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Priming Fresh and Aged Seed of Soybean (*Glycine max* L.)

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ABSTRACT

Background: High and stable production requires quality seed. Seed quality is the basis of efficient crop production and farmers need such seeds for optimum yield production. Therefore, various procedures are applied in seed production technology that aim to improve not only the germination of seeds but also the speed of its germination. These methods can reduce of seed aging and the effects of different agroecological factors.

Methods: For research the impact of priming used seed aged seven months (fresh seeds) and seed was nineteen months old (aged seed). The seed was treated with following solutions: potassium nitrate (1%), ascorbic acid (100mgl^{-1}) and potassium chloride (1%) for 6 hours and then germinated at 25°C in 8 days.

Result: The analysis showed that seed aging resulted in a decrease in its germination energy and germination. Moreover, time required for germination is prolonged, the lipid peroxidation intensity and content of free proline are increased and the amount of vitamin C is reduced. The results showed that the effect of priming is dependable on variety selection, seed age and treatments. Therefore, we can conclude that there is no universal use of one only primer, as it may not be suitable for each particular cultivar and can ultimately lead to a decrease in the germination energy and germination.

Key words: Aging of seeds, Germination, *Glycine max* L., Priming.

INTRODUCTION

Aging of the seed is natural process is a combination of biochemical reactions that lead to death of the seed (Hsu *et al.,* 2003). Genetic factors, temperature during storage, diseases, moisture content in the seed and also his initial quality have the main impact on seed aging (Rajjou and Debeaujon, 2008). High temperature, air moisture, conditions, higher intensity of lipid peroxidation during the storage is the main reasons for decreasing in quality (Shivasharanappa *et al.,* 2018). Aging has a bad impact on enzymes that are essential for embryo feeding and getting a normal seedling (Iqbal *et al.,* 2002). The application of a particular solution for seed priming can encourage biological activity and hence increase germination capacity (Schopfer *et al.,* 2001). Various methods are applied in production technology that aim to improve the quality of seeds, primarily in unfavorable environmental conditions and one of those methods is seed immersion (Miladinov *et al*., 2020; Arun *et al*., 2017).

There is not enough information in literature about priming effect on soybean seed germination energy that is naturally aged. So the main goal of this research was to test impact of the different treatment on germination energy on two varieties, with seeds that are different ages.

MATERIALS AND METHODS

The experiments were carried out on the seed of two soybean varieties, NS Zita and Victoria, which were selected at the Institute of Field and Vegetable Crops in Novi Sad. The seeds were produced during 2015 and 2016, at the time of the analysis, seed was seven months (fresh seeds) and seed was nineteen months old (aged seed). In the production conditions of Serbia, often passes seven months to the harvest time, until seeding, especially for the maturity group where the analyzed varieties belong. Therefore, in this study, such seed is called fresh seed. To minimize contamination, before priming, the seed was sterilized with 3% sodium hydrogen chlorate solution (NaOCl) for two minutes and after that washed under a stream of distilled water. For seed priming, following treatments were used: potassium nitrate-KNO_3 (1%), ascorbic acid-AsA (100 mgl^{-1}) and potassium chloride-KCl (1%).

The seed priming lasted for 6 h and seeds were dried to moisture of 11% (Miladinov *et al., 2015*). After drying, the seed was put on a pouring, 4 × 50 seeds for each variant tested. The seed was evenly placed on sterilized filter paper in Petri dishes with diameter of 9 cm and treated with 10 ml of distilled water. All Petri pots were closed with paraffin tape to prevent moisture loss and avoid contamination and then

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placed in the chambers at a temperature of 25°C for 8 days. Every day the germination of the seed was noted and the seed with 2mm or longer radicula was considered as emerged. The germination energy was determined after four and the germination after eight days.

The parameters of MGT and T50 were determined using the following formula:

- Mean germination time (MGT) calculated according the formula of Ellis and Roberts (1981):

$$MGT = \Sigma (ni/di)$$

Where,

ni: number of germinated seeds and di: day of counting.

- Time to 50% germination (T50) was determined using the formula of Coolbear *et al.,* (1984):

$$T50 = ti + (N/2 - ni)(tj - ti) / (nj - ni)$$

Where,

N is the final number of germinating seeds, nj and ni are represent the cumulative number of seeds germinated at times t j and t i , respectively, when ni<N/2<ni.

