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Effects of Root Rot in Soybean Cultivars with Diverse Susceptibility to the Disease on Plant Physiology, Yield, Amino Acids and Mycotoxins Profile in Climatic Conditions of Kazakhstan

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Abstract: Soybean is an important high-value crop but susceptible to soil-borne diseases. A field trial was conducted in Kazakhstan to evaluate the effects of soybean cultivars and *Fusarium* root rot severity on soybean crop physiology, chlorophyll fractions and amino acids profile. The highest yield was achieved in 2018 (up to 4 t ha^{-1}) for K-9648 cultivar. The greatest root rot severity (90%) and the lowest plant high (20.8 cm), number of beans per plant (54) were determined in 2020 and were related with the highest precipitations. Moreover, leaf position was crucial in determination of chlorophyll fractions, among which the highest values were achieved in 4rd leaf. Average concentration of non-essential, essential, aromatic and aliphatic amino acids were correlated with root rot development at flowering ($r = 0.73$) and maturing stage ($r = 0.54$), root rot severity ($r = 0.64$) and precipitations ($r = 0.86$). Thus, amino acids play the crucial role in defense proteins biosynthesis against biotic stress factors. Interestingly, despite *Fusarium* root rot occurrence, there were not determined mycotoxins in seeds, which indicate that they are not distributed to other organs from the place of their biosynthesis. This study indicated that among twelve soybean cultivars, Tanais and Isidor cultivars are at least susceptible to *Fusarium* root rot despite heavier rainfalls in 2020 and in this regard could be implemented to agriculture in the agro climatic conditions of Kazakhstan. However, in aspect of yield and desired amino acids profile, K-9648 cultivar was the most productive in 2018-2020.

Keywords: Amino Acids, Multispeq, Plant Physiology, Root Rot, Soybean

Introduction

Soybean is one of the most important agronomic crops drawing greater attention worldwide due to increasing demand on its products (e.g., oil, soy milk, soy protein, lecithin, tofu or soy sauce). Moreover, soybean is a rich source of protein and therefore is used as a feed in animal nutrition. Total Amino Acids (AA) content in soybean is up to 42% of dry mass (Assefa *et al*., 2018), far more than any other plant from legumes. However, its content may be diverse depending on cultivar, climatic conditions and fungal disease development. Soybean is susceptible to various diseases, mainly from fungal ones (Fones *et al*., 2017; Kalaitzandonakes *et al*., 2019; Li *et al*., 2019). Common fungal diseases are sudden death syndrome, damping-off and root rot, which are caused by *Fusarium oxysporum* (Cruz Jimenez *et al*., 2018; Chang *et al*., 2018; McLaren and Callahan. 2020), *F. equiseti* (Kuldybayev *et al*., 2019; You and Barbetti, 2017), *F. virguliforme* (Wang *et al*., 2019) under certain environmental conditions. Soil-borne diseases contribute to the decrease of roots length and weight, length of plant, weaken general plant condition and in consequence contribute to lower yield (Dutbayev *et al*., 2020c). Infections caused by *Fusarium* spp. can impact on photosynthesis (Dutbayev *et al*., 2020a) and therefore, understanding how soybean cultivars impact on photosynthetic properties is critical to develop management practices sustaining healthy and high-yield soybean (Abdulmajeed and Qaderi, 2019; Becklin *et al*., 2016; Nelson *et al*., 2018). Another negative effect of

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Science Publications

Fusarium root rot is mycotoxins secretion and contamination of soy-originated food and feed. Main mycotoxins determined in soybean are *fumonisins*, aflatoxins, deoxynivalenol (Gutleb *et al*., 2015).

Basic procedure preventing fungi development is fungicides application. However, in conjunction with worldwide tendency to limiting pesticides usage, ecological methods of cultivation are gaining in importance. Additionally, there were also implemented biological methods of protection, e.g., based on *Trichoderma* isolates, which inhibit *Fusarium virguliforme* growth, reduce root rot and induce defense-related genes in soybean seedlings (Pimentel *et al*., 2020) However, one of the milestones of effective, pesticides-free tillage is appropriate selection of soybean cultivars resistant to diseases in particular climatic conditions(Raza, 2019; Nyandoro *et al*., 2019; Sjarpe *et al*., 2020; Abdukadirova *et al*., 2016; Abugalieva *et al*., 2016).

Plant phenotyping is becoming increasingly important in plant biology and agriculture, however, the application of this approach is limited by long-term analytical techniques and processing of the results (Kuhlgert *et al*., 2016). One of the direction of non-disrupting analytical techniques is MultispeQ device designed for large-scale collection of high-quality plant health data and non-invasive evaluation of plant physiological conditions (Kuldybayev *et al*., 2019, 2020; Dutbayev *et al*., 2020b, 2020c; Zatybekov *et al*., 2018). It can be simply use for the determination of soybean cultivars photosynthetic status in the response to root rot. MultispeQ is dedicated for the evaluation of LEF (linear electron flow - the determinant of the electron transfers order), NPQt (non-photochemical quenching - chlorophyll protection system from the adverse effect of high light intensity), PSII (Photosystem II - the fraction of light energy captured by Photosystem II which is directed towards Photochemistry to make ATP and NADPH), Photo Synthetically Active Radiation (PAR).

