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EFFECT OF MULTIPLE ALLELES ON OXIDATIVE STABILITY AND GERMINATION OF SOYBEAN SEEDS SUBSEQUENT TO THE ACCELERATED AGEING TEST

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The purpose of this study was to determine changes of lipoxygenase activities, contents of antioxidants (tocopherol α, β-carotene and chlorophyll) fatty acids and soluble proteins, as well as, vigour in accelerated aged soybean seeds. The following soybean cultivars were used in the study: Williams 82, Goyou Kurakake and L93-7290.

Subsequent to the accelerated ageing test (AAT), 23% of seeds of the cultivar Goyou Kurakake retained normal germination, while grain of the two remaining cultivars, L93-7290 and Williams 82, completely lost germination ability. According to our results, the seeds of the cultivar Goyou Kurakake (the absence of lipoxygenases 2) is characterised by a significantly higher content of all observed antioxidants (tocopherol α 2.7 mg $100g^{-1}$, β-carotene 6.1 μg g^{-1}, chlorophyll 4.9 μg g^{-1}). The contents

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of β-carotene and chlorophyll decreased after AAT in the seed of the cultivar Goyou Kurakake by 42.8% and 60.5%, respectively, while the content of tocopherol α remained the same. Furthermore, the tocopherol α content was not changed after AAT neither in the cultivar L93-7290 nor in the cultivar Williams 82. The content of β-carotene after AAT remained the same in seeds of the cultivar Williams 82, while it decreased by 7.7% in the cultivar L93-7290. Since changes of the fatty acids content were not pronounced in the cultivar Goyou Kurakake after AAT it can be concluded that the antioxidative system had a crucial role in seeds protection against lipid peroxidation.

Key words: Soybean, lipoxygenase, accelerated ageing, antioxidants

INTRODUCTION

Aging is a universal physiological phenomenon occurring in living organisms. It usually progresses at a faster rate under stress of unfavourable environment, and it may normally proceed at a slower pace as programmed by a genetic information specific to the species under adapted conditions. Aging in stored seeds is a well known fact, and it has been becoming a problem in the modern agricultural practice. To understand this process research scientists use conditions of accelerated ageing tests (AAT) were seeds are deteriorated under controlled higher temperature and relative humidity for short durations. But this artificial ageing process might be (and probably is) physiologically different from natural sced deterioration. For example, PRISTLEY and LEOPOLD, (1983), reported a little increase in free radical levels of naturally aged soybean seeds but a doubling of free radicals in accelerated aged seeds. In contrast, LIKHLATCHEV *et al.* (1984), concluded that physiological changes in seeds subjected to accelerated ageing were the same as natural ageing, with the only difference being the rate at which they occur.

Perhaps the frequently cited cause of seed deterioration is lipid peroxidation. Lipid peroxidation begins with the generation of free radicals either by autoxidation or enzymatically by oxidative enzymes such as lipoxygenase. Lipid autoxidation may be the primary cause of seed deterioration at moisture contents below 6%. Above 14% moisture content, lipid peroxidation may again be stimulated by the activity of hydrolytic oxidative enzymes such as lipoxygenase, becoming more active with increasing water content. Biomembranes represent a key site of a direct injury by lipid peroxidation. Membranes possess an inherently larger surface area and are usually more unsaturated than storage lipids. The mechanism of lipid peroxidation is often initiated by oxygen around unsaturated or polyunsaturated fatty acids such as oleic and linolenic acids found in seed membranes. The result is the release of a free radical, often hydrogen (H·) from a methylene group of fatty acid adjacent to a double bond. In other cases, the free radical hydrogen may combine with other free radicals from carboxyl groups (ROOH) leaving a peroxy-free radical (ROO·) (MCDONALD, 1999). Once these

free radicals are initiated, they create profound damage to membrane and continue to propagate other free radicals which ultimately combine, terminating the destructive reactions. Free radicals also attack compounds other than fatty acids. Changes in protein structure of seeds have been observed and attributed to free radicals. The most reactive amino acids susceptible to oxidative damage appear to be cystine, histidine, tryptophane, methionine, and phenylalanine, usually in that order (LARSON, 1997).

