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ANTIOXIDANT PROPERTIES OF SOYBEAN SEEDLINGS GROWN IN DIFFERENT SOIL TYPES*

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SUMMARY: The purpose of this work was to assess change in antioxidant ability and accumulation of antioxidants in soybean seedlings, as well as some morphological parameters (plant height, shoot and root mass) during abiotic stress. Activity of antioxidant enzymes [superoxide dismutase (SOD; EC 1.15.1.1) and peroxidases (GPX-guaiacol peroxidase and PPX-pyrogallol peroxidase, EC 1.11.1.7)], phenylalanine ammonia lyase (PAL; EC 4.3.1.5), as well as content of non-enzymatic antioxidants (total phenolics, tannins and flavonoids) were analyzed in leaves of soybean seedlings grown in different soil types [mollic vertisol (humic); calcic chernozem (calcic) and haplic fluvisol (calcaric)]. Seedlings grown in fluvisol had significantly higher activity of SOD (7.5-8.6%) and PAL (39.2-43.0%) in leaves, as well as total phenolics and tannins content (8.1-80.7%) in comparison to those grown in chernozem and vertisol. However, soybean grown in chernozem had the most favourable conditions for growth and the highest total flavonoids content (2.3 mg rutin g^{-1} dry weight). Regardless the difference in response to higher and lower level of soil fertility, soybean seedlings grown in vertisol and fluvisol had similar morphological properties, which showed the great ability of their antioxidant systems to acclimate to change in soil quality.

Key words: *abiotic stress, antioxidants, Glycine max L., soil types*

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INTRODUCTION

The coordination between multiple pathways that reside in different cellular compartments is required due to acclimation of plants to changes in their environment, such as water and salt stresses, especially when the cell or the entire plant is exposed to a rapid decrease in water potential, or when additional environmental parameters are involved (Mittler et al., 2006). When different pathways are uncoupled, electrons that have a high-energy state are transferred to molecular oxygen (O_2) to form reactive oxygen species (ROS; Mittler, 2002). ROS, such as $^1O_2$, H_2O_2, $O_2^{·-}$ and HO·, are toxic molecules capable of causing oxidative damage to proteins, DNA and lipids (Apel and Hirt, 2004). Antioxidants such as ascorbic acid and glutathione (GSH), as well as ROS-scavenging enzymes such as superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT) and glutathione peroxidase (GPX) are essential for ROS detoxification (Apel and Hirt, 2004; Mittler et al., 2004; Dietz et al., 2006). Phenylalanine ammonia lyase (PAL, EC 4.3.1.5) is the entry-point enzyme into the phenylpropanoid pathway responsible for synthesis of plant phenylpropanoids or phenolics, many of which play important roles in plant defense and present important non-enzymatic antioxidants (Lawton and Lamb, 1987; Gerasimova et al., 2005; Lattanzio et al., 2006). Polyphenols and flavonoids suppress ROS formation by inhibiting enzymes, chelating trace elements involved in free-radical production, scavenging reactive species and upregulating or protecting antioxidant defenses (Choi et al., 2002; Grassmann et al., 2002).

In the northern Serbia, soybean, *Glycine max* L., represents one of the most frequently grown cultivar. It has been determined that variability of soil properties affects yield of soybean (Kravchenko and Bullock, 2000; Cox et al., 2003; Jiang and Thelen, 2004). The decline in soil moisture also results in a decrease in the diffusion rate of nutrients in the soil to the absorbing root surface (Hu and Schmidhalter, 2005). The purpose of this study was to investigate possible differences in activity of superoxide dismutase, peroxidases, phenylalanine ammonia lyase and contents of polyphenolics in leaves, as well as some morphological parameters of soybean seedlings grown in soils with different soil properties, with a view to assessing the ability of soybean antioxidant system to acclimate to change in soil quality without affecting the development at this stage of growth.