Taking under consideration facts above, the objective of our study was to evaluate the influence of root rot in twelve soybean cultivars with diverse susceptibility to the disease on plant physiology, yield, amino acids and mycotoxins profile in changing climatic condition of Kazakhstan.

Materials and Methods

Experimental Design

A field study in completely randomized design was conducted on twelve soybean cultivars with different susceptibility to *Fusarium* root rot. Seeds were sown in triplicate, on May 20th-24th, 2018-2020 in the Aktobe Agricultural Experimental Station (Aktobe, Kazakhstan; 50° 16′ 0″ N, 57° 13′ 0″ E). The dimensions of each plot were 6 × 2 m, with an inter-plot strip 30 cm wide. The soil of the experimental field was dark chestnut, medium loamy in texture. The humus content in the upper soil layer (0-20 cm) was 2.74%. The soybean cultivation was not fertilized and any chemical protection was applied. Soybean was harvested manually on September 7th-18th, 2018-2020. Temperature and precipitation data of the vegetative season are presented in Fig. 1.

Root Rot, Yield and Plant Physiology Assessment

Root rot development in soybean was determined at the flowering and maturing stage, visually from 25 randomly collected plants, according to the formula:

$$R = ab \times 100 / AK$$

where: “a” is the number of plants with the same damage; “b” is the corresponding lesion score; “A” is the number of plants in the count; “K” is the highest score of defeat. Root rot severity in 25 plants of each repetition was evaluated visually, in three-point scale (healthy <10%, low 10-20%, moderate and high >20%) according to EPPO (European and Mediterranean Plant Protection Organization) recommendations.

Plant height (length from the root neck to the tip of the central stem, cm), height of the lower bean (length from the root neck to the attachment point of the lowest bean, cm) was determined using tape measure, while the number of beans per plant, weight of 1000 beans (g) and yield (t ha^{-1}) was evaluated using seed meter (Arlab, Koszalin, Poland).

Photosynthetic Parameters Determination

Measurements of photosynthetic parameters were conducted on randomly collected plants (n = 25) from each plot at flowering and maturing growth stages, using Multispeq 1.0 device. It was equipped with a relative humidity, temperature and CO_2 sensor. The following parameters were examined: LEF (linear electron flow - the determinant of the electron transfer order), NPQt (non-photochemical quenching - chlorophyll protection system from the adverse effect of high light intensity), PSII (Photosystem II - the fraction of light energy captured by Photosystem II which is directed towards Photochemistry to make ATP and NADPH), PAR (photosynthetically active radiation) and relative chlorophyll content according to Kuhlgert *et al*. (2016).

Amino Acids Determination

Amino acids standards: Alanine, arginine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine were obtained from Sigma-Aldrich (St. Louis, USA). Individual stock solutions were prepared in 1% formic acid in water at a concentration of 1 mg mL^{-1}. Standard mixture was prepared at the concentrations 0.01-10 µg mL^{-1} and was stored at 4°C.

Soybean was milled in the laboratory mill, weighted (1 g) and mixed with 10 mL of water-methanol solution

(8:2, v/v) with 0.1% formic acid. Samples were vortexed for 5 min and centrifuged for 10 min at 10000 rpm. Extracts (1 mL) were filtered through a 0.22 µm hydrophilic PTFE filter, transferred into the vial and analyzed via LC-MS/MS followed by the validation according to the Document No. SANTE/11813/2017 (EC, 2017).

An Eksigent Ultra LC-100 (Eksigent Technologies, Dublin, CA, USA) liquid chromatography system was used (flow rate 0.5 mL min^{-1}) with KINETEX HILIC 1.7 µm, 2.1 × 50 mm (Phenomenex) column, kept at 40°C during analysis. The purified extract (2 µL) was injected into the LC-MS/MS. The mobile phases consisted of water + 0.2% formic acid + 20 mM ammonium formate (phase A) and acetonitrile (phase B). The experiment started at 5% A/95% B for 1 min, raised to 10% A/90% B in 2 min., then to 95% A/5% B in 3.5 min and was held for 1.5 min. Next, the mobile phase components were restored to the initial conditions and were held for 3 min. System MS/MS 6500 QTRAP (AB Sciex Instruments, Foster City, CA) was operated for mass spectrometric analysis, equipped with an Electrospray Ionization Source (ESI). The capillary voltage was maintained at 4000V for positive ion. The temperature of the turbo heaters was set at 400°C. Nitrogen was used as nebulizer Gas (GS1), auxiliary Gas (GS2) and Curtain gas (CUR) at a pressure of 50, 60 and 40 psi, respectively. Nitrogen was also used as the nebulizer and collision gas. All amino acids were detected in the Multiple Reaction Monitoring mode (MRM).

