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The Optimal Concentration of Primers for Enhancement of Germination Energy of Aged Soybean Seed

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Abstract: *Two varieties, Valjevka and Victoria, were chosen in order to investigate the influence of diverse primer concentrations on germination energy of aged soybean seed. The seed was produced in the period from 2012 to 2015, i.e. three-, two-, one-year-old, and fresh seed were analyzed. The following treatments were used for seed immersion: ascorbic acid (AsA) (100 ml/l, 250 ml/l, 500 ml/l), potassium nitrate (KNO_3) (1%, 3%, 5%), hydrogen peroxide (H_2O_2) (1%, 3%, 5%) and control (C) (non-treated seed). The application of the lowest concentration of primer solutions improved germination energy in both varieties, regardless of seed age. Treatment with 100 ml/l AsA and 1% H_2O_2 increased average values by 7%, while 1% KNO_3 was slightly less effective, with an increase of 5%. However, by applying solutions of inadequate concentration, the existing quality could be significantly decreased, with the germination energy reduction up to 13%. After determining the optimal concentration of all three primers and their application on seed of different age, it could be concluded that the effect of this treatment was not only seed age dependent, but it also depended on agro-meteorological conditions during vegetation, primarily at the time of flowering and seed filling stage. The best effects were accomplished at three-year-old, fresh, and two-year-old seed (9%, 7%, and 6%, respectively), i.e. years in which agro-meteorological conditions, above all precipitation level, were unfavorable during the most critical stages of soybean development.*

Keywords: *Germination energy, priming, seed age, soybean seed*

I. Introduction

Germination and vigor of soybean seed represent the most significant seed quality indicators, which influence seed utilization potential (Poštić et al., 2010). Seed aging induces enzyme inactivation, protein degradation, damage of cell membranes, acceleration of fatty acid oxidation, and nucleic acid damage (McDonald, 1999), which affects seed vitality, germination energy and vigor.

The general purpose of seed priming is to partially hydrate the seed to a point where germination processes are initiated but not completed (Ashraf and Foolad, 2005). Priming is the process of monitoring partial discharge of seeds, to begin the biochemical processes and metabolism of sugars and hydrolysis inhibitors during the first and second stage of germination before radicle emergence (Parmoon et al., 2013). Priming diminishes the effects of seed aging by reducing malondialdehyde (MDA) and free radical production, as well as through the maintenance of antioxidant activities (Basra et al., 2003). Also, most studies reported that priming improved the quality of aged seed by increasing enzyme activity, such as antioxidant enzymes and amylases (Ansari et al., 2013). The beneficial effects of seed priming have been demonstrated for many field crops, such as wheat (Parera and Cantliffe, 1994), sweet corn (Chiu et al., 2002), mung-bean (Khan et al., 2005), cucumber (Ghassemi-Golezani and Esmaeilpour, 2008a), lentil (Ghassemi-Golezani et al.,2008b) and winter rapeseed (Ghassemi-Golezani et al., 2010). However, seed priming decreased the germination percentage in barley and corn (Sharif et al., 2006), and reduced seedling emergence and number of plants per unit area in sunflower (Hussain et al., 2006).

Various seed priming techniques have been developed so far, including hydropriming (soaking in water), halopriming (soaking in inorganic salt solutions), osmopriming (soaking in solutions of different organic osmotica), thermopriming (treatment of seed with low or high temperatures), solid matrix priming (treatment of seed with solid matrices), and biopriming (hydration using biological compounds) (Ashraf and Foolad, 2005).

Ascorbic acid is one of the most important herbal antioxidants, which plays an important role in cell processes (Dumet and Benson, 2000). Seed priming with ascorbic acid increases performance and resistance to different external factors (Benson, 1990), while priming with potassium nitrate leads to the increase of potassium in cells (Parmoon, 2014). Potassium increases enzyme activity (antioxidant enzymes) and neutralizes negative effects of free radicals by influencing antioxidant enzymes (Hu and Schmidhalter, 2005). Potassium nitrate is highly effective in removing damages caused by aging (Parmoon et al., 2013). The available information suggest that hydrogen peroxide directly regulates the expression of numerous genes involved in plant defense and related pathways, such as antioxidant enzymes, defense proteins, and transcription factors

(Hung et al., 2005). There is not much information in literature about the influence of naturally aged soybean seed immersion on germination energy. Therefore, the objective of this research was to examine the impact of diverse primer concentration on germination energy of two soybean varieties of different seed age.