MATERIAL AND METHODS

Soil properties. The experiment was conducted on three different soil types (WRB, 2006): mollic vertisol (humic); calcic chernozem (calcic) and haplic fluvisol (calcaric). Soil samples were taken from chernozem, vertisol and fluvisol with soil probe from the surface horizon (0-30 cm) with three replicates approximately 10 m apart. Soil samples were air dried and sieved through a 2 mm sieve (0.2 mm for organic matter content analysis). Cylinders ($V=100\ cm^3$) was applied to take undisturbed soil samples for measuring bulk density. Soil texture was determined by sieving using the pipette method, with sodium pyrophosphate as a dispersing agent. Soil water retention was measured using a porous plate and pressure membrane apparatus at matric potentials of -33 and -1500 kPa. Soil pH value is determined potentiometrically in a 1:2.5 soil-distilled water (pH-H_2O) and soil-1M KCl solution (pH-KCl). Scheibler apparatus was applied for gasvolumetrically measuring of $CaCO_3$ content. Soil organic matter content was determined by a dichromate wet oxidation method.

Plant material. Seeds of soybean (*Glycine max* L.) cultivar Bečejka were obtained from the Institute of Field and Vegetable Crops, Novi Sad (Serbia). Seeds were sown at 10 cm depth. The experimental design was a randomized block with four replications. The sowing was done in an area of 34.5 × 16.5 m. The length of the experimental unit was 8 m and the width was 3.5 m. Plant material for biochemical and morphological analyses represented soy leaves (n=40 plants) sampled at the stage of the second trifoliolate (V2) (Fehr and Cavines, 1977).

Weather conditions of the experimental site. Average monthly air temperatures and precipitation on the experimental field at experimental sites in 2011 were presented in Figure 1. These results point to months of drought in this year.

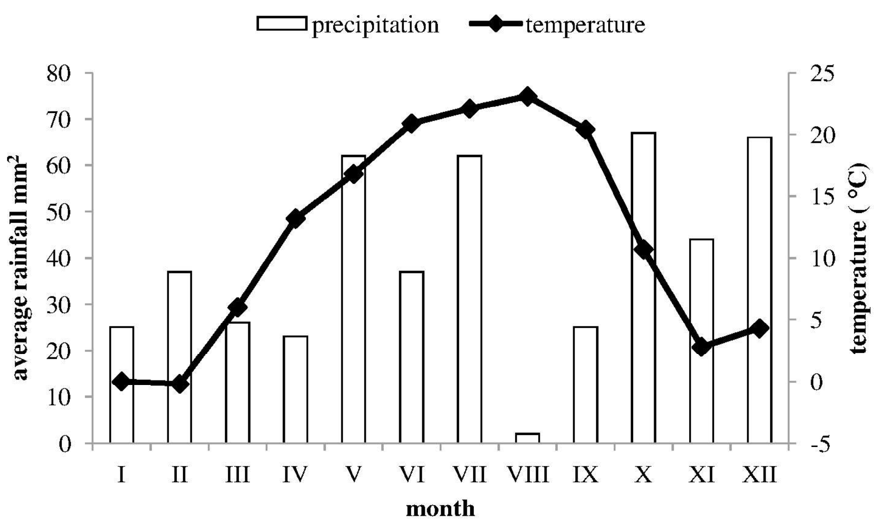

Figure 1. Average monthly air temperatures and precipitation (primary and secondary y-axis, respectively) in the experimental field (86 m above sea level).

Enzyme extracts. Superoxide dismutase (SOD; EC 1.15.1.1) activity was measured by monitoring the inhibition of nitroblue tetrazolioum (NBT) reduction at 560 nm (Mandal et al. 2008). Peroxidase (EC 1.11.1.7) activity was measured using guaiacol (guaiacol peroxidase; GPX) and pyrogallol (pyrogallol peroxidase; PPX) as substrates according to Morkunas and Gmerek (2007). Phenylalanine ammonia lyase (PAL; EC 4.3.1.5) activity was performed according to Gerasimova et al. (2005). SOD, PPX, GPX, PAL and activities were expressed in U g^{-1} fresh weight (fw).

Polyphenols contents. Total polyphenols and tannins content of acetone extract of dry leaves and roots were determined by the Folin-Ciocalteu procedure (Hagerman et al., 2000) and expressed as mg catechin g^{-1} dry weight (dw). The amount of flavonoids in methanolic extract of dry leaves and roots was performed by method described by Markham (1989) and expressed as mg rutine g^{-1} dw.