4×10 average clones were taken from each variant and biochemically analyzed after that. According to the method of Placer *et al*. (1966), extraction of malondialdehyde (MDA) from soybean seed was done by using solution of thiobarbituric acid (TBA), trichloroacetic acid (CCl_3COOH) and perchloric acid ($HClO_4$) and concentration was determined spectro photometrically at 532 nm. Soybean seed (0.5 g) were homogenized in mortar with 4.5 ml extraction solution and incubated in water bath at 90°C for 20 minutes. After incubation, solutions were cooled to stop the reaction and centrifuged for 10 min at 5500 r/min. MDA concentration *i.e.* intensity of lipid peroxidation was expressed as nmol of MDA g^{-1} of fresh weight. Extraction and determination of proline was done according to Bates *et al.* (1973). Seedlings (1 g) were extracted with 3% sulphosalicylic acid. Extracts (2 ml) were held for 1h in boiling water by adding 2 ml ninhydrin and 2 ml glacial acetic acid, after which cold toluene (4 ml) was added. Free proline content was measured by a spectrophotometer at 520 nm and calculated as mol g^{-1} DW against standard proline. According to Benderitter *et al.* (1998) 75 µl DNPH solution (2 g dinitrophenyl hydrazine, 230 mg thiourea and 270 mg copper sulphate ($CuSO_4 \cdot 5H_2O$) in 100 ml of 5 ml/L H_2SO_4) were added to 500 µl extract mixture (300 µl of an appropriate dilution of the extract with 100 µl 13.3% trichloroacetic acid (TCA) and water). The reaction mixture was subsequently incubated for 3 h at 37°C, then 0.5 ml of 65% H_2SO_4 (v/v) was added to the medium and the absorbance was measured at 520 nm in a UV spectrophotometer. The vitamin C content of the extracts was subsequently calculated using ascorbic acid as standard.

Data analysis was done using the statistical software package 'Statistica' (StatSoft, Inc., Tulsa, Oklahoma, USA). The obtained results were processed by the method of three factory analysis of variance. Individual testing was carried out using Tuckey test of probability $p \leq 0.05$ and $p \leq 0.01$.

RESULTS AND DISCUSSION

The results of the ANOVA carried out considering as main factors: variety, treatment and aging seed and their interactions are reported in Table 1. The examined parameters after priming were significantly influenced by all factors ($p \leq 0.05$).

Germination results

Research has shown that priming of fresh seeds in KNO_3, AsA and KCl germination energy, germination, mean germination time (MGT) and up to 50% of germinated seeds (T50%) increases, regardless of variety. The results are in line with research by other scientists who have found that by priming soybean seeds (Ahmadvand *et al*., 2012), corn (Basra *et al*., 2011) and chickpeas (Musa *et al*., 2001), germination and seed germination rates were improved. However, on the example of soybeans, it was determined that this pre-sowing measure reduces seed germination (Sibande *et al.* 2015). By priming the aged seed, the effect depended on the variety. Germination energy and seed germination were reduced in the cultivar NS Zita, while in the cultivar Victoria it was increased, but not significantly (Fig 1, 2). A similar conclusion was reached, but on bean and chickpea seeds, Karta *et al*. (2011). They determined that by priming fresh seeds, germination could increase more than with seeds that have been stored for a longer period, ie agedo seeds. Priming in KNO_3, AsA and KCl accelerates the germination of soybean seeds, regardless of its age and variety, even in cases where priming has an inhibitory effect on germination energy and seed germination

Table 1: A significant difference was found in the interaction between factors.

Source of variation	Seed soybean				Seedlings soybean		
	Germination energy	Germination	MGT	T50	Lipidperoxidation	Free proline	Vitamin C
Variety (A)	5,48**	5,82**	3,73*	3,19*	5,51**	5,63**	3,08*
Aging seed (B)	12,81**	13,05**	5,34**	5,11**	7,14**	6,19**	3,45*
Treatment (C)	5,33**	5,41**	3,11*	3,02*	4,22*	4,01*	5,84**
A x B	7,60**	7,89**	6,91**	6,83**	8,74**	7,25**	5,22**
A x C	5,12**	5,41**	3,81*	3,72*	6,92**	6,59**	3,41*
B x C	5,81**	6,11**	3,51*	3,27*	6,28**	6,11**	5,72**
A x B x C	4,91*	3,49*	1,81	1,75	5,91**	6,78**	3,15*

*p<0.05, **p<0.01.

