameter	Production Method			
	Organic		Conventional	
	Test		Test	
	Germination standard test	Accelerated ageing test	Germination standard test	Accelerated ageing test
Germination first count (%)	52,00±5,35aA	26,75±6,18bA	59,25±5,8aA	60,00±2,16aB
Germination (%)	54,25±6,02aA	29,25±2,22bA	73,00±4,24aB	63,25±2,63bB
Off-type seedlings (%)	7,00±1,83aA	9,00±1,83aA	4,75±0,96aA	10,75±0,96bA
Non-germinated seeds (%)	38,75±5,56aA	61,75±2,5bA	22,25±4,03aB	26,00±2,94aB
Length of seedling above-ground part (mm)	112,75±12,87aA	121,63±4,78aA	114,75±4,05aA	100,63±11,96aB
Root length (mm)	100,25±20,16aA	74,13±7,44aA	176,63±13,37aB	106,75±10,02bB
Fresh weight of seedling above-ground part (g)	8,76±0,55aA	8,93±0,97aA	7,96±0,38aA	8,27±0,61aA
Root fresh weight (g)	1,25±0,37aA	1±0,22aA	2,24±0,3aB	1,39±0,38bA
Dry weight of seedling above-ground part (g)	1,01±0,07aA	1,05±0,15aA	0,97±0,04aA	1,02±0,05aA
Root dry weight (g)	0,12±0,03aA	0,1±0,02aA	0,15±0,01aA	0,11±0,02bA

* Lowercase letters (a, b) indicate the statistical significance between the standard germination test and the accelerated ageing test (**n=4**, **SV±SD**), ***p<0.05*** (*Tukey's* test). Capital letters (A, B) indicate the statistical significance between organic and conventional production methods (**n=4**, **SV±SD**), ***p<0.05*** (*Tukey's* test).

After the application of the accelerated ageing test to determine vigour of conventionally produced soybean seeds (Table 1), a statistically significant reduction in seed germination (to 63.25%) was established, and also a slight difference in the first count in comparison to the standard laboratory test (60.00%). On the other hand, compared to the standard germination test, there was a statistically significant increase in the percentage of the off-type seedlings (10.75%), while the root length (106.75 mm), root fresh (1.39 g) and dry (0.11 g) weights were decreased. The exposure of seeds to the high temperature and high relative air humidity resulted in the increase in the number of non-germinated seeds and the occurrence of the off-type seedlings, thus seed germination was reduced. Compared to the standard germination test, the higher values of fresh (8.27 g) and dry (1.02 g) weights of the above-ground parts did not differ significantly after the application of the accelerated ageing test.

From the aspect of differences in the standard germination test between organically and conventionally produced soybean seeds, the statistical significance was recorded for germination (higher in conventionally produced seeds – 73.00%), non-germinated seeds (higher in organically produced seeds – 38.75%), root length (higher in conventionally produced seeds – 176.63 mm) and the root fresh weight (higher in conventionally produced seeds – 2.24 g). Comparing differences in the accelerated ageing test between organically and conventionally produced soybean seeds, the statistical significance was determined for the fist count (twice as large in conventionally produced seeds – 60%), germination (twice as large in conventionally produced seeds – 63.25%), non-germinated seeds (twice as large in organically produced seeds – 61.75%), length of the seedling above-ground part (longer in organically produced seeds – 121.63 mm) and the root length (longer in conventionally produced seeds – 106.75 mm).

Table 2 shows the values of correlation coefficients among the measurands. Mean values calculated from four replications, both production methods, both tests and both seed types, were used to determine the correlation coefficient. The highest positive correlation was recorded between the root length and the root fresh weight ($r = 0.998$), root length and the root dry weight ($r = 0.969$), as well as the first count and germination ($r = 0.964$). The highest negative correlations were determined between germination and non-germinated seeds ($r = -0.991$), first count and non-germinated seeds ($r = -0.981$), and also between the dry weight of the seedling above-ground part and the root dry weight ($r = -0.981$).

During the soybean seed ageing, biochemical changes occur and have a very strong impact on seed quality and vigour (Tatić, 2007). In addition, a specific chemical composition of seeds with 20–22% oil is suitable for degrading processes. Lipid autoxidation and the increase in free fatty acids during storage are the most common reasons for accelerated seed damages in oil plants (Lekić, 2003), while the accumulation of active oxygen species and free radicals is considered one of the most important factors of seed ageing (Bailly, 2004). Different storage conditions, primarily temperatures and air relative humidity, significantly affect soybean seed germination (Nkang and Umoh, 1997). According to Nkang and Umoh (1997), the optimal seed storage conditions are the temperature not higher than 25 °C and the relative air humidity ranging from 55% to 65%.

Balešević-Tubić et al. (2011) studied effects of ageing on vigour and biochemical changes in soybean seeds and established that extreme conditions of the temperature of 40 °C and the relative air humidity of 100% caused biochemical changes in seeds and reduced seed germination. After the 3-day accelerated ageing, the obtained seed germination was at the level of six-month natural ageing, both under controlled and conventional storage conditions. Seed germination after the 5-day accelerated ageing was equal to seed germination stored for 12 months under conventional storage conditions. According to Rastegar et al. (2011) the average germination period of deteriorated soybean seeds increased, which agreed with results obtained by Khaje Hoseini et al. (2003), who proved that deteriorated seeds needed more time to germinate.