Free radicals are suspected of assault on chromosomal DNA. Wilson and MCDONALD, (1986), ST. ANGELO *et al.* (1987), indicate in their papers that hydroperoxides and reactive free hydroperoxide radicals that are produced in the process of lipid oxidation also cause the destruction of the electro-transport system, accumulation of toxic components as well as co-oxidation of pigments.

Any practical approach reducing the process of lipid peroxidation might extend soybeans seed longevity. One approach would be to reduce the levels of enzymes known to increase lipid peroxidation, such as lipoxygenase. Lipoxygenase catalyses the addition of molecular oxygen to polyunsaturated molecules containing a *cis, cis*-1,4-pentadiene bond system. The role of these enzymes in higher plants has yet to be established. The most likely roles of the plant lipoxygenase are in the growth and development, senescence, defence systems of plants against attacks by pests and pathogens, and the synthesis of regulatory molecules in plants. The findings of MACCARRONE *et al.*, (2000) suggest that early activation of lipoxygenase is a key element in the execution of apaptosis induced by oxidative stress in plant cells. Moreover, cells contain a complex system of antioxidant defences to protect against the harmful consequences of activated oxygen species. Vitamin E or tocopherol nonenzymatically reduce plyunsaturated lipid peroxide free radicals. For example, one tocopherol molecule may afford antioxidant protection to several thousand fatty acid molecules (BEWLEY, 1986). Soybean oil contains a mixture of four tocopherol homologues (α-, β-, γ-, and δ-tocopherols) whose relative concentrations are: d-α-tocopherol (4-10%), d-β-tocopherol (1-3%), d-γ-tocopherol (60-66%), and d-δ-tocopherol (24-29%) (JUNG, and MIN, 1989). The tocopherol concentration is an important factor that influences the tocopherol antioxidant activity in bulk oils. Generally, antioxidant activity is the greatest at lower concentrations and decreases or may become prooxidant at higher concentrations. Enzymic oxidation of linoleic acid significantly decreases with the higher pigment concentration. Schewe et al. (1986), emphasise that pigments form an inactive complex with isoenzymes of lipoxygenase providing the production of the active enzyme-fat acid complex. Although the degradation process of chlorophylls is very expressed during maturation previous studies reported that chlorophyll contents can be strongly affected by both, the stage of maturity and the postharvest. The presence of chlorophyll is of interest for its potential role in oxidative stability. Chlorophyll inhibited diene conjugation throught a steric protection of fatty acid (ORTHOEFER and DUGAN, 1973). Besides chlorophyll, carotenoids, especially β-carotene are used as antioxidants to reduce oxidation of fats, due to their free radicals scavenging ability.

MATERIAL AND METHODS

Planting and characteristics of the seeds

Grain of soybean cultivars Williams 82, Goyou Kurakake and L93-7290 was used in these experiments. The seed of these cultivars is of the USDA SOYBEAN GERMPLASM COLLECTION origin, USDA-Agricultural Research Service, University of Illinois, and it has been multiplied at the Maize Research Institute, Zemun Polje, Yugoslavia since 1998. Grain used in this study was multiplied in 2001 and since then to 2003 it has been stored at the room temperature.

The cultivar Williams 82 belongs to the II maturity group and is of a standard chemical composition. Recessive genes for isoenzymes of lipoxygenase 2 and 3 were introduced by methods of selection to the cultivars Goyou Kurakake and L93-7290, and therefore the cultivars Goyou Kurakake and L93-7290 are characterised by the absence of lipoxygenases 2 and lipoxygenases 3, respectively. Besides, the grain of the cultivar Goyou Kurakake is large with a green colour of the testa.

The aim of these studies was to determine oxidation changes in soybean seeds that occurred during the process of accelerating ageing.