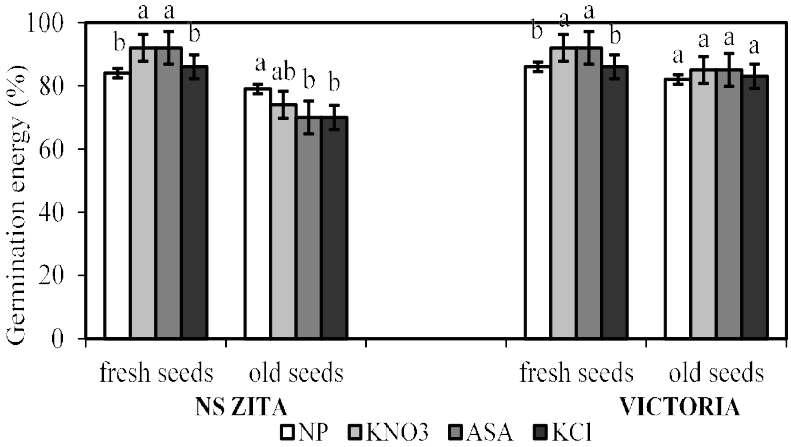

Fig 1: Effect of seed priming on germination energy (%) storage for a longer period.
(Values marked by the same letter are not significantly different at p=0.01 level of probability - Tuckey's Multiple Range Test).

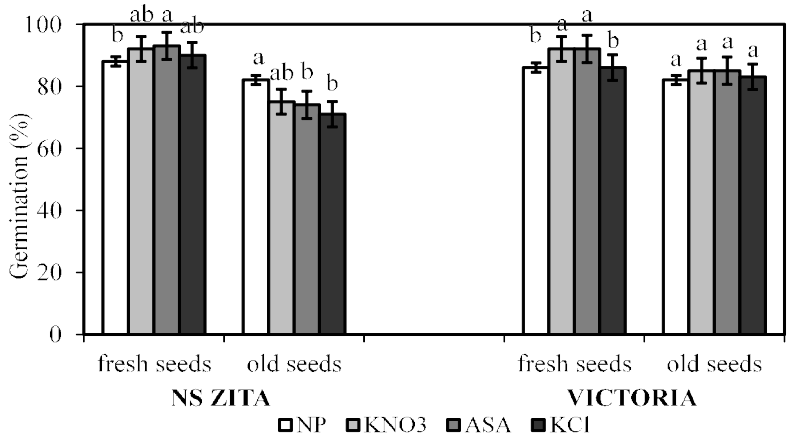

Fig 2: Effect of seed priming on germination (%) storage for a longer period.
(Values denoted by the same letter are not significantly different at p=0.01 level of probability - Tuckey's Multiple Range Test).

(Fig 3, 4). Observing the impact of the treatment, it was found that there is a difference in the efficiency of the treatment. KNO_3 had the best impact on seed quality. Even in the case of aged seeds of the NS Zita variety, in which the germination energy and seed germination were reduced by this pre-sowing measure, the effect of KNO_3 did not lead to a significant change in the germination energy and seed germination. The positive effect of KNO_3 can be attributed to the increased level of nitrate which stimulates the germination of seeds, because it acts as a signaling molecule (Benech-Arnold *et al*., 2000), better permeability of water and oxygen through cell membranes. (Baskin *et al*., 2000) and earlier nutrient mobilization (Bewley, 1997). In addition, KNO_3 induces the production of larger amounts of oxygen and phosphate, which are important in the germination process (Bliss *et al*., 1986).

Biohemical parameters

By primed of the seeds, there was a significant change in the intensity of lipid peroxidation. In fresh seeds, the content of MDA in soybean seedlings was equally reduced by priming (Fig 5). Similar results were found on maize seeds (Ya-jing *et al*., 2009). However, by immersion of the old seed, the effect depended on the variety. The results are partly in agreement with the research conducted by Siri *et al*. (2013). These authors concluded that the application of this pre-sowing measure reduces the MDA content and improves seed germination. They also point out that better seed germination is probably the result of increased overall antioxidant activity and improved cell membrane integrity. Bailly *et al*. (2000) concluded that the decrease in the intensity of lipid peroxidation is a consequence of increased enzyme activity. In this study, the priming of seeds in KNO_3, AsA and KCl and the content of free proline changed significantly depending on the variety, but also the age of the seeds (Fig 6). Bates *et al*. (1973) point out that the rapid degradation of free proline can provide ATP, which is necessary for oxidative phosphorylation and repair of damage caused by stress conditions. However, by soaking the seeds stored for a longer period, the effect depended on the soybean variety. In the cultivar NS Zita, in which the quality of seeds was reduced by primed, an increase in the

