



AGRICULTURAL ACADEMY
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**BEHAVIOUR OF *IN VITRO* RASPBERRY PLANTS
GROWN IN *IN VIVO* CONDITIONS**

ABSTRACT

of a dissertation for awarding the educational and scientific degree
“Doctor”

in Professional field 6. 1. “Plant Growing”

Scientific specialty: "Fruit Growing"

Scientific supervisor:
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The dissertation is written on 161 pages and includes 22 figures, 54 tables and 16 photos. The main text includes an introduction, reference review, aim and objectives of the research, material and methods, results and discussion, conclusions, contributions and recommendations for the practice.

The bibliography includes 283 literary sources, of which 36 in Cyrillic and 247 in Latin.

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I. Introduction

Raspberry (*Rubus idaeus*) is a half shrub, berry plant belonging to the family *Rosaceae*. The increased interest in it is due to the valuable economic and biological qualities, on the one hand the specific taste, high content of sugars, vitamins, organic acids, pectin and polyphenols, and on the other the high content of antioxidants that have anticancer effects against cancer cells.

Raspberry is a fruit species that is widespread on almost all continents because of its plasticity and taste. It is widely used in the foothills and mountain regions of Bulgaria, where soil and climatic conditions are largely favourable for its development. In these places the climate is cool and the soil and atmospheric humidity are higher.

The advantages of raspberries over other fruit species are, such as earlier onset of fruit bearing; fast return on investment, high purchase price and the ability to apply modern growing techniques.

This stimulates producers to create new raspberry plantations, a better intensification of the existing cultivars and to introduce new ones.

II. Reference review

Reference review allows to get an idea of the agrobiological characteristics and requirements of raspberries; vegetative and reproductive manifestations; resistance to low temperatures; susceptibility to diseases and pests; content of mineral elements and biologically active compounds in raspberry leaves; biochemical composition of fruits and methods of storage and processing.

III. Aim and objective of the research

The aim of the present dissertation is to monitor the behaviour of cultivars and candidate cultivar of raspberries produced *in vitro* and grown *in vivo* under normal (0.50 m) and ultra dense (0.30 m) planting distances.

The following **objectives** were performed to achieve the aim:

1. Analysis on the content of basic nutrients from the soil profile of the raspberry plantation;
2. Monitoring the course of the phenological phases of the raspberry cultivars and the candidate cultivar;
3. Establishment of the values of the elemental composition and pigments in the leaf samples of the raspberry cultivars and

the candidate cultivar during the periods of blossoming, harvesting and after fruit harvesting;

4. Study of vegetative and reproductive manifestations of the raspberry cultivars and the candidate cultivar;

5. Study of the biochemical composition of fruits;

6. Determining the quality characteristics and colour parameters of fruits;

7. Characterization of cultivar characteristics and manifestations of the cultivars and the candidate cultivar at normal and ultra dense planting distances.

IV. Material and methods

1. Material

The research work on the topic was conducted during the period (2018-2020) in a collection plantation of the Research Institute of Mountain Stockbreeding and Agriculture, Troyan. The object of the study are the widely distributed raspberry cultivars, such as “Willamette” and “Meeker”, the Bulgarian cultivar ‘Samodiva’ and the candidate cultivar ‘Magdalena’. Planting material was produced by clonal propagation (*in vitro*). Clonal plants were cultured on MS basic nutrient medium enriched with 0.1 mg/l IBA, 0.1 mg/l GA3 0.3 mg/l BAP (Kondakova et al. in press). MS medium with halved salt concentration and addition of 0.3 mg/l IBA was used to induce rhizogenesis. In order to study the behaviour of raspberry plants *in vivo*, in the conditions of the region of the Fore-Balkans, an open-air field experiment has been set.

The experiment is set in two variants with six replications, each one linear meter of the intra-row area.

- I var. - planting at 0.50 m in the intra-row area;

- II var. - planting at 0.30 m in the intra-row area;

The plants were planted in pits measuring 0.30/0.30/0.30 m, with the addition of granulated chicken manure of 0.200 kg. In both variants the inter-row spacing is 3.00 m. Inter-row spacings are naturally grassed, with the application of the necessary mowing of the grass, while the intra-row area is maintained in black fallow by tillage.

All plants are grown on gray forest soil under irrigated conditions and supporting structure. Fertilizing was applied: 20 kg/da

ammonium nitrate (annually), 15 kg/da triple superphosphate and 7.5 kg/da potassium chloride (first and third year).

2. Studied indicators:

2.1. Determining the main nutrient stock of soil:

- **Soil reaction (pH)** - determined potentiometrically in water and in KCL.
- **Nitrogen (mg/kg)** - according to the method of Bremner and Keeney in 2018 and according to Kjeldahl method, BDS - EN ISO 5983, in 2019 and 2020.
- **Phosphorus (mg/100g)** - according to the method of P. Ivanov
- **Potassium (mg/100g)** - according to the method of P. Ivanov
- **Humus (%)** - according to Tyurin

2.2. Phenological observations

The following phenophases were reported:

- **Beginning of vegetation** - the moment when the buds from the upper third of the shoots of raspberries are cracked.
- **Beginning of blossoming** - up to 5% of blossoms are open;
- **Beginning of full blossoming** - up to 5% of blossoms are open;
- **End of blossoming** - about 25% of blossoms are open;
- **Beginning of fruit ripening** - 25% of the plants for each cultivar have single ripe fruits;
- **Beginning of large scale fruit ripening** - period of mass fruit harvesting;
- **End of fruit ripening** - last fruits ripen, which are economically profitable harvest.
- **End of vegetation** - when more than 75% of the leaves have fallen.

2.3. Vegetative indicators

The measurements were performed after the end of the vegetation:

- **Number of shoots per linear meter** - the shoots are listed in each repetition;

- **Height of shoots (cm)** - all shoots from each replication were measured;

- **Thickness of shoots (mm)** - measured at 10 cm from the soil surface;

2.4. Reproductive indicators

- **average fruit weight (g)** - samples of 30 fruits were taken during one of the first and two of the mass harvestings;

- **Average yield of 1m (g);**

2.5. Content of mineral elements and biologically active compounds in leaf samples during the phenophases of blossoming, fruit harvesting and after harvesting by the following methods:

➤ **Methods for determining the content of mineral elements in raspberry leaves**

- **nitrogen content** (according to Kjeldahl method, BDS - EN ISO 5983);

- **phosphorus content** (Colorimetric method of Guericke and Kurmis, AOAS, 2007);

- **potassium content** (by the method of Atomic absorption spectrophotometry, AOAS, 2007).