Accelerating ageing test

The accelerating ageing test is an official soybean seed vigour test. Although the test has been in use since the 1960s (TeKrony, 1993), it was long before it was standardised. After a great number of studies, all factors to be controlled during the analyses were determined along with precise testing conditions, hence the test was included into the ISTA Rules in 2003. The principal characteristic of the AAT is the exposure of seed to stress conditions: temperature of 41 ± 0.3°C and high relative humidity (>95%) for three days (72 h ± 15 min). Seed moisture is a very import parameter of checking AAT accuracy, which should range from 28 to 30%. After this treatment, seed is germinated according to the standard ISTA method (Ista, 2003). The following categories are used for the evaluation: normal seedlings, abnormal seedlings, dead seed, hard seed and fresh seed.

Beside the analysis of seed germination ability performed after the process of accelerating ageing, the analyses of activities of isoenzyme lipoxygenase were performed together with analyses of contents of fatty acids, vitamin E (tocopherol α), β-carotene and chlorophyll. Moreover, the degree of their changes was also determined.

Chemical analyses

The lipoxygenase isoenzyme activity was determined after Axelrod with 0.2 M sodium phosphate buffer (pH 6.5). The solution of linoleic and arachidonic acids was used as a substrate. The mixture containing (2.975-X) mL 0.2 M of borate buffer, pH 9.0, 0.025 mL of sodium linoleate substrate and X ml of enzyme was placed into a quartz cuvette and changes of absorption at 234 nm were recorded in the course of five minutes in order to determine lipoxygenase 1 (Lx1). Hydroperoxide products of the reaction catalysed by lipoxygenase 2 (Lx2) had the

absorption maximum at 238 nm. The essential substrate solution of arachidonic acid for the assay of lipoxygenase 2 was diluted with 0.2 M sodium phosphate buffer (pH 6.1) and 0.1 mL of enzyme was added. With the purpose of determination lipoxygenase 3 activity (Lx3), a reaction mixture containing (2.8-X) mL 0.2 M of sodium phosphate buffer (pH 6.5), 0.2 mL of sodium linoleate substrate and X ml of enzyme was used. Changes of absorption were recorded at 280 nm for five minutes. The activity of lipoxygenase isoenzyme was expressed in the unit of μmol ml^{-1} min^{-1} that was defined as the quantity of enzyme that generated 1 μmol of conjugated diene per minute under standard assay conditions. The extinction coefficient for diene for linoleic acid at 234 nm and arachidonic acid at 238 nm of 2.5 x $10^4 M^{-1}cm^{-1}$ was used to compute activities of isoenzyme lipoxygenases 1 and 2, while the activity of isoenzyme of lipoxygenase 3 was computed by the application of the extinction coefficient of 2.2 x $10^4 M^{-1}cm^{-1}$ (AXELROD *et al.* 1981; ZHU *et al.* 1996).

Gas chromatography (the appliance Varian 14000, with FID detector) was applied to determine higher fatty acids from oil previously extracted by the method of Soxhlet using diethyleter as the diluent. The extraction of fatty acids was done by BF_3-method after VAN WIJANGARDENU (1967). The 300 x 0.32 cm metal columns were used as a stationary phase 20% LAC-3R-728 in Chromosorb W/AW (80-100 mesh).

The tocopherol α content was determined by the HPLC method (JAKOVLJEVIĆ, 1995).

The chlorophyll content was determined by the method after MAC KINNEY (1941). The extraction of chlorophyll was done by 80% acetone, while the content was calculated on the basis of absorbencies obtained at 663 nm and 645 nm.

The determination of β-carotene was based on its previous chromatographic separation form other pigments and on spectrophotometrical measurements. The extraction was done by the mixture of petroleum ether and acetone (70 : 30) in the water bath and with Ahlin condenser. The chromatography column is prepared in the following way: a filter paper disc is placed on the plate of sintered glass; the paper disc is then covered by the mixture of absorbents: aluminium oxide, sodium sulphate and magnesium oxide (3:2:1). After chromatography, measurements are done at the absorption maximum (447 to 449 nm), depending on the acetone content in the solution (TURČIĆ i sar. 1976).