II. Material And Methods

The experiment was conducted in 2015 at the Institute of Field and Vegetable Crops Novi Sad. Testing was performed on seed of two soybean varieties from different maturity groups: Valjevka and Victoria. The seed was produced in the period from 2012 to 2015 on experimental fields RimskiŠančevi, i. e. three-, two-, one-year-old, and fresh seed was tested. Material was stored in a conventional seed storing facility, with storage conditions dependent on environmental factors.

Due to variations in agro-meteorological conditions between the analyzed years, precipitation level values during soybean vegetation, flowering and pod filling phase were used for the purpose of testing (Table 1).

The following treatments were used for immersion:

1. Ascorbic acid AsA (100 ml/l, 250 ml/l, 500 ml/l)
2. Potassium nitrate KNO_3(1%, 3%, 5%)
3. Hydrogen peroxide H_2O_2(1%, 3%, 5%)
4. Control C (non-treated seed).

Seed was immerged in primer solutions for 6 h (Miladinov et al.,2015), and then dried until it reached 11% of moisture content. After that, 4x50 seeds of each variety were taken and tested at 25 °C for the period of 5 days using standard laboratory methods (ISTA, 2009).

Statistical analysis of collected results was performed using the analytics software package Statistica 9, while results were sorted on the basis of DUNCAN test for significance threshold of 0.05 and 0.01.

III. Results

Based on the obtained results, it can be concluded that the quality of seed, i.e. germination energy declined during seed aging. It was observed, however, that one-year-old seed had higher germination energy compared to fresh seed, because 2014 from the agronomic aspect was more favorable for soybean production than 2015. Valjevka variety had higher germination energy than Victoria variety in all analyzed years. However, seed of Valjevka variety showed more rapid decline of quality than seed of Victoria variety (Table 2).

Immersing the seed in solutions with the lowest concentrations led to the improvement of germination energy in both varieties regardless of seed age. The application of 100ml/l AsA and 1% H_2O_2 increased the quality by an average of 7% compared to the control variant, while the use of 1% KNO_3 was less effective, with an increase of 5%. With an enhancement of primer concentration the quality declined, so at the highest concentration germination energy was reduced by 2-5% compared to non-treated seed (Table 3).

Immersing the seed of Valjevka and Victoria varieties in 100ml/l AsA increased the quality by 8% and 7%, respectively. While Victoria variety had a positive reaction even with the application of the highest concentration of this primer, the same treatment led to significant decrease in quality of seed of Valjevka variety. Similar results were registered with the use of H_2O_2, where germination energy was enhanced by 7% and 6%. However, the seed of both varieties were more sensitive to higher concentrations of this primer compared to AsA treatment, and therefore the decline in the quality was more significant. The use of KNO_3had a lower effect, improving the quality only by 4% and 5%. Besides that, increase of this primer concentration led to the largest decline in quality. Seed of Valjevka variety was more susceptible to concentration increase, i.e. the quality was reduced by 7% (4% more compared to seed of Victoria variety).

The lowest concentrations of primer exhibited the highest effects in both soybean varieties regardless of seed age. By applying 100ml/l of AsA on Valjevka variety, the best results were achieved on three-year-old and fresh seed, with the quality increase of 11% and 12%. The similar effect was noticed at seed of Victoria variety, with the germination energy enhancement by 8% and 12%. The smallest improvement in quality occurred in one-year-old soybean seed. The application of 1% H_2O_2 on the seed of Valjevka variety gave the best results on three-year-old and fresh seed, increasing germination energy by 12% and 9%. However, Victoria variety showed a different reaction, i.e. the increase of germination energy in all cases, except for fresh seed. Priming with 1% KNO_3 had the effect only on the three- and two-year-old seed of Valjevka variety (7% and 5%), while fresh seed of Victoria variety also showed a positive reaction to the application of this pre-sowing treatment, with increase of germination energy by 8%. The obtained results indicate that the effects of seed immersion are not only largely dependent on primer concentration, but also on the variety and seed age. Priming of fresh seed of Valjevka variety with 1% KNO_3 was not effective, although the application of the stated primer on fresh seed of Victoria variety increased germination energy by 8%. On the other hand, the use of 1% H_2O_2 on the seed of Valjevka variety increased the quality by 9%, while Victoria variety exhibited only insignificant germination energy improvement of 2%.