➤ **Methods for determining the content of biologically active compounds in raspberry leaves.**

- **Content of chlorophyll "a", chlorophyll "b" and β carotene - spectrophotometrically**

2.6. Study on the biochemical composition of raspberry fruits

The biochemical analyzes of fruits were carried out in the chemical laboratory of RIMSA Troyan and the following indicators were determined:

- **Dry matter by Re (%)** - determined with a refractometer;

- **Dry weight (%);**

- **Sugars - total, invert and sucrose (%)** - by the method of Schoorl and Regenbogen;

- **acids (%)** - by titration with 0.1 n NaOH;

- **ascorbic acid (mg/%)** - by the method of Fialkov;

- **tanning substance (%)** - by the method of Levental;

- **anthocyanins (mg /%)** - by the method of Fuleki and Francis;
- **pectin** - by the method of Melitz;
- **total polyphenols (mgGAE/100 g)** - the method is adapted to the method of Singleton and Rossi (1965).

2.7. Study on the qualitative characteristics of fruits

The qualitative characteristics of fruits were performed in the laboratory of the Institute of Food Preservation and Quality, Plovdiv and the following indicators were determined: Пловдив и са определени следните показатели:

- **pH; appearance; colour; consistency; aroma; taste; general tasting evaluation; general sensory evaluation;**
- **6:):) colour by the scale of Gardner** - instrumental with laboratory apparatus "GOLORGRAD2000", the company BYK-GARDNER INC. USA. The indicators are reported according to the CIE Lab system. The colour coordinates L, a and b are taken during the measurement: L - colour brightness; + a - red color; -a- green color; + b - yellow color - b- blue color.

2.8. Statistical processing

The following statistical analyzes were used to process the data obtained from the experimental work of the studied cultivars and candidate cultivar of raspberries: variational-statistical one-factor and two-factor analysis of variance, correlation and regression analysis (Lidanski 1988), using software product MS Excel - 2010.

Experimental data were processed by analysis of variance (one-factor and two-factor), multiple comparisons of averages were performed by Duncan's test.

3. Soil - climate characteristics

The experiments were set on the territory of RIMSA - Troyan on a slope with eastern exposure with an altitude of 460 m.

3.1. Soil characteristics

The soil on which the experiment was conducted is light gray forest, typical of Troyan region.

The mechanical composition of the soil was determined by Rutkowski method. As a result of the performed analyzes it was established that

the variety of the soil by mechanical composition is heavy sandy-clay to clay, with moderately erosion with low humus content (Table 1).

Table 1. The mechanical composition of the soil was determined by Rutkowski method.

Cultivars	Soil layers / Variety of soil, Content of physical clay, %		
	0-20 cm	20-40 cm	40-60 cm
“Willamette”	Clay (60)	Clay (60)	Clay (90)
“Willamette”	Clay (48)	Clay (42)	Clay (78)
“Meeker” - 0.50m	Clay (60)	Heavy sandy-clay (30)	Clay (36)
“Meeker” - 0.30m	Clay (54)	Clay (48)	Clay (60)
‘Samodiva’ - 0.50m	Clay (60)	Clay (66)	Clay (80)
‘Samodiva’ - 0.30m	Clay (78)	Clay (60)	Heavy sandy-clay (30)
candidate cultivar ‘Magdalena’ - 0.50m	Clay (36)	Clay (60)	Clay (54)
candidate cultivar ‘Magdalena’ - 0.30m	Clay (54)	Clay (54)	Heavy sandy-clay (30)

3.2. Climate characteristics

3.2.1. Temperature

The climate is moderately continental with a pronounced mountain influence. The average annual temperature for the three-year period is 11.2 °C, and for the period April - October, when the raspberry vegetation is 16.5 °C. The lowest average monthly temperature during the experiment was reported in January (0.5 °C), and the highest in August - 20.6.

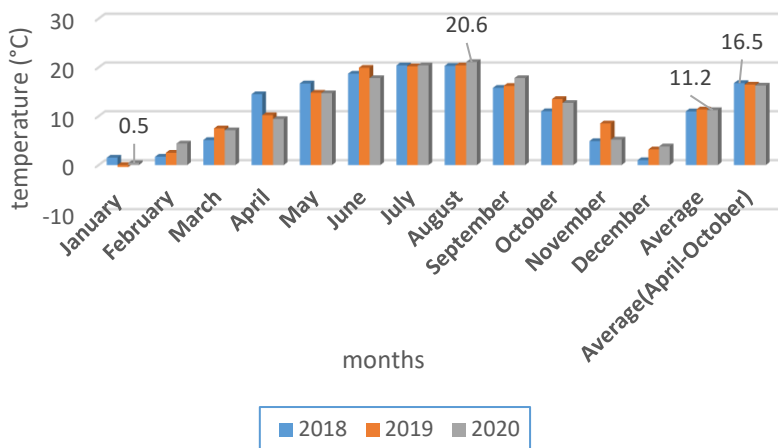


Figure 1. Average monthly air temperature (°C) for the experimental period (2018-2020)

3.2.2. Precipitation

The precipitation regime is continental. For the period of the study 2018-2020, the average annual precipitation was 787.97 l/m². And in April-October of the experimental period are 573.17 l/m². Most precipitation was reported in 2018. - 919.4 l/m², and at least in 2020 - 679.6 l/m².

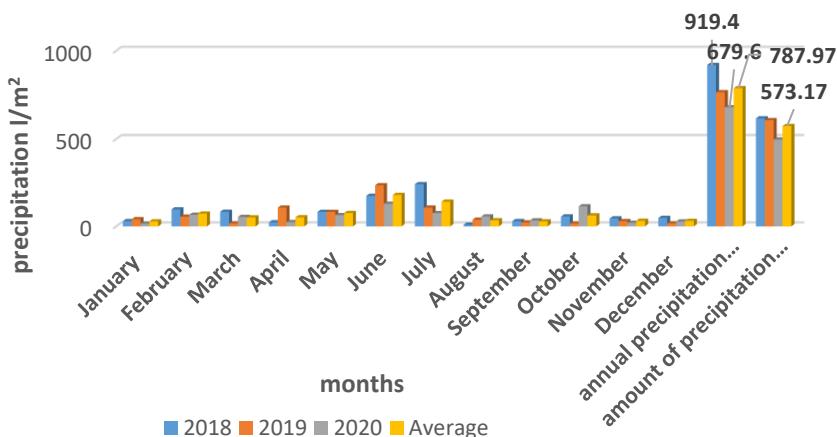


Figure 2. Average monthly amount of precipitation (l/m^2) during the experimental period (2018-2020)

V. Results and discussion

1. Phenological observations

An idea of the course of the individual phenophases for the studied raspberry genotypes under the conditions of the Troyan region is given by the prepared phenological calendar (Table 2).