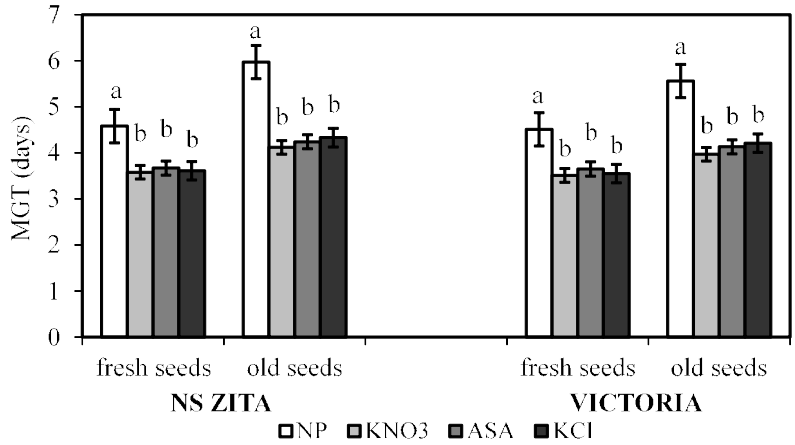

Fig 3: The influence of priming on the MGT (days) storage for a longer period.
(Values marked by the same letter are not significantly different at p=0.01 level of probability - Tuckey's Multiple Range Test).

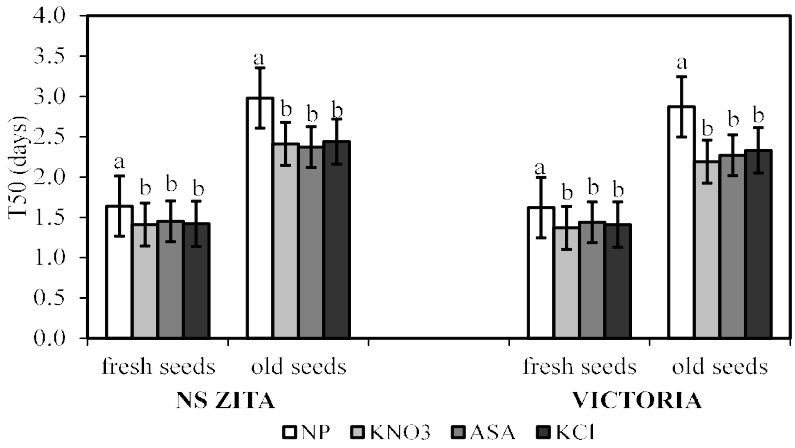

Fig 4: Influence of priming on the T50 (days) stored for a longer period.
(Values marked by the same letter are not significantly different at p=0.01 level of probability - Tuckey's Multiple Range Test).

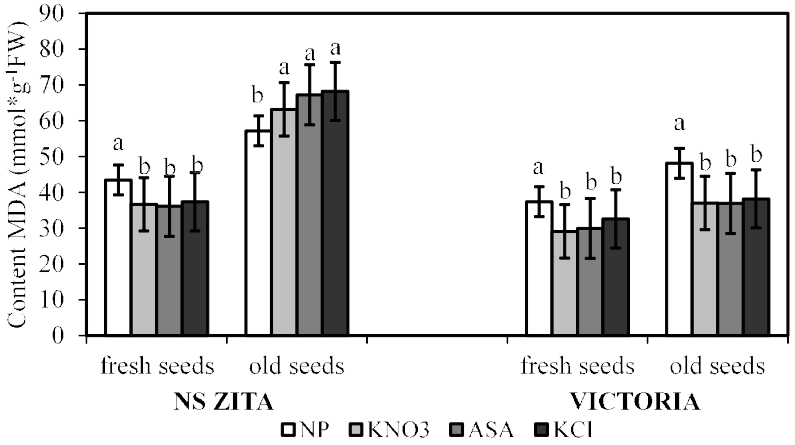

Fig 5: The influence of priming on the MDA content (mmol/gFW) stored for a longer period.
(Values marked by the same letter are not significantly different at p=0.01 level of probability - Tuckey's Multiple Range Test).