The content of salt soluble proteins was determined after the method of Lowry, (1951), after extraction on the Na-phosphate buffer and protein precipitation with 10% TCA. The sample absorbance was measured at 750 nm. The albumin solution from a bovine serum was used as a standard. The standard curve was applied to calculate the unknown concentration.

Statistical analyses

All chemical analyses were performed with four replicates and obtained results were statistically analysed. Statistical significance of differences of means of observed chemical parameters was determined by the LSD test after the analysis of variance for trials set up according to the RCB design was performed.

RESULTS AND DISCUSSION

In the majority of the combinations, partial dominance was the most Standard germination in the control samples (prior to AAT) was low in all three observed genotypes. However, considering that seed was stored at the room temperature for two years, it can be concluded that the sample of the cultivar Goyou Kurakake had a considerably high percent of germinated seeds (70%), which is higher by approximately 43% than the percentage of germinated seeds in the cultivars L93-7290 and Williams 82. Furthermore, the germination test showed that there were 7% of hard seeds in the sample of the cultivar Goyou Kurakake which could germinate under certain prolonged conditions of humidity and temperature. Subsequent to AAT, 23% of seeds of the cultivar Goyou Kurakake retained normal germination while seeds of cultivars L93-7290 and Williams 82 completely lost germination ability (Table 1). It is important to indicte that subsequent to AAT, a small number of hard seeds was recorded in the sample of the cultivar Goyou Kurakake, which leads to a conclusion that the seed of this cultivar has a harder, less permeable testa, so the conditions of higher humidity and temperature during AAT significantly affected on process of seed imbibition.

Table 1. - Germination of soybean seeds prior to and subsequent to AAT

Cultivar	Germination prior to AAT (%)				Germination subsequent to AAT (%)			
	Normal seedlings	Abnormal seedlings	Dead seed	Hard seed	Normal seedlings	Abnormal seedlings	Dead seed	Hard seed
Goyou Kurakake	70	17	6	7	23	5	70	2
L93-7290	46	27	27	0	0	1	99	0
Williams 82	48	16	36	0	0	0	100	0

The results obtain by the germination test will be quite comprehensible if the essential facts of oxidation stability of soybean seed are analysed. The cultivar Goyou Kurakake seed is characterised by the lack of lipoxygenase 2 that has a very important role in the process of peroxidation of fatty acids. Based on our analysis a certain activity of lipoxygenase 2 in the seed of this genotype was recorded in both, control sample and a sample after AAT, but the explanation of this phenomenon is still in the domain of assumption. As lipoxygenase isoenzyme activity was recorded indirectly over the amount of produced peroxides, it is possible that peroxides recorded at 238 nm were not a result of lipoxygenase 2 activity, but activities of other two lipoxygenases (Lx1, Lx3) present in seed. Besides, it was possible that isoenzymes were activated due to external factors during storage, as according to some studies mutant genotypes (lx_2lx_2) contained a certain level of Lx2 proteins, although it could not have been detected on the electrophoretic gel (DAVIS and NIELSEN, 1986). The Lx2 activity in the control sample of the cultivar Goyou Kurakake was lower by 30.2%, i.e. 43.8% than in the control sample of the cultivars L93-7290 and Williams 82, respectively. The Lx1

activity did not significantly change after AAT in seeds neither of the cultivar Goyou Kurakake nor the cultivar L93-7290. It is observable that the Lx1 activity in the control sample of the cultivar Goyou Kurakake was lower by about 42% than in the control sample of cultivars L93-7290 and Williams 82. Subsequent to AAT, the Lx3 activity in the seed of all three observed cultivars was higher than in the control samples. Although the Lx3 activity subsequent to AAT was extremely high (6.462 μmol ml^{-1} min^{-1}) in the seed of the cultivar Goyou Kurakake, the increase of the activity in relation to the activity in the control sample was the lowest in the seed of this cultivar and amounted to 87.5% (Table 2).