During the study period, the beginning of the vegetation of raspberry cultivars begins in March. At the earliest on March 4, at the first variant of the candidate cultivar ‘Magdalena’ in 2019, and at the latest on April 10, 2020 in the same variant but of ‘Meeker’ cultivar.

Blossoming in 2018, for all cultivars begins on April 30; in 2019 in the first ten days of May, and in 2020 on 15 May with the exception of the candidate cultivar.

Full blossoming of all cultivars and candidate cultivar takes place in May to early June.

Harvesting begins in June - at the earliest in 2018 - on June 5-6; in 2019 in the period 11 - 24 June, and in 2020 in the period 12-22 June.

In 2018 the phenophases of flowering, fruit ripening and harvesting of the cultivars take place earlier, compared to the following 2019 and 2020. The end of the vegetation in 2018 and 2019. occurs in the months of November-December, and in 2020 from October to December.

Table 2.Phenological calendar for raspberry genotypes for the period 2018-2020

Cultivars	Bud bursting	Beginning of blossoming	Full blossoming	End of blossoming	Beginning of fruit harvesting	Harvesting	End of fruit harvesting	End of vegetation
2018								
“Willamette” - 0.50m	25.03	30.04	04.05	14.05	01.06	06.06	25.06	09.12
“Willamette” - 0.30m	25.03	30.04	03.05	14.05	31.05	05.06	27.06	09.12
“Meeker” - 0.50 m	27.03	30.04	05.05	15.05	30.05	05.06	27.06	07.12
“Meeker” - 0.30m	27.03	30.04	07.05	15.05	01.06	06.06	27.06	07.12
‘Samodiva’ - 0.50m	30.03	30.04	06.05	14.05	31.05	05.06	25.06	02.12
‘Samodiva’ - 0.30	30.03	30.04	05.05	14.05	31.05	05.06	25.06	20.11
candidate cultivar ‘Magdalena’ - 0.50m	22.03	30.04	04.05	15.05	29.05	05.06	25.06	23.12
candidate cultivar ‘Magdalena’ - 0.30m	22.03	30.04	04.05	14.05	28.05	06.06	25.06	20.12
2019 r.								
“Willamette” - 0.50m	29.03	11.05	18.05	03.06	14.06	18.06	15.07	12.12
“Willamette” - 0.30m	29.03	08.05	18.05	02.06	13.06	18.06	15.07	14.12
“Meeker” - 0.50m	29.03	16.05	27.05	06.06	13.06	18.06	15.07	18.11
“Meeker” - 0.30m	29.03	08.05	27.05	10.06	14.06	24.06	15.07	19.11
‘Samodiva’ - 0.50m	16.03	08.05	15.05	30.05	12.06	16.06	15.07	18.11
‘Samodiva’ - 0.30m	28.03	11.05	18.05	30.05	13.06	17.06	15.07	18.11
candidate cultivar ‘Magdalena’ - 0.50m	04.03	07.05	15.05	03.06	10.06	13.06	15.07	30.11
candidate cultivar ‘Magdalena’ - 0.30m	05.03	07.05	14.05	05.06	06.06	11.06	15.07	30.11
2020 r.								
“Willamette” - 0.50m	30.03	15.05	19.05	06.06	17.06	19.06	22.07	08.12
“Willamette” - 0.30m	03.04	15.05	19.05	03.06	18.06	19.06	27.07	08.12
“Meeker” - 0.50m	10.04	18.05	26.05	13.06	16.06	22.06	27.07	14.10
“Meeker” - 0.30	04.04	16.05	24.05	13.06	16.06	22.06	27.07	16.10
‘Samodiva’ - 0.50m	27.03	15.05	18.05	04.06	17.06	20.06	24.07	15.10
‘Samodiva’ - 0.30m	27.03	15.05	20.06	03.06	17.06	20.06	22.07	15.10
candidate cultivar ‘Magdalena’ - 0.50m	18.03	12.05	14.05	30.05	07.06	12.06	22.07	06.12
candidate cultivar ‘Magdalena’ - 0.30m	15.03	11.05	14.05	30.05	07.06	12.06	22.07	06.12

2. Vegetative indicators

The number and degree of development of young raspberry plants are important parameters in the assessment of vegetative and reproductive potential. Based on these indicators, the application of various agro-technical measures (pruning) is determined, as well as the system for growing raspberries (with or without a supporting structure).

2.1. Shoot formation

The average number of shoots of one linear meter for the three-year period of the study is the highest in the second variant of “Willamette” - 31.06, followed by the first variant of the candidate cultivar ‘Magdalena’ (28.06), and with the smallest number of shoots is the first variant of ‘Samodiva’ - 11.89 pcs. (Figure 3).

2.2. Height of shoots

According to the indicator average height of the shoots for the period 2018-2020, we note that the second variant of “Meeker” has formed the highest shoots - 174.92 cm, and the same variant of the candidate cultivar ‘Magdalena’ the lowest - 108.72 cm (Figure 4).

2.3. Thickness of shoots

Shoot thickness is an indicator that significantly represents the structure and shape of plants in each cultivar. The average values for the three-year study period in terms of shoot thickness show similar values for the raspberry genotypes, as the two variants of ‘Samodiva’ are distinguished with the thickest shoots with 8.05 mm, 7.73 mm, and the two thinnest ones are registered in “Willamette” with 6.44 mm and 6.35 mm (Figure 5).

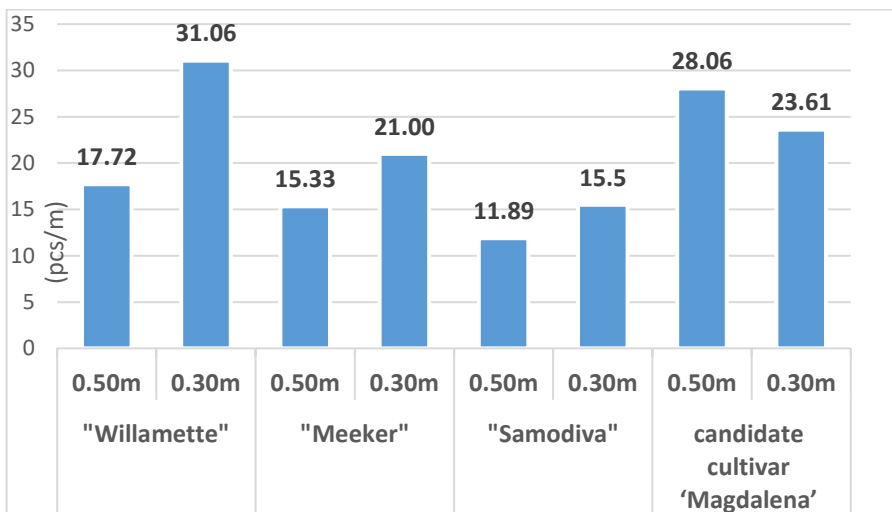


Figure 3. Average number of shoots (pcs/m) by variants of raspberry genotypes for the period (2018-2020)