Mycotoxins Determination

Mycotoxins: Deoxynivalenol (DON) and its acetylated forms (3-AcDON, 15-AcDON), Nivalenol (NIV), Zearalenone (ZON), Diacetoxyscirpenol (DAS) Fusarenon X (FusX), T-2, HT-2 and Fumonisins (Fum B1, Fum B2, Fum B3), Neosolaniol (NEO) were obtained from LGC (Wasel, Germany). Individual stock solutions were prepared in acetonitrile/water (1:1, v/v) at a concentration of 1 mg mL^{-1} and were used to obtain a standard mixture at the 0.1-1000 $\mu g mL^{-1}$ concentrations. The standard mixture was stored at -18°C.

Mycotoxins were extracted using QuEChERS method and analyzed via LC-MS/MS based on Nugmanov *et al*. (2018 protocol), followed by the validation according to the Document No. SANTE/11813/2017 (EC, 2017).

Statistical Analysis

Data statistical processing was performed using the Statistica 12.0 program. Analysis of Variance (ANOVA) was conducted and Pearson's correlation coefficients were indicated based on Principal Component Analysis (PCA). The statistical significance was established as $p \leq 0.05$.

Results

Climatic Conditions and Root Rot Development

Aktobe region is located almost in the center of the Eurasian continent. This determines the features of a sharply expressed continental climate with high continentality, which increases from the northwest to the southeast. In the summer, dry overheated tropical air masses are removed from the south to the region from the deserts of Central Asia and Iran and from the north - northern, arctic air masses from beyond the Urals. The average temperatures in vegetative seasons were 20.2, 21.0, 21.2°C, while precipitations were 19.5, 15.1, 31.1 mm in 2018-2020, respectively (Fig. 1). Our results indicated the year climatic conditions more impacted on soybean root rot index than cultivar dependent resistance. The development of soybean root rot was observed from seedling stage. The analysis of general distribution of root rot severity during plant flowering showed diversified values between 2018-2019 (10.8-30.3, 25-56%, respectively) and 2020 (6.9-12.5%). Meanwhile, during soybean maturing the root rot development was significantly higher in 2018 and 2020 (19.4-50.5%) but lower in 2019 (35.2-60.5%) (Fig. 2).

Crop Physiology

Results of the study indicated the highest yield in 2018 (up to 4 t ha-1), which was correlated with climatic conditions, root rot severity and development. In three-year study, generally the greatest soybean yield was determined in healthy plants and gradually decreased achieving the lowest values in plants with middle and high root rot severity (up to 1.4 t ha-1 in 2020 for Cheremosh cultivar). Low level of root rot caused yield loses up to 5.4%, while for middle and high level yield losses varied between 12.1-38.7% (Table 1). In 2018-2020 the highest weight of 1000 beans was determined in Cheremosh, Tanais and Samer 5 soybean cultivars, with 121.9-278 g losses from Fusarium root rot between 79.4 and 105.6 g for each 1000 beans. The average weight of 1000 beans was the lowest in Anastasya, Samer 1, Samer 3 and Swapa cultivars (95.6-167.3 g) with losses caused by root rot 11-60.7 g per each 1000 beans. These indexes at Belor, Isidor, Maple Ridge and Samer 2 were 115.3-195.4 g with losses 33.9-82.7 g. There were observed significant higher differences in root rot development in maturing stage (up to 60.5% in 2019) (Fig. 2).

Root rot severity was significantly correlated with precipitation (r = 0.61), sum of free amino acids (r = 0.61) and root rot development at flowering stage (r = 0.67) (Table 2 and Fig. 3) and impacted on reduced plant height (up to 21.6 cm), number of beans per plant (up to 8.2), weight of 1000 beans (up to 92.5 g) and yield but contributed to the increase of lower bean height (up to 13.2 cm) (Table 1).

Photosynthetic Properties

In 2018-2020, leaf position impacted to chlorophyll fractions, LEF and PAR. It was determined higher values of PhiNPQ (0.34), NPQt (1.2) and LEF (174) in 4th leaf, while PAR reached greater values in 3rd (48). The general distribution of these variables was in normal range, thus ANOVA was used ($p<0.001$). Interaction of root rot and genotypes significantly impacted to Phi2 chlorophyll fractions numerical variables. There were not observed significant differences of PhiNPQ, NPQt, LEF, PAR chlorophyll fractions between soybean cultivars. The impact of soybean leaf position on photosynthetic parameters in 2018-2020 is given in the Table 3.