Table 2. - Lipoxygenase isoenzyme activity prior to and subsequent to theAAT

Cultivar	Lx1 (μmol ml^{-1} min^{-1})		Lx2 (μmol ml^{-1} min^{-1})		Lx3 (μmol ml^{-1} min^{-1})	
	Prior to AAT	Subsequent to AAT	Prior to AAT	Subsequent to AAT	Prior to AAT	Subsequent to AAT
Goyou Kurakake	5.83[b]	5.24[c]	0.185[c]	0.152[c]	1.458[c]	6.462[a]
L93-7290	9.26[a]	9.47[a]	0.265[b]	0.253[b]	0.894[c]	4.786[b]
Williams 82	9.20[a]	4.57[d]	0.355[a]	0.291[b]	0.949[c]	4.559[b]
LSD 0.05	0.399		0.063		1.038	

[a-d] Significance among the means at P<0,05

The content of polyunsaturated fatty acids was not changed in the seed of the cultivar Goyou Kurakake subsequent to the AAT, but it did pronouncedly changed in the seeds of cultivars L93-7290 and Williams 82. Subsequent to the AAT the decrease of linoleic acid amounted to 5%, in the seeds of cultivars L93-7290 while the decrease of linolenic acid amounted to 31% in the seeds of cultivars Williams (Table 3). LIN and PEARCE, (1990) reported that linoleic and linolenic fatty acid contents decreased with soybean seeds ageing and free linoleic and linolenic fatty acids increased two-fold during deterioration of soybean seeds (Lin and Pearce, 1990). However, PRIESTLY and LEOPOLD, (1979), studied lipid peroxidation during soybean accelerated ageing (40°C, 100% RH, five days). They detected a slight decrease in the amount of phospholipid (4%) and an increase in total lipids (20%). Significantly, no change in the proportions of fatty acids was found. The autors concluded that lipid peroxidation did not take place during accelerated ageing of soybean.

The decrease of linoleic and linolenic acids in seeds of the cultivar Goyou Kurakake was not observed after the AAT, although all three isoenzymic forms had a high activity. Lipoxygenase isoenzymes present in this seeds participated, to the greatest extent, in the catalysation of the pigment oxidation process. Pigments had strong antioxidative effects and considering lower activation energy they easily bound with lipoxygenase enzymes forming inactive complexes and avoiding catalytic lipoxygenase activity in the processes of peroxidation of fatty acids. Although chlorophyll degrades during soybean seed maturation, the green testa and high content of chlorophyll are a genetic trait of the

cultivar Goyou Kurakake. According to obtained results the total content of chlorophyll in mature grain of this cultivar amounted to 4.9 μg g^{-1}, while chlorophyll almost completely degraded during maturation in cultivars L93-7290 and Williams 82. The fact that the chlorophyll content decreased by 60.5% in seeds of the cultivar Goyou Kurakake after the AAT points out that this pigment compound had an extremely antioxidative role (Table 4).

Table 3. - Content of fatty acids prior to and subsequent to the AAT

Fatty acids (%)	Goyou Kurakake		L93-7290		Williams 82		LSD 0.05
	Prior to AAT	Subsequent to AAT	Prior to AAT	Subsequent to AAT	Prior to AAT	Subsequent to AAT	
Palmitic	11.1[c]	12.7[c]	11.0[c]	14.7[a]	11.3[d]	14.3[b]	0.141
Stearic	4.8[a]	4.1[b]	3.7[c]	3.7[c]	4.9[a]	3.8[c]	0.141
Oleic	25.6[a]	25.1[b]	22.2[d]	21.1[f]	22.6[c]	21.5[e]	0.115
Linolic	50.8[c]	50.4[f]	54.9[a]	52.3[d]	53.0[b]	52.5[e]	0.163
Linolenic	7.5[d]	7.5[d]	8.1[b]	8.2[b]	11.3[a]	7.9[e]	0.145
Arachidonic	0.2[a]	0.2[a]	0.0[b]	0.0[b]	0.0[b]	0.0[b]	0.083