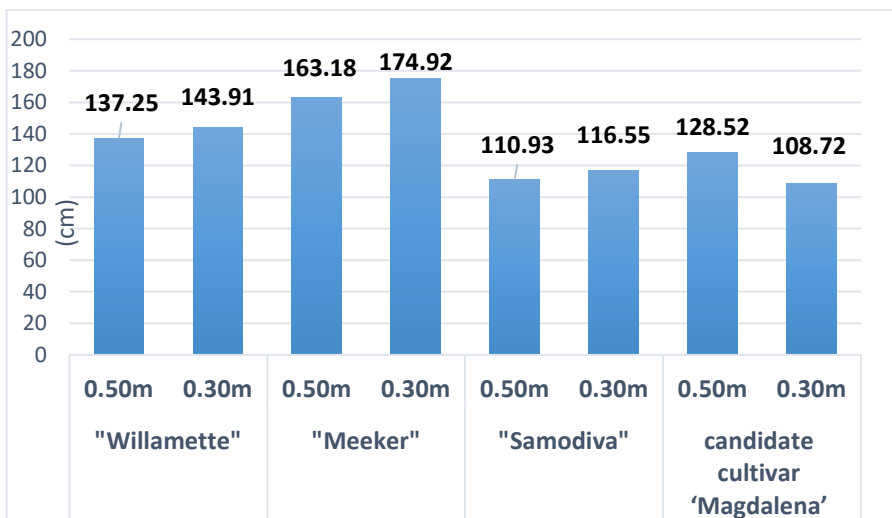


Figure 4. Average height of shoots (cm) by variants of raspberry genotypes for the period (2018-2020)

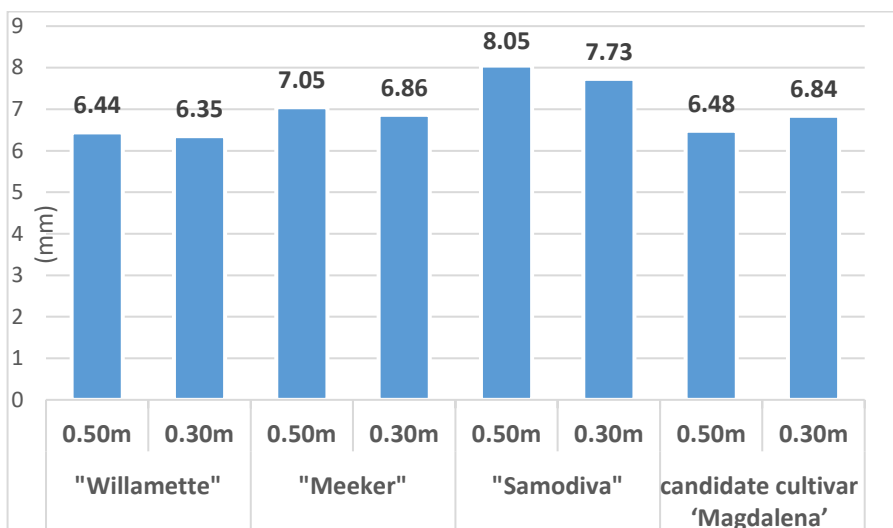


Figure 5. Average thickness of shoots (mm) by variants of raspberry genotypes for the period (2018-2020)

3. Reproductive indicators of raspberry cultivars and candidate cultivar

During the three-year period, the reproductive indicators of the average weight of the fruit (g) and the average yield per 1 m² of the raspberry cultivar: "Willamette", "Meeker", "Samodiva" and the candidate cultivar 'Magdalena' were monitored.

The highest average fruit weight from the three-year study period was reported in both "Meeker" variants 2.87 g and 2.80 g. It can be found that fruits with a higher average weight predominate at shorter planting distances. The only exception is "Meeker" (Figure 6).

The highest average yield from the study period was registered in the second variant of "Willamette" with 2022.83 g, followed by the candidate cultivar 'Magdalena' from the same variant with 1869.55 g (0.30 m). It was observed again that the shorter the planting distances, the higher the yields. The only exception is 'Samodiva' cultivar (Figure 7).

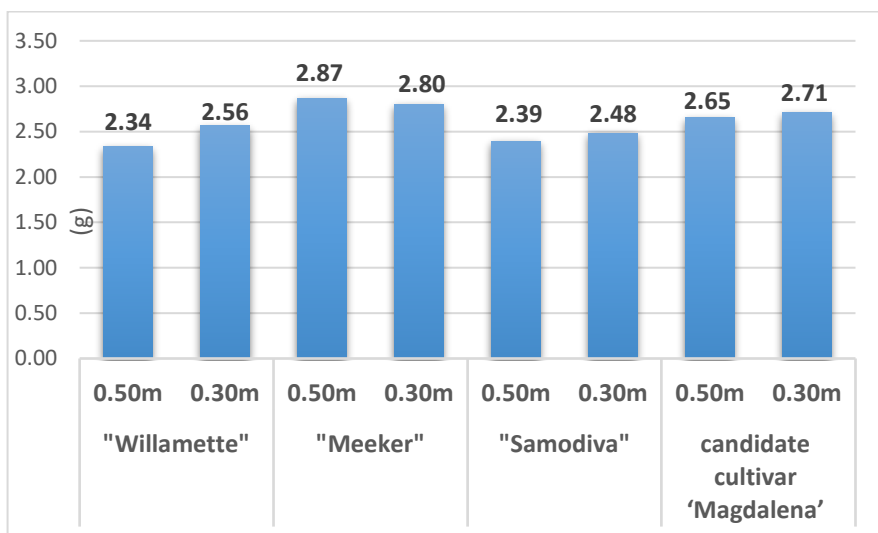


Figure 6. Average fruit weight by variants (g) for the period 2018-2020.