Amino Acid and Mycotoxin Profile of Soybean Cultivars

Among analyzed soybean cultivars the greatest value of the sum of Free Amino Acids (FAA) was obtained in K-9648 cultivar (5024.2 µg kg^{-1}). Most of cultivars had high values of the sum of FAA (above 1000 µg kg^{-1}), except Cheremosh (725.8 µg kg^{-1}) and Samer 2 (862.5 µg kg^{-1}). Moreover, among tested cultivars, K-9648 was the most abundant in non-essential, essential and aliphatic FAA (2684.6, 2339.6 and 468.7 µg kg^{-1}, respectively). However, Samer 1 cultivar had the highest value of aromatic FAA (956 µg kg^{-1}) (Fig. 4). All groups of FAA were positively correlated with temperature (r = 0.53), precipitation (r = 0.86), root rot development (r = 0.77) and severity (r = 0.64) (Table 2).

Despite diversified level of root rot development, the level of following mycotoxins: DON, 3-AcDON, 15-AcDON, NIV, ZON, DAS, FusX, T-2, HT-2, FumB1, FumB2, FumB3, NEO was under LOD (limit of detection, <0.001 µg kg^{-1}) in soybean samples.

Table 1: Physiological parameters of 12 soybean cultivars in 2018-2020

Cultivar	Year	Root rot severity	Number of plants, %	Plant height, cm	Height of lower bean, cm	Number beans per plant	Weight of gram 1000 beans weight	Yield (t ha^{-1})
Anastasya	2018	healthy	22	44.3	7.3	30.3	156.0	3.4
		low	41	38.2	7.7	17.7	147.3	3.2
		middle and high	37	35.8	8.9	15.2	100.3	2.9
	2019	healthy	21	45.3	7.7	30.3	156.3	3.1
		low	38	43.3	7.6	27.4	145.4	2.8
		middle and high	41	35.1	9.5	15.2	95.6	2.4
	2020	healthy	2	41.95	5.1	40	124.9	3.1
		low	20	35.5	5.8	18.2	116.2	2.9
		middle and high	78	30	10.1	11.7	101.5	2.5
Belor	2018	healthy	13	47.3	6.0	43.0	179.7	3.5
		low	7	35.9	6.8	26.0	166.3	3.3
		middle and high	80	27.9	9.6	10.5	130.0	3.1
	2019	healthy	17	47.5	6.8	43.0	179.5	3.3
		low	18	45.4	6.5	36.0	166.5	3.1
		middle and high	65	27.0	6.6	10.0	130.0	2.7
	2020	healthy	4	30.75	7.3	49.0	165.1	3.1
		low	17	22.26	6.6	12.5	142.4	2.8
		middle and high	79	21.6	7.1	10.1	128.4	2.6
Cheremosh	2018	healthy	23	37.8	5.7	34.2	220.8	2.3
		low	24	33.0	5.6	24.2	203.2	2.0
		middle and high	53	23.7	6.4	9.3	167.3	1.8
	2019	healthy	21	37.9	5.7	35.2	220.8	2.1
		low	25	33.0	5.6	33.2	213.5	1.8
		middle and high	54	23.7	6.4	9.3	167.3	1.5
	2020	healthy	20	34.5	5.9	32.7	215.8	1.8
		low	24	32.7	6.1	30.1	154.7	1.6
		middle and high	56	21.2	6.3	8.6	141.6	1.4
Isidor	2018	healthy	33	31.1	5.3	24.5	152.7	3.6
		low	17	31.7	5.0	24.7	143.3	3.3
		middle and high	50	22.3	6.0	9.0	118.0	2.9
	2019	healthy	33	32.5	5.3	25.5	155.4	3.4
		low	25	32.0	5.2	25.1	165.1	3.2
		middle and high	42	21.7	6.5	8.9	115.3	2.7
	2020	healthy	30	30.0	5.4	23.5	143.9	3.2
		low	22	27.3	5.6	20.8	136.1	2.9
		middle and high	48	20.8	5.8	7.8	123.5	2.7
Maple Ridge	2018	healthy	20	34.6	6.1	24.6	194.4	3.5
		low	24	32.2	6.7	17.7	180.3	3.2
		middle and high	56	29.2	8.9	9.7	128.7	2.8
	2019	healthy	18	35.6	8.0	23.2	187.6	3.2
		low	21	33.1	9.0	17.7	175.4	2.9
		middle and high	61	28.9	8.5	13.7	121.7	2.5
	2020	healthy	2	39.4	0.5	52.0	195.4	2.8
		low	16	34.1	5.5	35.1	131.0	2.5
		middle and high	82	31.9	6.6	16.9	112.7	2.3
Samer 1	2018	healthy	25	34.2	9	30.0	156.3	3.2
		low	33	33.1	8.7	29.0	146.7	2.8
		middle and high	42	29.0	7.8	22.6	134.0	2.5
	2019	healthy	35	35.5	8.9	30.1	158.3	2.9
		low	25	34.1	8.8	29.5	153.7	2.6
		middle and high	40	30.0	7.7	21.6	131.0	2.2
	2020	healthy	14	39.5	8.8	25.0	146.1	2.8
		low	33	32.2	8.4	23.0	144.8	2.4