[a-f] Significance among the means at $P < 0.05$

Besides chlorophyll, β-carotene also affected the reduction of lipid peroxidation during seed accelerating ageing of the cultivar Goyou Kurakake. Although the β-carotene content was high in control samples of all three observed genotypes it did not change after AA in cultivars L93-7290 and Williams 82 which leads to a conclusion that the lipoxygenase activity was aimed at peroxidation of polyunsaturated fatty acids. During AA, β-carotene induced the inhibition of the lipoxygenase activity in the seed of the cultivar Goyou Kurakake. Subsequent to the AAT, the β-carotene content decreased by 42.8% in this cultivar in relation to the control sample (Table 4).

Table 4. - Content of tochopherol α, β-carotene and chlorophyll prior to and subsequent to the AAT

Cultivar	Tocopherol α (mg 100g^{-1})		β-carotene (μg g^{-1})		Chlorophyll (a + b) (μg g^{-1})	
	Prior to AAT	Subsequen to AAT	Prior to AAT	Subsequen to AAT	Prior to AAT	Subsequen to AAT
Goyou Kurakake	2.7[b]	2.85[a]	6.12[a]	3.49[d]	4.90[a]	1.94[b]
L93-7290	1.5[d]	1.5[d]	5.74[b]	5.30[c]	0.35[c]	0.21[d]
Williams 82	1.8[c]	1.7[c]	5.17[c]	5.13[c]	0.25[d]	0.20[d]
LSD 0.05	0.141		0.282		0.057	

[a-f] Significance among the means at $P < 0.05$

According to COHEN *et al.* (1985), the increased concentration of chlorophyll and the β-carotene at a fixed linoleate concentration induces the inhibition of forming products of peroxidation, i.e. the inhibition of the lipoxygenase 2 activity. The reduction of conjugated dienes at 0.75 μg and 7.5 μg of chlorophyll amounted to 22.9% and 95.8%, respectively. Redukcija konjugovanih diena pri 0.75 μg hlorofila bila je 22.9%, a pri kontentraciji od 7.5 μg hlorofila 95.8%. According to these authors the inhibitory effect of chlorophyll on lipid oxidation can be a result of forming an inactive complex of lipoxygenase and pigment, which reduces the concentration of free enzyme for the reaction with fatty acids.

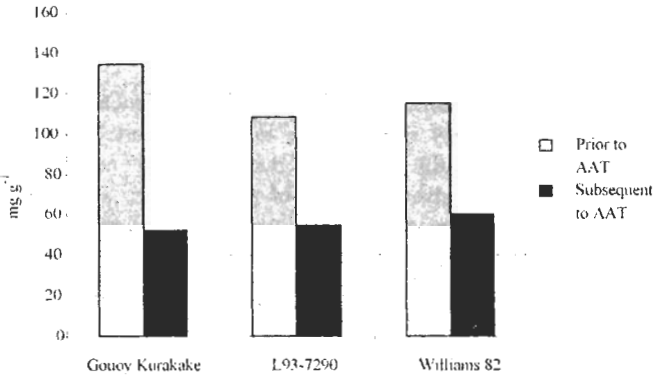

Figure 1. Content of salt soluble proteins prior to and subsequent to the AAT

Accelerating ageing conditions did not affect the tocopherol β content in either of the three observed genotypes. However, according to our results tocopherol β content in the control sample of the cultivar Goyou Kurakake was higher by 45%, i.e. 34% than in the control sample of the cultivars L93-7290 and Williams 82, respectively (Table 4). NISHIBA and SUDA (1998) investigated the degradation of antioxidative compounds such as vitamin E, vitamin C and lutein in agueous homogenate prepared from normal soybean seeds (Lx1,2,3) or lipoxygenase-lacking soybean seeds (triple-null mutant, Lx0) during 60 min. In contrast to our results, in the Lx1,2,3 soybean homogenate, tocopherol levels rapidly decreased during the first 5 min after which time only a slight change in the retention was observed. Among tocopherol homologues, the α-tocopherol decrease was most intensive and its retention reached about 40% in few minutes. In the contrast, their decrease was not observed in the Lx0 soybean homogenate.