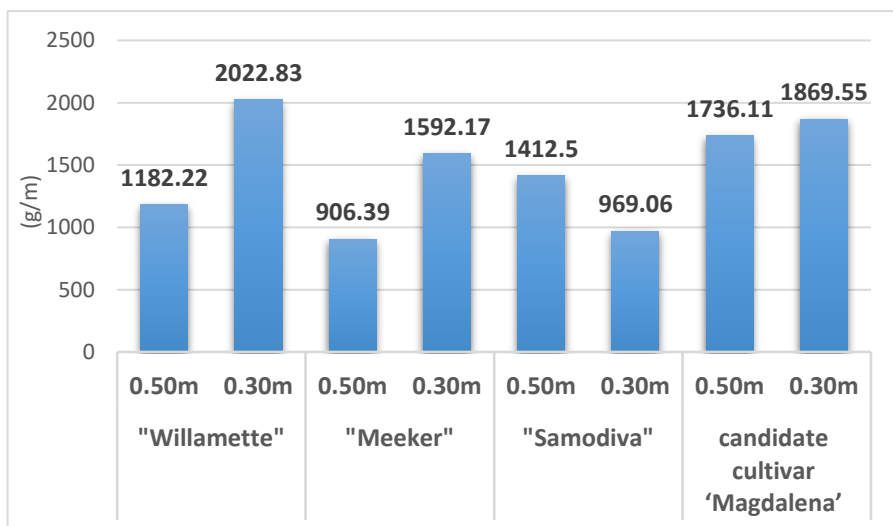


Figure 7. Average fruit yield by raspberry genotypes for the period (2018-2020).

Table 3.Biochemical composition of fresh fruit of raspberry cultivars and candidate cultivar for the period 2018 - 2020

Indicators Cultivars	Dry weight (%)	DM by Re (%)	Total sugars (%)	Inverted sugar (%)	Sucrose (%)	Acids (as malic) (%)	Ascorbic acid (mg %)	Tanning substances (%)	Anthocyanins (mg %)	Pectin (%)
“Willamette” - 0.50m	11.70	8.50	2.20	1.53	0.63	0.71	22.85	0.14	31.99	1.69
“Willamette” - 0.30m	12.19	9.70	3.50	1.38	2.01	0.65	18.75	0.10	22.85	1.60
“Meeker” - 0.50m	14.38	8.00	2.45	1.70	0.71	0.58	17.01	0.07	23.66	1.62
“Meeker” - 0.30m	13.47	9.67	2.73	1.13	1.52	0.56	19.36	0.06	21.87	1.04
“Samodiva” - 0.50m	12.20	7.00	2.80	1.92	0.84	0.65	16.43	0.06	21.61	1.33
“Samodiva” - 0.30m	11.40	6.00	2.47	1.77	0.60	0.67	15.25	0.08	14.08	1.55
candidate cultivar “Magdalena” - 0.50m	11.74	8.17	3.55	1.72	1.74	0.65	14.67	0.10	15.16	1.45
candidate cultivar “Magdalena” - 0.30m	12.10	8.50	3.02	2.15	0.82	0.75	14.67	0.07	26.13	1.33
x ±SE	0.36	0.44	0.17	0.11	0.20	0.02	1.00	0.01	2.03	0.08
St Dev	1.01	1.25	0.49	0.31	0.56	0.06	2.83	0.03	5.73	0.21
VC %	8.32	15.23	17.28	18.91	50.31	9.42	16.29	30.85	25.84	14.73
Significance level (P) among variants	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s
Significance level (P) among variants and candidate cultivar	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s

4. Biochemical composition and raspberry fruit

In 2018-2020 the main biochemical components of fresh raspberries from the four studied genotypes and their variants were identified (Table 4). The following generalizations can be made from the average results: the fruits are low in sugar. Organic acids are present in values below one percent for cultivars and variants. There is a significant variability of ascorbic acid between genotypes, with the highest content of the first variant of “‘Willamette’”, and the lowest candidate cultivar ‘Magdalena’. In the case of anthocyanins, divergent results were reported for the cultivars and variants, as the highest value again is in the fruits of the first variant of “‘Willamette’”.

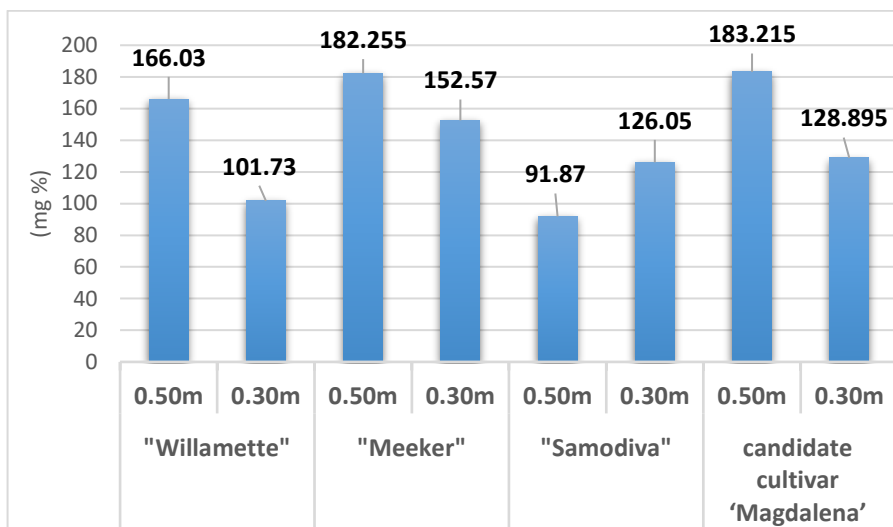


Figure 8. Content of total polyphenols in raspberries in 2018 and 2020.

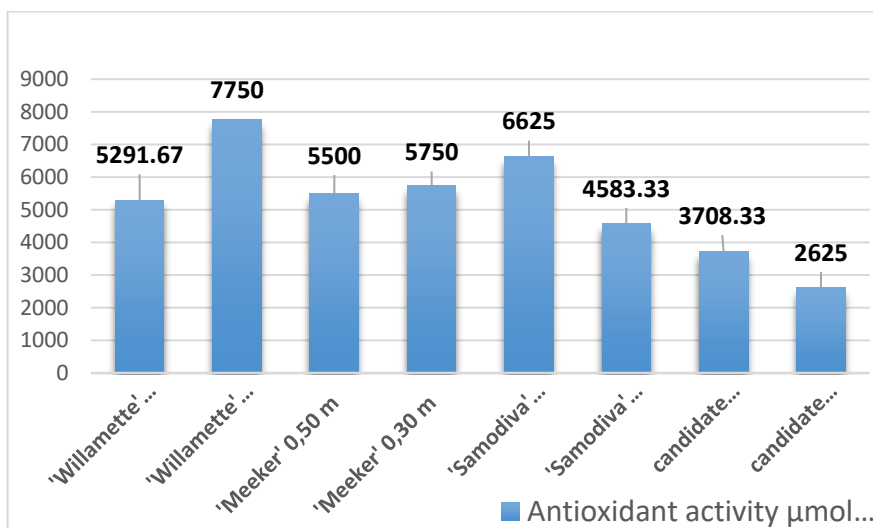


Figure 9. Antioxidant activity in fruits of raspberry genotypes in 2018.