The application of appropriate primer concentration can increase germination energy to a certain extent. However, by using primer solutions of inadequate concentration, the existing quality could be significantly decreased. The seed of Valjevka variety was more sensitive to higher concentrations of primers, i.e. this variety showed larger quality decline. The application of 500 ml/l AsA reduced germination energy up to 7%, while the highest quality decrease of 13% was recorded by applying 5% KNO_3. Using 5% KNO_3 and 5% H_2O_2, Victoria variety manifested a smaller decline, by 5% and 7%, respectively.

After the analysis and determination of the most adequate primer concentrations, their influence on germination energy of differently aged seed was identified. The analysis of the collected data indicated that priming of old seed could improve its quality to a certain level. The highest improvement of 9% was obtained in three-year-old seed. Two-year-old seed showed increase of germination energy by 6%, while in the case of one-year-old and fresh seed, the increase was 3% and 7%, respectively (Table 4).

IV. Discussion

Natural process of seed aging led to the decrease in germination energy and quality depended on the production year and variety. The reduction of quality is mainly due to the respiratory process in which free radicals are formed (Marcos-Filho, 2005). In soybean seed, such free radicals can cause changes that affect predominantly fatty acids, causing lipid peroxidation which influences the structure and function of cell membrane, thus changing its permeability, and in addition leads to modifications in enzyme activity and synthesis of nucleic acids (Marcos-Filho, 2005). The quality decrease is particularly characteristic for seed with high oil content (Balešević-Tubić et al., 2007), such as soybean, which contains from 15% to 25% of oil, and 32% to 49% of protein (Đorđević et al., 2013). Seed priming leads to regeneration of the nucleic acid production process, increases protein synthesis, repairs mitochondria (McDonald, 1999) and activates the mechanism of antioxidants (Siri, 2013). Apart from antioxidant activity improvement, priming of old seed reduces the level of lipid peroxidation (Parmoon, 2014), leading to the increase in seed quality (Bailly et al., 1998). It is known that seed quality improvement depends on the degree of deterioration caused by aging, and that germination energy increase can be achieved by immersing the seed in a particular solution, but to a very limited extent (Alvarado & Bradford, 1988; Wattanakulpakin et al., 2012). Butler et al. (2009) found that old seed priming could not improve its quality, because the damage caused by seed aging was irreversible. The effect of this treatment also depends on plant species whose seed is subjected to this process. In the studies of onion (Patil and Manjare, 2013) and melon (Warley and Fernando, 2004), the stated treatment provided good results on old seed, while in vetch (Karta et al., 2011) the best results were achieved with the fresh seed. It was determined that both analyzed soybean varieties had a good response to this pre-sowing treatment, so that germination energy increased by 8% and 7% at adequate primer concentrations. The effect of seed immersion was also analyzed in two wheat varieties, where positive reaction of seed quality was recorded (Jabbarpour, 2014).

Ascorbic acid is an antioxidant molecule that acts as the primary substrate in a cyclic way included in detoxification and neutralization of superoxide radicals and oxygen (Noctor and Foyer, 1998). The exogenous application of ascorbic acid can influence a number of different processes in plants, including seed germination, as it increases the adsorption of this molecule in different tissues and participates in the biosynthesis of other hormones, such as gibberellin acid and ethylene, which are essential for the germination process (Arrigoni and Detullio, 2000). Effect of ascorbic acid on the old seed depends on the plant species. In the studies of sunflower the quality increase of 12,23% was registered, while there was no effect on safflower seed (Dolatabadion et al., 2008). However, the use of hydrogen peroxide induced positive resultsin safflower seed, since this molecule affects the oxidation of inhibitor that is located in the seed coat, and thereby improves its quality (Dolatabadion et al., 2008). Seed priming with hydrogen peroxide exhibited positive effects in many different plant species (Zeinalabedini et al. 2009; Barba-Espín et al. 2010; Bahin et al. 2011). Stimulating action of this primer to enzyme peroxidase activity induced the quality increase of wheat seed (Liheng et al., 2009). However, the application of higher concentrations of the stated primer caused damage to plant cells and had toxic effects to cell membrane (Kathiresan et al., 2006). Seed priming with potassium nitrate increases the content of this element in cells (Parmoon, 2014). Potassium amplifies enzyme activity and neutralizes the negative effects of free radicals (Hu and Schmidhalter, 2005). Priming seed welsh ports KNO3 solution reduced the negative effect of aging on the seed germination (Dong et al, 2014). In the studies of gladiolus seed priming with potassium nitrate, the best results were achieved by using concentration of 1%. Higher concentrations of primers led to the decrease of germination energy (Ramazanet al., 2010). Immersing the old seed of Sylybum marianumin in potassium nitrate solution gave the best results with the application of 3% solution, with the quality increase of 11,3% (Parmoon, 2014).