Statistical analyses. Assessed values of the biochemical (determinations made in triplicates) and morphological (measures obtained from 40 plants) analyses were expressed as means ± standard error and tested by ANOVA followed by comparison of the means by Duncan's multiple range test ($P<0.05$). Correlation between PAL activity and contents of total polyphenols, tannins and flavonoids was assessed by correlation analyses and expressed by coefficient of correlation (r). Data were analyzed using STATISTICA for Windows version 11.0.

RESULTS AND DISCUSSION

Soybean yield components are highly quantitative traits with low heritability, and influenced by differences arising from soil heterogeneity and environmental factors (Manavalan et al., 2010). Soil types used in this experiment have substantially different characteristics (Table 1).

Table 1. Basic properties of investigated soil types.

Soil type		Chernozem	Vertisol	Fluvisol
Elevation (m)		80	75	80
Location	N	45°20'31"	45°18'07"	45°14'50"
	E	19°51'26"	21°03'32"	19°51'02"
Depth (cm)		0-30	0-30	0-30
Texture (%)	CS	0	1.6	1.8
	FS	36.2	26.7	39.4
	S	30.2	31.6	36.7
	C	33.6	40.1	22.1
OM (%)		2.54	3.66	1.87
$CaCO_3$ (%)		0.38	0.33	3.71
pH	KCl	6.01	5.69	7.17
	H_2O	7.02	6.48	8.01
N (%)		0.127	0.183	0.094
BD (g cm^{-3})		1.57	1.22	1.43
Water retention (kPa)	-33	27.5	32.8	25.1
	-1500	15.3	16.3	11.8

(CS: Coarse sand; FS: Fine sand; S: Silt; C: Clay; OM: Organic matter; BD: Bulk density)

Chernozem is considered as moderate humic, slightly calcareous, neutral soil, with coarser texture than vertsol, while fluvisol showed coarser texture than chernozem, low humic content, medium calcareous and alkaline pH reaction. Observed vertisol is a fine-textured, very humic, non-calcareous and acidic soil. Chernozem and vertisol have more favourable soil structure than fluvisol (Ćirić et al., 2012), which provides better conditions for plant growth. Investigated vertisol and chernozem are developed at loess terraces, while fluvisol is formed at Danube river plain. Considering presented properties of soil we can emphasize that vertisol and chernozem has high level of fertility. Fluvisol has lower fertility due the low organic matter content, relatively high pH reaction and unfavourable structure.

Under optimal growth conditions, ROS are mainly produced at a low level in organelles such as chloroplasts, mitochondria and peroxisomes, while during stress, the rate of ROS production is dramatically elevated. ROS accumulation during stress greatly depends on the balance between ROS production and ROS scavenging (Mittler et al., 2004), that in turn depends on changes in growth conditions (light intensity, drought, salt and temperature stress, etc.), as well as the severity and the duration of the stress and the ability of the tissue to rapidly acclimate to the energy imbalance. Superoxide anion ($O_2^{\cdot-}$) is usually rapidly dismutated, either non-enzymatically or via SOD, to relatively stable H_2O_2, which can produce $^{\cdot}$OH radical in the presence of divalent metal ions as Fe^{2+} (Delledonne, 2005). Activity of SOD was markedly increased in soybean leaves grown in fluvisol (Figure 2), contrary to that grown in chernozem and vertisol where peroxidases were significantly active (PPX and GPX, respectively). The $^{\cdot}$OH radical initiates chain reaction, including LP, enzyme inactivation and degradation of nucleic acids (Mehdy, 1994). Furthermore, plant peroxidases trigger conversion of H_2O_2 to water and oxygen, which gives them an important role in plant defense system (Almagro et al., 2009). PAL has been extensively studied in plants because of its decisive function in the biosynthesis of many secondary metabolites (Liu, 2006). Our results showed that PAL activity in leaves of soybean grown in fluvisol was significantly higher comparing to PAL activity in leaves of soybean grown in chernozem and vertisol (Figure 2).