Table 1: Continue

		middle and high	53	31.0	11.5	17.8	135.1	2.1
Samer 2	2018	healthy	30	26.7	9.6	23.1	153.0	2.7
		low	30	27.4	8.7	19.3	148.3	2.4
		middle and high	40	25.8	10.6	12.7	125.7	2.2
	2019	healthy	26	27.7	10.5	22.5	155.2	2.5
		low	33	27.6	9.7	21.5	144.3	2.2
		middle and high	41	25.1	9.3	12.9	121.3	1.8
	2020	healthy	2	34.3	7.2	23.8	258.6	2.4
		low	24	31.9	8.9	21.7	255.1	2.1
		middle and high	73	28.7	13.2	10.8	254.5	1.7
Samer 3	2018	healthy	20	44.1	6.7	30.9	130.2	3.3
		low	21	41.8	7.0	27.8	127.7	3
		middle and high	59	30.6	12.5	11.8	97.7	2.8
	2019	healthy	20	45.2	7.5	35.8	135.4	2.9
		low	18	41.6	12.5	28.7	124.6	2.6
		middle and high	62	31.6	7.0	12.9	92.5	2.3
	2020	healthy	15	37.4	6.1	27.3	142.7	2.7
		low	17	31.4	7.4	21.2	136.2	2.5
		middle and high	68	28.1	10.8	17.4	114.5	2.2
Samer 5	2018	healthy	49	35.7	5.7	32.0	278.0	2.5
		low	25	35.6	5.5	35.7	243.7	2.3
		middle and high	26	31.8	7.0	19.6	130.3	2.1
	2019	healthy	25	35.3	6.7	32.0	278.0	2.4
		low	41	35.4	6.5	35.7	263.7	2.2
		middle and high	34	31.7	5.4	18.5	130.8	1.9
	2020	healthy	4	28.3	4.7	11.5	136.4	2.4
		low	33	26.8	6.1	17.4	135.2	2.1
		middle and high	63	24.5	6.7	11.2	128.2	1.8
Swapa	2018	healthy	46	35.5	4.75	18.5	167.3	2.8
		low	31	35.8	5.2	17.6	149.3	2.5
		middle and high	22	30.0	12.5	8.2	114.0	2.3
	2019	healthy	41	35.8	4.8	19.5	167.3	2.7
		low	34	35.1	4.7	18.1	159.3	2.4
		middle and high	25	29.1	10.8	8.2	114.0	2.1
	2020	healthy	1	38.5	1.0	48.0	119.8	2.7
		low	23	32.9	5.2	25.5	123.7	2.5
		middle and high	76	30.2	7.8	15.8	100.9	2.2
Tanais	2018	healthy	28	44.3	5.0	38	189.1	2.6
		low	29	46.8	6.4	42.6	164.7	2.4
		middle and high	43	33.5	8.2	20.2	134.7	2.3
	2019	healthy	30	45.5	6.6	45.0	191.5	2.7
		low	29	45.1	6.4	44.0	184.7	2.4
		middle and high	41	34.6	5.2	19.3	137.3	2.2
	2020	healthy	26	43.7	6.8	41.6	185.6	2.6
		low	24	39.3	7.2	45.5	172.8	2.4
		middle and high	50	32.7	7.6	16.1	128.9	2.1
K-9648	2018	healthy	21	55.1	5.2	53.9	184.3	4.0
		low	37	55.2	5.0	54.0	180.0	3.8
		middle and high	42	43.2	7.7	38.7	135.3	3.6
	2019	healthy	19	58.2	5.2	32.0	187.0	3.9
		low	40	56.2	5.1	20.6	179.0	3.7
		middle and high	31	39.8	8.1	10.9	125.4	3.4
	2020	healthy	4	37.1	4.0	54.0	155.3	3.7
		low	6	26.	5.7	52.8	139.7	3.5
		middle and high	90	25.2	8.2	36.4	121.9	3.2
ANOVA t-test, P-value			cultivar	<0.05	<0.05	<0.05	<0.05	<0.05
			root rot severity	<0.05	<0.05	<0.05	<0.05	<0.05
ANOVA t-test, P-value			climatic conditions	<0.05	<0.05	<0.05	<0.01	<0.05
			root rot severity	<0.05	<0.05	<0.05	<0.05	<0.05

Table 2: Pearson's correlation coefficient of the impact of cultivar on soybean physiology, root rot and amino acids