The average content of total polyphenols in 2018 and 2020 show a wide range of variability in cultivars and variants. Fruits from the larger planting distances of ‘Meeker’ and the candidate cultivar ‘Magdalena’ contain the highest amount of polyphenols and the fruits from the plants of the first variant of Samodiva have the lowest content. Antioxidant activity was reported only in 2018. There is a significant variability in the values and the second antioxidant activity has the second variant of ‘Willamette’, followed by the first variant of ‘Samodiva’.

5. Qualitative characteristics of raspberries

Raspberry fruit is very interesting for consumers because of its pleasant aroma and colour, low caloric content and high nutritional value, with health benefits that are manifested in the large amount of antioxidants. In order to distinguish the quality of raspberry fruit on the market, it is important to make sensory and qualitative characteristics.

Table 4. Qualitative characteristics of raspberry fruit

cultivar indicator	pH	Apperance	colour	Consistency	aroma	taste	Total taste evaluati on	Total sensory evaluation	L	a	b
“Willamette”-0.50m	3.45	5.00	5.00	5.00	4.00	4.50	4.50	4.63	22.60	44.22	15.22
“Willamette”-0.30	3.53	5.00	5.00	5.00	5.00	4.75	4.75	4.94	27.91	39.51	14.14
“Meeker”-0.50m	3.43	4.50	4.75	5.00	4.75	4.75	4.75	4.73	25.94	42.77	16.25
“Meeker”-0.30m	3.47	5.00	5.00	5.00	4.50	4.75	4.75	4.88	23.75	38.45	12.38
‘Samodiva’-0.50m	3.49	5.00	5.00	5.00	4.75	4.75	4.75	4.88	20.68	45.47	14.81
‘Samodiva’-0.30m	3.48	4.50	4.75	4.75	4.75	4.75	4.75	4.70	26.34	34.65	10.09
candidate cultivar ‘Magdalena’ - 0.50m	3.36	4.75	5.00	5.00	4.75	4.75	4.75	4.83	29.29	39.60	20.93
candidate cultivar ‘Magdalena’ - 0.30m	3.43	5.00	5.00	5.00	5.00	4.75	4.75	4.93	28.10	42.31	14.04
$\bar{x} \pm SE$	0.02	0.08	0.04	0.03	0.11	0.03	0.03	0.04	1.06	1.24	1.11
St Dev	0.05	0.23	0.12	0.09	0.32	0.09	0.09	0.11	2.99	3.51	3.14
VC %	1.45	4.75	2.43	1.81	6.82	1.91	1.91	2.28	11.69	8.59	21.32

In the first experimental year a qualitative characteristic of fruits was made. According to the indicator, the pH values are almost equal and have a high acid character. The appearance of fruit is rated as 5.00 for most genotypes. Exceptions are the first variant of ‘Meeker’ and ‘Magdalena’ and the second of ‘Samodiva’. In the colour indicator, this trend is maintained. In terms of consistency, except for ‘Samodiva’ (0.30 m), the other cultivars are rated with a maximum score of 5.00. The results for the taste indicator show that no maximum score has been obtained. The highest fruit brightness was reported in candidate cultivar ‘Magdalena’ in both variants and the lowest was in ‘Samodiva’ (0.50 m).

6. Dynamics of soil nutrients

In the first experimental year, an agrochemical analysis of the soil during the period of blossoming and harvesting of plants in three soil layers of the intra-row and inter-row spacing of the plantation was made.

In the blossoming phenophase, the intra-row spacing of the three soil layers are determined by the degree of acidity - strongly acidic, with a range from 4.5 to 5.0. The nitrogen content is highest in the surface soil layer, decreasing in depth. The phosphorus content is lowest in the lowest soil layer. The phosphorus content in the next soil layer is significantly higher (20-40 cm), decreasing again on the surface (Table 5).

The nitrogen content in the inter-row space in all three soil depths has almost the same values, which are lower than those in the intra-row spacing. The highest content of phosphorus is reported in the upper soil layer, as the element gradually decreases in depth. In all three soil depths the potassium content is almost equal, both in the inter-row spacing and in the row. The reported results for the humus content in the intra-row and inter-row area show that its values from the soil profile are low (Table 6).

Table 5.Composition of agrochemical indicators of the soil layers of the intra-row spacing during blossoming of raspberries in 2018.

Soil layers cm		pH		$\sum$ N- NH ₄ +NO ₃	P ₂ O ₅	K ₂ O	Humus
		H ₂ O	KCl	mg/kg	mg/100 g		%
0-20 cm	Minimum	5.00	4.40	14.40	2.60	10.00	0.81
	Maximum	5.00	4.50	34.60	8.30	16.90	1.44
	Mean	5.00	4.47	27.67	5.37	12.63	1.15
	St error	0.00	0.03	6.64	1.65	2.15	0.18
	St Dev	0.00	0.06	11.49	2.85	3.73	0.32
	CV%	0.00	1.34	41.53	53.07	29.53	27.83
20-40 cm	Minimum	4.90	4.30	15.00	1.70	8.00	0.26
	Maximum	5.00	4.50	31.70	10.00	18.20	1.71
	Mean	4.97	4.37	21.90	6.20	12.87	0.92
	St error	0.03	0.07	5.03	2.42	2.95	0.42
	St Dev	0.06	0.12	8.72	4.19	5.12	0.73
	CV%	1.21	2.75	39.82	67.58	39.78	79.35
40-60 cm	Minimum	4.50	3.90	12.10	0.90	14.00	0.18
	Maximum	4.90	4.20	16.10	1.40	22.50	1.11
	Mean	4.60	4.00	12.70	1.17	17.93	0.53
	St error	0.13	0.10	1.25	0.15	2.47	0.29
	St Dev	0.23	0.17	2.16	0.25	4.29	0.50
	CV%	5.00	4.25	17.00	21.37	23.90	94.30

Table 6. Composition of agrochemical indicators of the soil layers of the inter-row spacing during blossoming of raspberries in 2018.