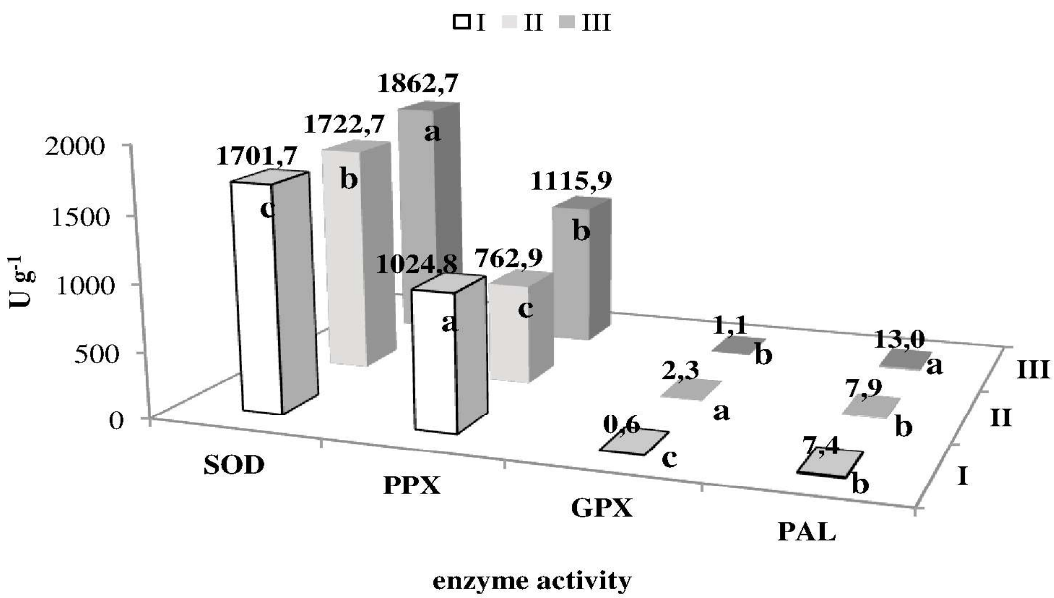

Figure 2. Activity of superoxide dismutase (SOD), peroxidases [pyrogallol (PPX) and guaiacol (GPX)] and phenylalanine ammonia lyase (PAL) in leaves of soybean seedlings grown in chernozem (I), vertisol (II) and fluvisol (III). Values marked with different letter differ significantly at $P<0.05$ (Duncan's test).

Also, total polyphenolics and tannins content were higher in leaves of soybean seedlings grown in fluvisol and vertisol, in comparison to that grown in chernozem, but positive correlation between PAL activity and polyphenolics content (total polyphenolics $r=0.90$, total tannins $r=0.69$ and total flavonoids $r=0.72$) was determined only in plants grown in vertisol (Figure 3). However, total flavonoids content assessed in leaves of soybean grown in chernozem, was also in a positive correlation with PAL activity (r=0.86) (Figure 3).

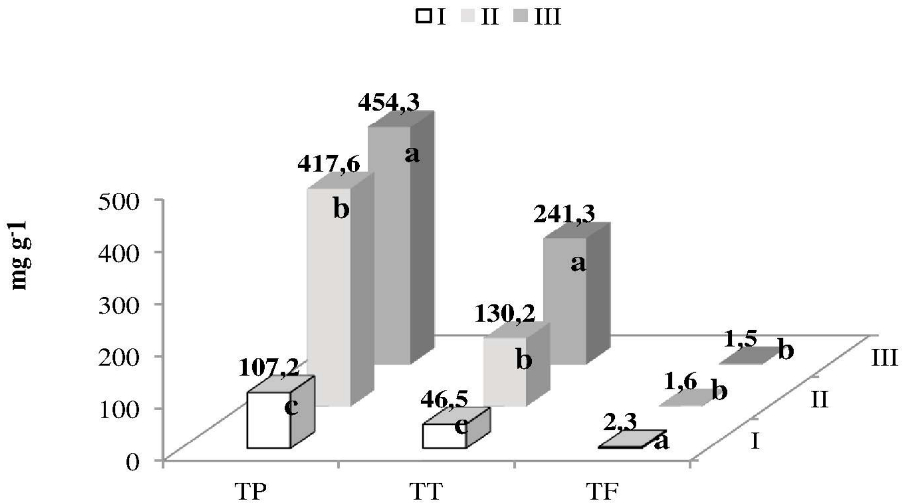

Figure 3. Total polyphenols (TP), tannins (TT) and flavonoids (TF) contents in leaves of soybean seedlings grown in chernozem (I), vertisol (II) and fluvisol (III). Values marked with different letter differ significantly at $P<0.05$ (Duncan's test).