V. Conclus on

Besides the favorable effect which could be accomplished by seed immersion, this pre-sowing treatment could also provoke quality decrease, if solutions of inadequate concentration were applied. Seed priming can cause germination energy increase regardless of seed age, but only to a limited extent. By analyzing ascorbic acid, potassium nitrate and hydrogen peroxide application, the absence of a universal solution was determined, i.e. the efficiency of a certain primer depended on the variety, seed age, and agro-meteorological conditions during the production year. The best effect of the aforementioned treatment was achieved in three-year-old, fresh, and two-year-old seed (9%, 7%, 6%, respectively), produced under unfavorable agro-meteorological conditions, especially with low precipitation level, in the most critical phases of soybean seed development. The weakest result was achieved in one-year-old seed produced in 2014, where precipitation level was 510.9 mm during the vegetation period, with precipitation level of 219.6 mm during soybean flowering and seed filling.

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TABLE

Table 1. Precipitation level (mm)

Year	Precipitation level (mm)	
	Vegetation period	Flowering and pod filling phase
2012	213.7	51.2
2013	334.7	61.1
2014	510.9	219.6
2015	336	29

Table 2. Soybean seed germination energy (%)

Production year	Variety			
	Valjevka		Victoria	
	After harvest	2015	After harvest	2015
2012	86	23	82	21
2013	96	42	90	52
2014	92	86	86	82
2015	81		79	

Table 3. The influence of priming on germination energy of soybean seed (%)

Variety (A)	Seed age (B)	Treatment (C)									
		AsA (ml/l)			KNO_3 (%)			H_2O_2 (%)			Cont.
		100	250	500	1	3	5	1	3	5	
Valjevka	Three-year-old	33**	28*	22	30**	22	17*	35**	23	22	23
	Two-year-old	49**	45	35**	53**	42	40	50**	47*	39	42
	One-year-old	91*	89	81*	86	83	73**	86	83	82*	86
	Fresh seed	91**	88**	80	81	75**	74**	90**	81	79	81
	Mean (A*C)	66**	63*	55*	62*	56	51**	65**	59	56	58
Victoria	Three-year-old	29**	26*	26*	27*	19	19	32**	27**	20	21
	Two-year-old	57*	51	51	57*	52	51	57*	53	51	52
	One-year-old	85	84	82	85	80	79	93**	79	79	82
	Fresh seed	91**	87**	82	87**	74*	74*	81	76	72**	79
	Mean (A*C)	66**	62*	60	64*	56*	56*	66**	61	56*	59
Mean (C)		66**	63*	57	63*	56	54*	66**	60	56	59

*significant difference for 0.05

**significant difference for 0.01

LSD	Variety x Treatment	Treatment
0.05	2.98	4.73
0.01	3.87	5.52

Table 4. The effect of interaction between treatment and seed age on germination energy (%)

Seed age (C)	Treatment (B)				Mean (B)
	ASA 100 ml/lH_2O	KNO_3 1%	H_2O_2 1%	C	
Three-year-old	31**	29**	34**	22	31**
Two-year-old	53**	55**	54**	47	54**
One-year-old	88	85	89	84	87
Fresh seed	91**	84*	86**	80	87**
Mean B*C	66	63	66	58	

*significant difference for 0.05
**significant difference for 0.01

LSD	Seed age	Seed age x Treatment
0.05	4.11	3.23
0.01	6.77	5.12