	Year	Temperature	Precipitation	Non-Essential FAA	Essential FAA	Aromatic FAA	Aliphatic FAA	Sum of FAA	Root rot development at flowering	Root rot development at maturity	Root rot severity	Plant heigh	Heigh of lower bean	Number of beans per plant	1000 beans weight	Yield
Year	1.00	0.91	0.70	0.75	0.77	0.75	0.68	0.76	0.62	0.08	0.53	-0.25	-0.07	-0.16	0.10	-0.31
Temperature	0.91	1.00	0.36	0.51	0.53	0.51	0.47	0.52	0.30	-0.25	0.35	-0.18	-0.16	-0.11	0.05	-0.32
Precipitation	0.70	0.36	1.00	0.83	0.86	0.83	0.76	0.85	0.90	0.63	0.61	-0.25	0.12	-0.18	0.14	-0.14
Non-essential FAA	0.75	0.51	0.83	1.00	0.99	0.97	0.93	1.00	0.73	0.50	0.61	-0.23	-0.02	-0.14	-0.09	0.07
Essential FAA	0.77	0.53	0.86	0.99	1.00	0.99	0.93	1.00	0.77	0.52	0.61	-0.20	0.00	-0.12	-0.10	0.02
Aromatic FAA	0.75	0.51	0.83	0.97	0.99	1.00	0.89	0.98	0.74	0.51	0.58	-0.17	0.02	-0.09	-0.14	0.02
Aliphatic FAA	0.68	0.47	0.76	0.93	0.93	0.89	1.00	0.93	0.72	0.54	0.64	-0.14	0.02	-0.08	-0.05	0.07
Sum of FAA	0.76	0.52	0.85	1.00	1.00	0.98	0.93	1.00	0.75	0.51	0.61	-0.21	-0.01	-0.13	-0.10	0.04
Root rot development at flowering	0.62	0.30	0.90	0.73	0.77	0.74	0.72	0.75	1.00	0.75	0.67	-0.15	0.14	-0.12	0.01	-0.10
Root rot development at maturity	0.08	-0.25	0.63	0.50	0.52	0.51	0.54	0.51	0.75	1.00	0.50	0.04	0.26	-0.02	-0.08	0.29
Root rot severity	0.53	0.35	0.61	0.61	0.61	0.58	0.64	0.61	0.67	0.50	1.00	-0.31	0.02	-0.30	0.09	0.08
Plant heigh	-0.25	-0.18	-0.25	-0.23	-0.20	-0.17	-0.14	-0.21	-0.15	0.04	-0.31	1.00	0.18	0.79	-0.15	0.37
Heigh of lower bean	-0.07	-0.16	0.12	-0.02	0.00	0.02	0.02	-0.01	0.14	0.26	0.02	0.18	1.00	-0.09	0.09	0.03
Number of beans per plant	-0.16	-0.11	-0.18	-0.14	-0.12	-0.09	-0.08	-0.13	-0.12	-0.02	-0.30	0.79	-0.09	1.00	-0.03	0.38
1000 beans weight	0.10	0.05	0.14	-0.09	-0.10	-0.14	-0.05	-0.10	0.01	-0.08	0.09	-0.15	0.09	-0.03	1.00	-0.37
Yield	-0.31	-0.32	-0.14	0.07	0.02	0.02	0.07	0.04	-0.10	0.29	0.08	0.37	0.03	0.38	-0.37	1.00

Table 3: Average chlorophyll fractions in soybean depending on leaf position in 2018-2020 ($p \leq 0.05$)

	Chlorophyll fractions	Leaf position	µM of photons m^{-2} s^{-1}
	PhiNPQ	3rd leaf	0.25
		4th leaf	0.34
p-value			0.023
	NPQt	3rd leaf	0.7
		4th leaf	1.2
p-value			0.033
	LEF	3rd leaf	120
		4th leaf	174
p-value			0.012
	PAR	3rd leaf	48
		4th leaf	35
p-value			0.028

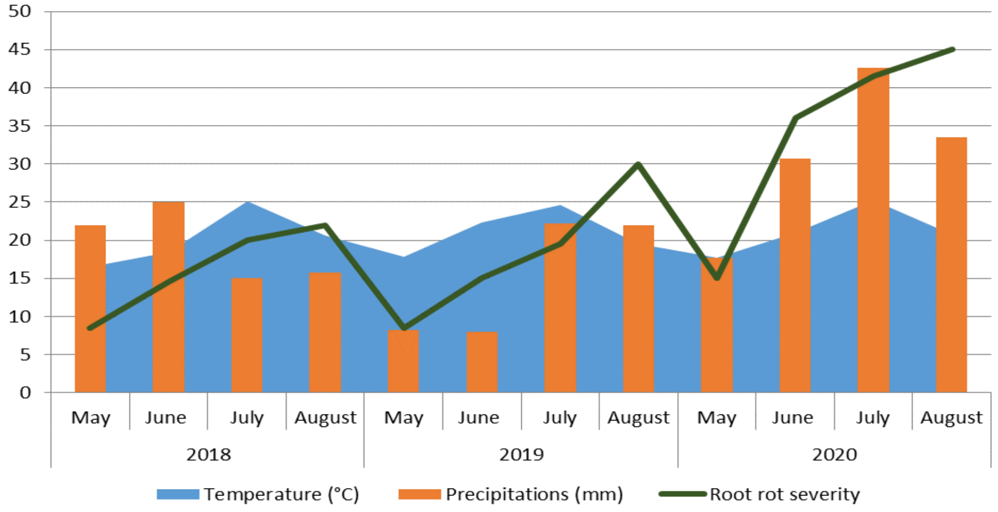

Fig. 1: Temperature, precipitations and root rot severity in soybean, in 2018-2020