According to morphological analyses, soybean seedlings grown in chernozem had significantly higher values of shoot and root mass in comparison to those grown in vertisol and fluvisol (Table 2), yet different soil types did not changed the height of these plants (Table 2).

Table 2. Morphological properties of soybean seedlings grown in different soil types. Results represent a mean ± standard error
(SE) and coefficient of variation (CV) (n=40). Results marked with different letters differ significantly at $P<0.05$ (Duncan's test).

	Chernozem			Vertisol			Fluvisol		
	x	SE	*CV*	*x*	SE	*CV*	*x*	SE	*CV*
Plant height (cm)	13,4 a	0,4	9,4	12,0 a	0,5	14,7	14,7 a	0,4	9,1
Shoot mass (g)	10,7 a	1,6	49,8	2,4 b	0,1	16,1	2,7 b	0,2	20,2
Root mass (g)	2,0 a	0,2	41,0	0,36 b	0,02	21,1	0,81 b	0,07	28,6

CONCLUSION

Leaves of soybean plants grown in soil with low level of fertility (fluvisol) had enhanced activity of SOD and PAL, while those grown in soil with higher fertility (chernozem and vertisol) had enhanced activity of peroxidases, which could point to different response of soybean antioxidant system to abiotic factors when grown in soils of different quality. Chernozem stimulated accumulation of flavonoids in soybean leaves and development of soybean plants. Regardless the difference in response to higher and lower levels of soil fertility, soybean seedlings grown in vertisol and fluvisol had similar morphological properties, which showed the great ability of their antioxidant systems to acclimate to change in soil quality.

REFERENCES

ALMAGRO, L., GOMEZ ROS, L.V., BELCHI-NAVARRO, S., BRU, R., ROS BARCELO, A., PEDRENO. M.A.: Class III peroxidases in plant defence reactions. J. Exp. Bot., 60:377–390, 2009.

APEL, K., HIRT., H.: Reactive oxygen species: metabolism, oxidative stress, and signal transduction. Annu. Rev. Plant Biol., 55:373–399, 2004.

CHOI, W.C., KIM, S.C., HWANG, S.S., CHOI, B.K., AHN, H.J., LEE, M.Y., PARK, S.H., KIM, S.K.: Antioxidant activity and free radical scavenging capacity between Korean medicinal plants and flavonoid by assay-guided comparison. Plant Sci., 163:1161-1168, 2002.

COX, M.S., GERARD, P.D., WARDLAW M.C., ABSHIRE, M.J.: Variability of selected soil properties and their relationships with soybean yield. Soil Sci. Soc Am. J., 67(4)1296-1302, 2003.

ĆIRIĆ, V., MANOJLOVIĆ, M., NESIĆ LJ., BELIĆ M.: Soil dry aggregate size distribution: effects of soil type and land use. J. Soil Sci. Plant Nutri., 12(4)689-703, 2012.

DELLEDONNE, M.: NO news is good news for plants. Curr. Opin. Plant Biol., 8:390-396, 2005.

DIETZ, K.J., JACOB, S., OELZE, M.L., LAXA, M., TOGNETTI, V., DE MIRANDA, S.M., BAIER, M., FINKEMEIER, I.: The function of peroxiredoxins in plant organelle redox metabolism. J. Exp. Bot., 57:1697–1709, 2006.

GERASIMOVA, N.G., PRIDVOROVA, S.M., OZERETSKOVSKAYA, O.L.: Role of L-phenylalanine ammonia lyase in the induced resistance and susceptibility of potato plants. App. Biochem. Microbiol., 41:103–105, 2005.

GRASSMANN, J., HIPPELI S., ELSTNER, E.F.: Plant`s defence and its benefits for animals and medicine: role of phenolics and terpenoids in avoiding oxygen stress. Plant Physiol. Biochem., 40:471-478, 2002.

JIANG, P., THELEN K.D.: Effect of soil and topographic properties on crop yield in a north-central corn–soybean cropping system. Agron. J., 96(1)252-258, 2004.