The content of salt soluble proteins was the highest (134.3 mg g^{-1}) in the control sample of the cultivar Gouoy Kurakake, which was higher by 19.4% and 14.4% than in the control samples of the cultivars L93-7290 and Williams 82, respectively. Subsequent to AAT, the content of salt soluble proteins decreased in all three observed soybean genotypes. According to THOMAS *et al.*, (1989), hydrolysis of neutral lipids to free fatty acids occurs in the course of storage, and considering that protein solubility decreases in the acid medium, the opinion of

these authors is that hydrolysis of lipids during storage is one of the principle reasons for the extraction decrease of soluble proteins. According to our results, the content of salt soluble proteins subsequent to AAT of seeds of the cultivar Gouoy Kurakake was dereased by 61.3% in relation to the content in the control sample, while the decrease of this content amounted to 49.5% and 47.7% in cultivars L93-7290 and Williams 82, respectively (Fig. 1). The reactivity of products made by lipid oxidation with amino acids could also be one of the reasons for protein solubility decrease. According to GARDNER, (1979), chemical changes occurring during the interaction of hydroperoxide and proteins encompass protein-protein bonding, protein sectioning, protein-lipid bond and damaging of amino acids.

CONCLUSIONS

Peroxidation of fatty acids is a complex process which predominantly depends on the activity of very reactive free radicals, whose performance is unpredictable and cannot be fully monitored due to a great number of chain reactions. In order to reduce adverse effects of peroxidation, a great attention should be paid to this issue and especially to the possibility to control this process.

Based on gained results it can be concluded that beside the reduction of activities of isoenzyme lipoxygenase, the content of antioxidants has a very important role in the protection against lipid peroxidation and maintenance of soybean seed vigour during ageing. The seed of the cultivar Goyou Kurakake characterised by the lack of Lx2 and high contents of tochopherol α, β-carotene and chlorophyll, in contrast to the seed of the cultivars L93-7290 and Williams 82, maintained to a great extent (23%) germination ability after AA.

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UTICAJ MULTIPLIH ALELA NA OKSIDACIONU STABILNOST I KLIJAVOST SEMENA SOJE NAKON UBRZANOG STARENJA

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I z v o d

Svrha ovih istraživanja bila je da se odrede promene aktivnosti izoenzima lipoksigenaze, sadržaja antioksidanasa (tokoferola α, β-karotina i hlorofila), masnih kiselina i rastvorljivih proteina, kao i životne sposobnosti semena soje nakon ubrzanog starenja. Za istraživanja su korišćene sledeće sorte soje: Williams 82, Goyou Kurakake and L93-7290.

Nakon testa ubrzanog starenja 23% semena sorte Goyou Kurakake zadržalo je normalnu klijavost, dok je seme sorti Williams 82 i L93-7290 potpuno izgubilo klijavost. Na osnovu naših rezultata sorta Goyou Kurakake, koja se odlikuje odsustvom lipoksigenaze 2, imala je značajno viši sadržaj ispitivanih antioksidanasa (tokofherol α 2,7 mg $100g^{-1}$, β-karotina 6,1 μg g^{-1}, hlorofila 4,9 μg g^{-1}). Sadržaj β-karotina i hlorofila, nakon testa ubrzanog starenja, smanjio se za 42,8% odnosno 60,5%, dok je sadržaj tokoferol α ostao nepromenjen. Sadržaj tokoferol α ostao je nepromenjen i u semenu sorti Williams 82 i L93-7290 nakon ubrzanog starenja. Nakon testa ubrzanog starenja u semenu sorte Williams 82 sadržaj β-karotina je ostao isti, dok je u semenu sorte L93-7290 smanjen za 7,7%. S obzirom da nakon testa ubrzanog starenja nije došlo do promene sadržaja masnih kiselina kod sorte Goyou Kurakake, može se zaključiti da antioksidacioni sistem ima značajnu ulogu u zaštiti od lipidne peroksidacije.

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