Soil layers cm		pH		Σ N- NH ₄ +NO ₃	P2O5	K ₂ O	Humus
		H ₂ O	KCl	mg/kg	mg/100 g		%
0-20 cm	Minimum	4.90	4.30	13.20	3.10	11.30	0.87
	Maximum	5.00	4.50	17.90	3.70	15.60	1.32
	Mean	4.93	4.40	15.17	3.37	12.77	1.08
	St error	0.03	0.06	1.41	0.18	1.42	0.13
	St Dev	0.06	0.10	2.44	0.31	2.45	0.23
	CV%	1.22	2.27	16.10	9.20	19.19	21.30
20-40 cm	Minimum	4.40	3.90	13.80	1.10	9.50	0.18
	Maximum	4.90	4.40	17.30	2.00	17.70	0.64
	Mean	4.70	4.13	15.17	1.60	12.37	0.39
	St error	0.15	0.15	1.08	0.26	2.67	0.13
	St Dev	0.26	0.25	1.87	0.46	4.62	0.23
	CV%	5.53	6.05	12.33	28.75	37.35	58.97
40-60 cm	Minimum	4.30	3.70	13.20	0.20	13.40	0.08
	Maximum	4.90	4.10	15.60	1.80	22.50	0.60
	Mean	4.50	3.83	14.00	0.90	18.73	0.37
	St error	0.20	0.13	0.80	0.47	2.74	0.15
	St Dev	0.35	0.23	1.39	0.82	4.75	0.26
	CV%	7.77	6.00	9.93	91.11	25.36	70.27

During the raspberry harvesting in 2018, the soil reaction in all three soil layers is defined as very strongly acidic with values from 4.5 to 5.0. The nitrogen content during fruit harvesting has almost doubled compared to the blossoming phenophase, but there is a reverse trend in phosphorus and potassium content, ie these two elements decrease. In terms of organic matter content in all three soil layers it is in unsatisfactory quantities for the plants.

Table 7.Composition of agrochemical indicators of the soil layers of the intra-row spacing during blossoming of raspberries in 2018.

Soil layers cm		pH		$\sum$ N- NH ₄ +NO ₃	P2O5	K ₂ O	Humus
		H ₂ O	KCl	mg/kg	mg/100 g		%
0-20 cm	Minimum	4.40	3.80	34.60	1.40	6.20	0.90
	Maximum	5.20	4.60	49.50	7.60	14.80	1.64
	Mean	4.76	4.20	43.64	3.26	10.16	1.20
	St error	0.17	0.17	3.09	1.12	1.42	0.14
	St Dev	0.38	0.38	6.91	2.51	3.17	0.32
	CV%	7.98	9.04	15.83	76.99	31.20	26.67
20-40 cm	Minimum	4.60	3.90	31.70	0.50	5.30	1.06
	Maximum	4.90	4.40	69.10	4.40	10.40	1.37
	Mean	4.78	4.12	41.80	2.60	8.00	1.18
	St error	0.05	0.09	6.89	0.83	0.96	0.05
	St Dev	0.11	0.19	15.40	1.85	2.15	0.11
	CV%	2.30	4.61	36.84	71.15	26.88	9.32
40-60 cm	Minimum	4.40	3.70	25.30	0.40	6.90	0.72
	Maximum	4.80	4.20	46.10	1.50	13.60	1.39
	Mean	4.58	3.92	36.64	0.92	9.54	0.96
	St error	0.09	0.10	3.33	0.21	1.47	0.12
	St Dev	0.20	0.22	7.45	0.47	3.29	0.28
	CV%	4.37	5.61	20.33	51.10	34.49	29.17

7. Content of mineral elements and biologically active compounds in leaf samples

Leaf diagnostics is a widely used method in fruit growing, which establishes a direct relationship between the mineral composition of the leaves and the growth and reproductive manifestations of plants. With its application it is possible to determine and control the need for different types of fertilizers.

7.1. Content of mineral elements in leaf samples

In 2018 after a leaf diagnosis during blossoming of the plants, it was found that the two variants of the candidate cultivar ‘Magdalena’ have the highest content of nitrogen and phosphorus and the two variants of the variety ‘Willamette’ have the highest content of potassium (Table 8).

Table 8.Mineral composition of raspberry genotype leaves during the period of full blossoming of plants in 2018

Cultivars/indicators	N (%), $\bar{x} \pm S_x$	P (%), $\bar{x} \pm S_x$	K (%), $\bar{x} \pm S_x$
“Willamette”-0.50m	0.91 $\pm$ 0.05	0.23 $\pm$ 0.02	0.60 $\pm$ 0.07
“Willamette”-0.30m	1.09 $\pm$ 0.09	0.21 $\pm$ 0.01	0.60 $\pm$ 0.06
“Meeker”-0.50m	1.07 $\pm$ 0.05	0.27 $\pm$ 0.02	0.45 $\pm$ 0.03
“Meeker”-0.30m	0.96 $\pm$ 0.09	0.21 $\pm$ 0.03	0.45 $\pm$ 0.03
‘Samodiva’-50m	0.90 $\pm$ 0.14	0.25 $\pm$ 0.02	0.40 $\pm$ 0.02
‘Samodiva’-30m	0.92 $\pm$ 0.02	0.24 $\pm$ 0.01	0.40 $\pm$ 0.01
candidate cultivar ‘Magdalena’ - 0.50m	1.12 $\pm$ 0.14	0.30 $\pm$ 0.01	0.40 $\pm$ 0.00
candidate cultivar ‘Magdalena’ - 0.30m	1.12 $\pm$ 0.10	0.29 $\pm$ 0.04	0.30 $\pm$ 0.00
St. Dev	0.10	0.03	0.10
VC %	9.71	13.33	23.11
Minimum	0.90	0.21	0.30
Maximum	1.12	0.30	0.60
Proof of differences (P) among variants	n.s	n.s	n.s
Proof of differences (P) among genotypes	n.s	P<0.05	P<0.05

* n.s - non-significant

During the period of fruit harvesting in 2018, a larger number of elements were studied. From the analysis we find that the leaves of “Willamette” of the first variant have the highest content of copper and magnesium, and the second variant of zinc. Both variants of “Meeker” have the highest content of phosphorus, but the lowest of nitrogen and calcium. ‘Samodiva’ in the first variant has the most nitrogen and iron, and the second variant has calcium and magnesium. Candidate cultivar ‘Magdalena’ in the second variant has the highest content of calcium and manganese. It can be found that all cultivars

from shorter planting distances contain the same high amount of potassium. (Table 9).