KRAVCHENKO, A.N., BULLOCK, D.G.: Correlation of corn and soybean grain yield with topography and soil properties. Agron. J., 92(1)75-83, 2000.

LATTANZIO, V., LATTANZIO, V.M.T., CARDINALI, A.: Role of phenolics in the resistance mechanisms of plants against fungal pathogens and insects. In Phytochemistry: Advances in Research, ed Imperato, Research Signpost: India, 2006.

LAWTON, A.M., LAMB., J.C.: Transcriptional activation of plant defense genes by fungal elicitor, wounding and infection. Mol. Cell Biol., 7:335-341, 1987.

LIU, R., XU, S, LI, J., HU, Y., LIN, Z.: Expression profile of a PAL gene from *Astragalus membranaceus* var. *Mongholicus* and its crucial role in flux into flavonoid biosynthesis. Plant Cell Rep., 25:705–710, 2006.

MANDAL, S., MITRA, A., MALLICK, N.: Biochemical characterization of oxidative burst during interaction between *Solanum lycopersicum* and *Fusarium oxysporum* f. *sp. lycopersici*. Physiol. Mol. Plant P., 72:56-61, 2008.

MEHDY, M.C.: Active oxygen species in plant defense against pathogens. Plant Physiol., 105:467-472, 1994.

MITTLER, R.: Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci., 7:405–410, 2002.

MITTLER, R., KIM, Y., SONG, L., COUTU, J., COUTU, A., CIFTCI-YILMAZ, S., LEE, H., STEVENSON B., ZHU, J.K.: Gain- and loss-offunction mutations in Zat10 enhance the tolerance

of plants to abiotic stress. FEBS Letters, 580:6537–6542, 2006.
MITTLER, R., VANDERAUWERA, S., GOLLERY M., VAN BREUSEGEM, F.: Reactive oxygen gene network of plants. Trends Plant Sci., 9:490–498, 2004.
MORKUNAS, I. GMEREK J.: The possible involvement of peroxidase in defense of yellow lupine embryo axes against *Fusarium oxysporum*. Journal Plant Physiol., 164:185-194, 2007.
WRB: A Framework for International Classification, Correlation and Communication. Food and Agriculture Organization of the United Nations, Rome, 2006.

ANTIOKSIDANTNE KARAKTERISTIKE KLIJANACA SOJE GAJENIH NA RAZLIČITIM TIPOVIMA ZEMLJIŠTA

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Izvod

Cilj ovog rada bio je da se utvrdi da li akumulacija antioksidanata i promene u antioksidantnoj sposobnosti u klijancima soje utiču na morfološke parametre (visinu biljke i masu nadzemnog i podzemnog dela biljke). Analizirane su promene u aktivnosti antioksidantnih enzima [superoksid-dismutaze (SOD; EC 1.15.1.1) i peroksidaza (GPX-gvajakol i PPX-pirogalol peroksidaze, EC 1.11.1.7), fenilalanin amonijum-lijaze (PAL; EC 4.3.1.5), kao i sadržaju neenzimskih antioksidanata (ukupnih fenola, tanina i flavonoida) u listu klijanaca soje gajene na različitim tipovima zemljišta [ritska crnica, černozem i fluvisol]. U listu klijanaca soje gajenih na fluvisolu zabeležena je značajno veća aktivnost superoksid-dismutaze (7.5-8.6%) i fenilalanin amonijum-lijaze (39.2-43.0%, kao i sadržaj ukupnih fenola i tanina (8.1-80.7%) u odnosu na klijance gajene na černozemu i ritskoj crnici. Ipak, klijanci soje gajeni na černozemu imali su najbolje uslove za rast i razvoj, kao i najveći sadržaj flavonoida (2.3 mg rutina g^{-1} suve mase). Pored toga, morfološke karakteristike klijanaca soje gajenih na ritskoj crnici i fluvisolu se nisu značajno razlikovale, što navodi na zaključak na izuzetno izraženu adaptabilnost antioksidantnog sistema ovih biljaka na promene u kvalitetu zemljišta.

Ključne reči: abiotički stres, antioksidanti, Glycine max L., tipovi zemljišta

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