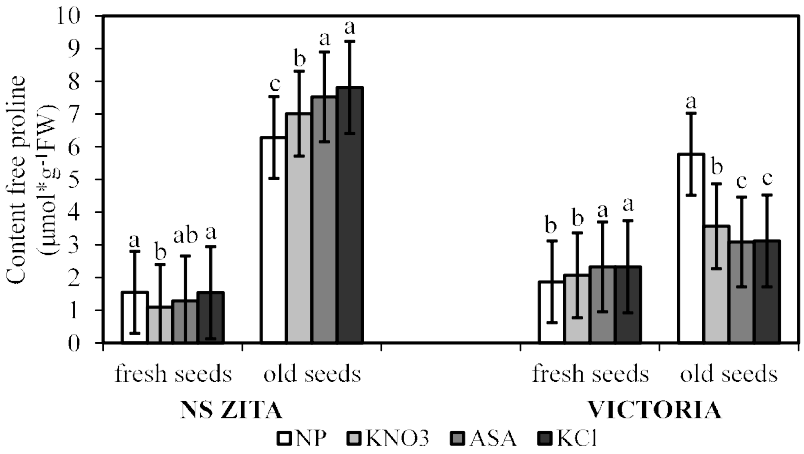

Fig 6: The influence of priming on the free proline content (µmol/gFW) stored for a longer period.
(Values denoted by the same letter are not significantly different at p=0.01 level of probability - Tuckey's Multiple Range Test).

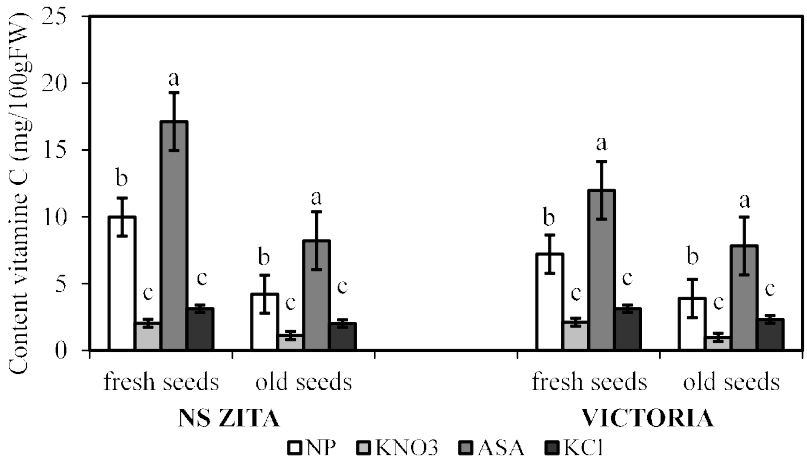

Fig 7: The influence of priming on the vitamin C content (mg/100gFW) stored for a long period.
(Values marked by the same letter are not significantly different at p=0.01 level of probability - Tuckey's Multiple Range Test).

content of free proline was found. However, in the variety in which the quality was improved by primed of the seeds, but not significantly, the content of free proline was reduced. The results are in agreement with research conducted by Heuer (2003). He points out that, in addition to the beneficial effect, free proline can also have a negative effect, if it accumulates in a higher concentration. Increased content of free proline consequently have a disturbance in the relations between inorganic ions and the consequence of all that is reduced seed quality. Priming of soybean seeds in KNO_3 and KCl reduced the concentration of vitamin C, while the use of AsA increased its amount (Fig 7).

CONCLUSION

Analysis showed that seed aging resulted in decrease in its quality. Time required for germination is prolonged, lipid peroxidation intensity and the content of free proline are increased and amount of vitamin C is reduced. The results showed that the effect of this pre-sowing practice was dependent on variety, seed age and treatment. Some varieties reacted very well to priming, while others have inhibited reactions, so seed quality was significantly reduced. By priming the seed, germination was speed up, regardless of its age and variety while the intensity of lipid peroxidation is reduced. The effect of priming on the content of free proline for soybean seed was dependent on the variety and age of the seed, while the highest influence on vitamin C concentration had the treatment. Therefore, we can conclude that there is no universal use of one single primer, as it may not be suitable for each particular variety and can ultimately lead to a decrease in the quality of seed.

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