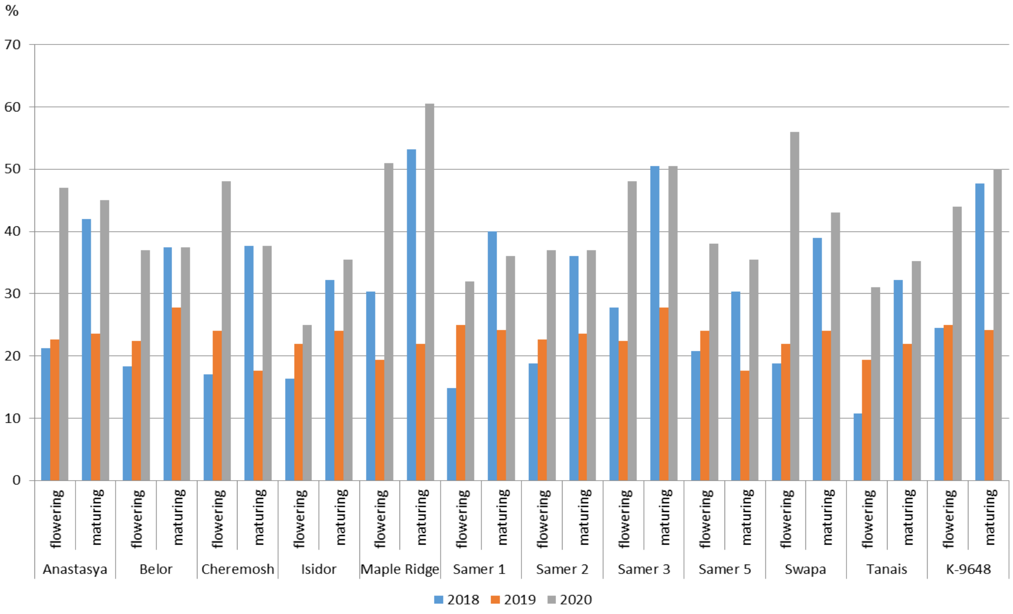

Fig. 2: Fusarium root rot development in flowering and maturing stages, in 2018-2020

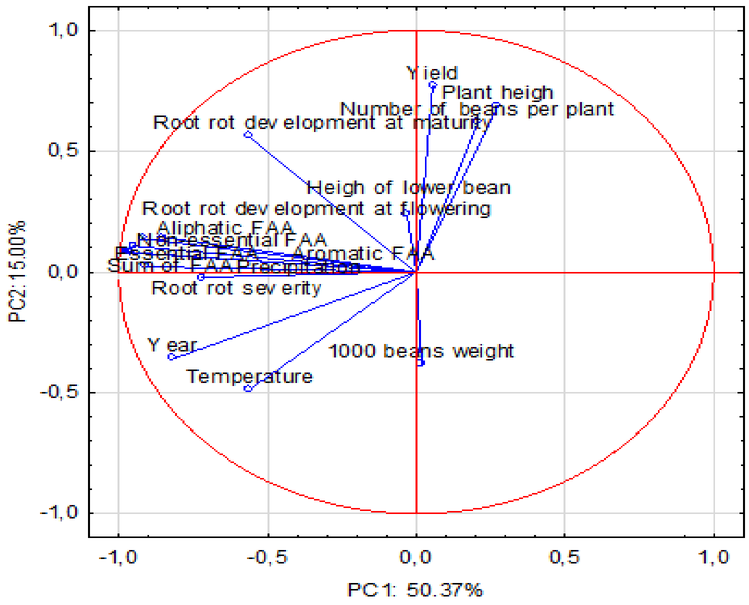

Fig. 3: Principal component analysis of the impact of cultivar on soybean physiology, root rot and free amino acids

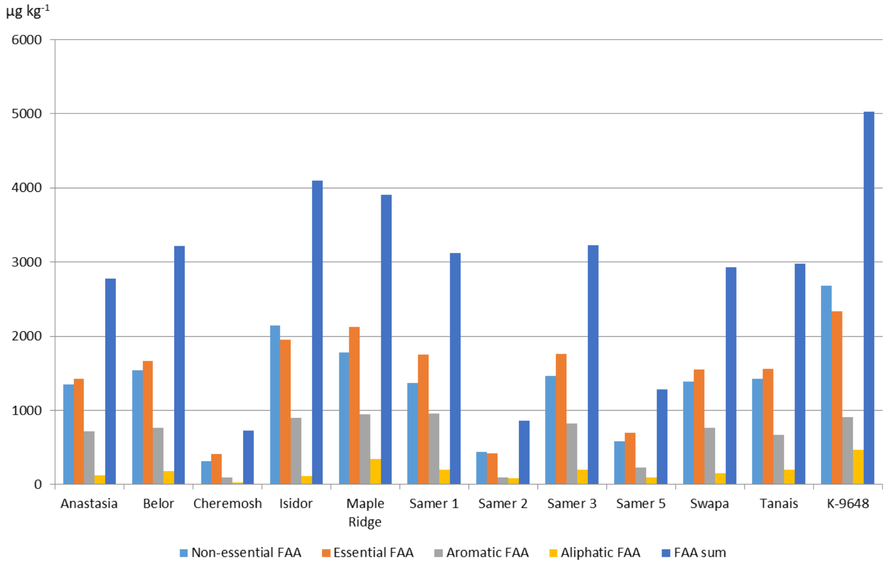

Fig. 4: Amino acids concentration in soybean cultivars (average values from 2018-2020)