Table 2. Correlation matrix of analysed parameters

Correlations	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, %	1.000	**0.964**	-0.232	**-0.981**	-0.758	0.687	-0.807	0.668	-0.773	0.636
Germination %		1.000	-0.418	**-0.991**	-0.595	0.854	**-0.913**	0.841	-0.896	0.800
Off-type seedling, %			1.000	0.295	-0.459	-0.734	0.371	-0.712	0.775	-0.865
Non-germinated seed, %				1.000	0.692	-0.792	**0.907**	-0.781	0.831	-0.716
Length of seedling above-ground part, mm					1.000	-0.122	0.471	-0.118	0.181	0.006
Root length, mm						1.000	**-0.903**	**0.998**	**-0.962**	**0.969**
Fresh weight of seedling above-ground part, g							1.000	**-0.913**	0.831	-0.779
Root fresh weight, g								1.000	**-0.945**	**0.955**
Dry weight of seedling above-ground part, g									1.000	**-0.981**
Root dry weight, g										1.000

The hypocotyl length is an important seed property, on which the emergence of the entire plant depends. This trait can be crucial for deeper sowing (Prijić and Jovanović, 1989). In addition, the root length also reflects the intensity of the initial growth of the seedling. According to Srebrić et al. (2010), the best germination of soybean seeds was achieved on chernozem, then on eutric cambisol, and the lowest on pseudogley. The variation in the length of primary roots and hypocotyls was present on different types of soils. The shortest, i.e. longest (8.3 cm) seedling hypocotyls were recorded on pseudogley, i.e. sand, respectively, under optimal conditions (20/30 °C). Significant differences in the primary root length were determined among all treatments within each genotype (genotype S1, S2, S3, S4), and then among genotypes within each treatment. Precipitation sums and distribution, and especially the occurrence of drought, reduce quality and yield of soybean seeds. Vujaković et al. (2006) stated that by applying the standard laboratory method, soybean seeds produced under irrigation conditions had higher germination than seeds produced by dry land farming conditions. These authors tested vigour by the application of Hiltner test, cold test and the accelerated ageing test, and established that the highest values of this parameter were obtained by the accelerated ageing test. According to the study carried out by Maksimović et al. (2004), germination of all observed soybean genotypes (Proteinka, Novosađanka and Vojvođanka) was higher than the minimum value (75%) prescribed by the Regulation of Seed Testing Quality of Agricultural Crops (Official Gazette of RS, issue 47/87). On that occasion, using the standard laboratory method, seed germination under rainfed conditions amounted to 88–90%, while this value was higher under irrigation conditions and ranged from 92 to 98%.

CONCLUSION

The comparison of differences between the accelerated ageing test and the standard germination test of soybean seeds showed the statistically significant decrease in the first count (26.75%) and germination (29.25%), and the increase of the percentage of non-germinated seeds (61.75%) after the application of the accelerated ageing test. The following seed traits were statistically significantly reduced after the application of the accelerated ageing test in comparison to the standard germination test: germination, root length (106.75 mm), root fresh weight (1.39 g) and root dry weight (0.11 g), while the percentage of the off-type seedlings (10.75%) was increased. From the aspect of differences in the standard germination tests between organically and conventionally produced soybean seeds, statistically significantly higher were germination (73.00%), root length (176.63 mm) and the root fresh weight (2.24 g) in seedlings of conventionally produced seeds, while percentage of non-germinated seeds (38.75%) was higher in organically produced seeds. By monitoring the differences in the accelerated ageing test between organically and conventionally produced soybean seeds, it was determined that the first count (60%), germination (63.25%) and the root length (106.75 mm) were statistically significantly

higher in conventionally produced seeds, while higher percentage of non-germinated seeds (61.75%) and the length of seedling above-ground parts (121.63 mm) were recorded in organically produced seeds.

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ОРИГИНАЛНИ НАУЧНИ РАД

УТИЦАЈ НАЧИНА ПРОИЗВОДЊЕ НА ЖИВОТНУ СПОСОБНОСТ СЕМЕНА СОЈЕ

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РЕЗИМЕ: Тест убрзаног старења семена један је од најважнијих тестова за испитивање животне способности семена, који омогућава утврђивање степена очувања клијавости и одређивање дужине периода чувања семена у складишту. Циљ овог рада био је да се испита утицај два различита начина производње – органског и конвенционалног – на животну способност семена соје сорте *Каћа*, применом теста убрзаног старења. Семе је излагано стресним условима температуре од 45 ºC и влажности ваздуха (100%) у трајању од 72 часа. Након примене теста дошло је до повећања броја неклијалог семена органске соје, чиме је смањен проценат клијавости. У односу на стандарни лабораторијски метод, након теста убрзаног старења дужина надземног дела клијанца (121,63 mm), маса свежег надземног дела клијанца (8,9 g) и маса осушеног надземног дела клијанца (1,05 g) били су већи, док су дужина (100,25 mm), свежа (1,26 g) и сува маса (0,1 g) корена били већи код стандардног лабораторијског метода. Након теста убрзаног старења забележен је већи проценат атипичних клијанаца (10,75%) и неклијалог семена (26%) код конвенционалне соје, док је с друге стране дошло до смањења дужине надземног дела клијанца (100,63 mm), дужине корена (106,75 mm) и свеже масе корена (1,39 g).

КЉУЧНЕ РЕЧИ: тест убрзаног старења, конвенционална производња, органска производња, соја