Discussion

Assessment of yield is very important for drawing up strategies for combating various plant diseases Our results determined that cultivar susceptibility to *Fusarium* root rot is diverse what allows indication of most resistance cultivars in climatic conditions of Kazakhstan. There were noticed soybean yield losses up to 68% as a result of soybean cultivars inoculation by the *Rhizoctonia solani* (Chang *et al*., 2017). In this study soybean yield losses due to *Fusarium* root rot varied from 30 to 53.2%, depending on the cultivar. Moreover, our study revealed gradual reduction of 1000 beans weight, plant high and number of beans per plant which was also confirmed by Zhang *et al.* (2010). However, in contrast to Cruz Jimenez *et al.* (2018), root rot development and severity was more dependent from greater precipitations that temperature.

The process of photosynthesis as the basis for the productivity of any culture can be influenced not only by abiotic factors but also biotic ones (Kuldybayev *et al.*, 2019; Passari *et al*., 2019; Aphalo, 2017; Bauriegel *et al*., 2011). These pathogens, mainly soil-borne cause root rot of plants (Chang *et al*., 2020). Metabolites of fungal pathogens cause carbon starvation by suppressing photosynthesis (Xing *et al*., 2020). Among the physiological indicators, chlorophyll a fluorescence is the most valuable parameter of photosynthesis (Kalaji *et al*., 2017), which provides information on the efficiency of Photosystem II and I as components of the light harvesting complex under environmental stress (Olšovská, 2001). This parameter can inform about the nature of the disease and the level of resistance or susceptibility of plants to a certain strain of the fungus already in the first days of infection (Matorin *et al*., 2018; Chilvers, 2019). Protective mechanisms of the photosynthetic apparatus have special properties. One of them is NPQt or non-photochemical quenching which opposes excess light (Critchley, 1998) and act as a safe mechanism for dissipating significant levels of excitation energy of chlorophyll (Murchie and Lawson, 2013). Previously it was noticed that soybean cultivar has a significant effect on the Linear-Electron Flow (LEF) after *F. equiseti* inoculation (Kuldybayev *et al*., 2020). According to the statistical analysis, the results can be explained possibly by the presence of metabolic products in plants affected by the pathogen. Lack of mycotoxins despite root rot occurrence was resulted by the collection of soybean seeds for mycotoxins analysis, while *Fusarium* presence was observed only on roots. It confirmed that despite plant colonization by fungi, mycotoxins are secreted only in parts of their occurrence and do not penetrate to other organs.

There was determined that some proteins (Defense-Related; DR) have defense role against biotic stress (Souza *et al*., 2017), thus higher FAA concentration in our study is probably related with greater biosynthesis of defense proteins from FAA during root rot development in soybean seedlings. Moreover, higher fractions of arginine, tryptophan and aspartic acid testify to the biosynthesis of antimicrobial peptides (Mishra and Wang, 2012). Interestingly, lower precipitations in 2018-2019 caused reduction of FAA probably as the result of metabolism disorders in the environment of water deficit and their engagement in the protein against abiotic stress synthesis.

Conclusion

Selection of appropriate soybean cultivars customized to climatic conditions and resistant to *Fusarium* root rot is challenging aspect of pesticides reduction in agriculture. Results of the study indicated that among twelve soybean cultivars, Tanais and Isidor cultivars are at least susceptible to *Fusarium* root rot despite heavier rainfalls in 2020 and in this regard could be implemented to agriculture in the agroclimatic conditions of Kazakhstan. However, in aspect of yield, K-9648 cultivar was the most productive in 2018-2020. Moreover, chlorophyll fraction was comparable between soybean cultivars, however, for PhiNPQ, NPQt and LEF higher values were obtained in 4th leaf. Furthermore, there was indicated that significant accumulation of amino acids was induced by root rot development, severity and precipitations. Thus, amino acids play the crucial role in defense proteins biosynthesis against biotic stress factors. Interestingly, despite *Fusarium* root rot occurrence, there were not determined mycotoxins in seeds, which indicate that they are not distributed to other organs from the place of their biosynthesis.

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Author’s Contributions

All authors equally contributed in this study.

Ethics

This article is original and contains unpublished material. The corresponding author confirms that all other authors have read and approved the manuscript and no ethical issues have been involved.

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