Table 9.Mineral composition of raspberry genotype leaves during the period of fruit harvesting of plants in 2018

Cultivars/ indicators	N (%) $\bar{x} \pm S_x$	P (%) $\bar{x} \pm S_x$	K (%) $\bar{x}$ $\pm S_x$	Ca (%) $\bar{x} \pm S_x$	Mn mg/kg $\bar{x} \pm S_x$	Fe mg/kg $\bar{x} \pm S_x$	Zn mg/kg $\bar{x} \pm S_x$	Cu mg/kg $\bar{x} \pm S_x$	Mg (%) $\bar{x} \pm S_x$
“Willamette”- 0.50m	0.98 ± 0.13	0.25 ± 0.01	0.60 ± 0.06	0.79 ± 0.03	172.00 ± 23.64	144.00 ± 16.04	38.00 ± 2.08	11.00 ± 2.89	0.33 ± 0.01
“Willamette”- 0.30m	1.15 ± 0.02	0.22 ± 0.00	0.70 ± 0.00	0.73 ± 0.01	153.67 ± 9.56	119.67 ± 5.46	51.67 ± 9.17	9.33 ± 2.96	0.29 ± 0.01
“Meeker”- 0.50m	0.85 ± 0.08	0.28 ± 0.01	0.60 ± 0.06	0.61 ± 0.04	155.33 ± 17.37	126.00 ± 30.86	42.67 ± 0.88	9.00 ± 3.79	0.28 ± 0.01
“Meeker”- 0.30m	0.86 ± 0.15	0.26 ± 0.01	0.70 ± 0.00	0.65 ± 0.00	139.00 ± 16.01	124.67 ± 37.23	42.67 ± 2.67	5.00 ± 1.53	0.29 ± 0.01
‘Samodiva’- 0.50m	1.45 ± 0.10	0.22 ± 0.01	0.67 ± 0.03	0.87 ± 0.04	225.00 ± 43.58	234.33 ± 15.60	42.67 ± 0.67	1.33 ± 0.33	0.29 ± 0.01
‘Samodiva’- 0.30m	1.13 ± 0.08	0.25 ± 0.01	0.70 ± 0.00	0.98 ± 0.09	237.00 ± 44.52	127.67 ± 52.15	45.33 ± 1.33	1.33 ± 0.33	0.33 ± 0.03
candidate cultivar ‘Magdalena’- 0.50m	1.15 ± 0.01	0.23 ± 0.01	0.53 ± 0.03	0.79 ± 0.07	215.00 ± 9.07	181.67 ± 29.31	39.00 ± 0.58	1.33 ± 0.33	0.28 ± 0.01
candidate cultivar ‘Magdalena’- 0.30m	1.07 ± 0.13	0.21 ± 0.01	0.70 ± 0.02	0.99 ± 0.55	284.67 ± 37.32	152.67 ± 12.72	35.00 ± 1.73	1.00 ± 0.00	0.30 ± 0.15
St. Dev	0.19	0.02	0.06	0.14	50.70	39.24	5.09	4.26	0.02
VC %	17.95	9.62	9.85	17.75	25.64	25.93	12.15	86.64	6.36
Minimum	0.85	0.21	0.53	0.61	139.00	119.67	35.00	1.00	0.28
Maximum	1.45	0.28	0.70	0.99	284.67	234.33	51.67	11.00	0.33
Proving the differences (P) among variants	n.s	n.s	P<0.05	n.s	n.s	n.s	n.s	n.s	n.s
Proving the differences (P) among genotypes	P<0.05	P<0.05	n.s	n.s	n.s	n.s	n.s	P<0.05	n.s

* n.s - non-significant

During the blossoming period in 2019 “Willamette” of the first variant has the highest content of nitrogen, phosphorus and potassium, ‘Samodiva’ (0.30) of calcium, zinc and copper, and candidate cultivar ‘Magdalena’ of the first variant of iron and copper, and the second variant of manganese. It can be noted that the nitrogen content is significantly higher in the variants of the four genotypes, due to the applied fertilizing (Table 10).

Table 10. Mineral composition of leaves of raspberry genotypes during the period of full blossoming of plants in 2019.

Cultivars/indicators	N (%), x ±S _x	P (%), x ±S _x	K (%), x ±S _x	Ca (%), x ±S _x	Mn (mg/kg), x ±S _x	Fe (mg/kg), x ±S _x	Zn (mg/kg), x ±S _x	Cu (mg/kg), x ±S _x
“Willamette”- 0.50m	3.37 ±0.01	0.18 ±0.07	0.36 ±0.01	2.35 ±0.05	379.04 ±64.18	81.27 ±3.82	27.76 ±0.02	24.71 ±0.22
“Willamette”- 0.30m	2.94 ±0.00	0.15 ±0.02	0.38 ±0.00	2.06 ±0.12	430.77 ±8.66	69.71 ±5.10	27.98 ±1.24	13.30 ±0.29
“Meeker”-0.50m	2.92 ±0.21	0.12 ±0.01	0.32 ±0.01	2.17 ±0.12	234.80 ±1.43	71.04 ±5.35	24.10 ±1.19	15.04 ±0.62
“Meeker”-0.30m	2.72 ±0.21	0.09 ±0.00	0.37 ±0.02	2.12 ±0.06	277.13 ±26.9	64.64 ±2.29	24.80 ±0.41	13.76 ±1.56
“Samodiva”-0.50m	3.16 ±0.03	0.13 ±0.02	0.31 ±0.02	1.86 ±0.06	447.04 ±10.37	82.11 ±4.84	24.67 ±2.06	22.38 ±0.83
“Samodiva”-0.30m	3.17 ±0.22	0.06 ±0.03	0.34 ±0.03	2.59 ±0.05	312.79 ±18.87	84.83 ±10.76	29.26 ±5.66	26.10 ±4.07
candidate cultivar “Magdalena”- 0.50m	2.34 ±0.32	0.17 ±0.01	0.33 ±0.04	2.3 5±0.01	456.11 ±14.75	86.54 ±8.95	26.45 ±7.08	26.08 ±0.42
candidate cultivar “Magdalena”- 0.30m	2.01 ±0.01	0.17 ±0.07	0.33 ±0.03	2.30 ±0.28	497.38 ±3.98	74.92 ±6.64	21.10 ±0.97	18.60 ±2.24
St. Dev	0.46	0.04	0.02	0.22	94.73	7.52	2.63	5.51
VC %	16.2	31.34	6.58	9.99	24.97	9.71	10.19	27.55
Minimum	2.01	0.06	0.32	1.86	234.8	64.64	21.10	13.30
Maximum	3.37	0.18	0.38	2.59	497.38	86.54	29.26	26.10
Proving the differences (P) among variants	n.s	n.s	n.s	n.s	n.s	n.s	n.s	p<0.05
Proving the differences (P) among genotypes	p<0.01	n.s	n.s	n.s	p<0.001	n.s	n.s	p<0.01

* n.s - non-significant

Table 11.Mineral composition of raspberry genotype leaves during the period of fruit harvesting of plants in 2019.

Cultivars/indicators	N (%), $\bar{X} \pm S_x$	P (%), $\bar{X} \pm S_x$	K (%), $\bar{X} \pm S_x$	Ca (%), $\bar{X} \pm S_x$
‘Willamette’-0.50m	2.66 ± 0.21	0.16 ± 0.01	0.36 ± 0.01	2.09 ± 0.01
‘Willamette’-0.30m	1.98 ± 0.02	0.11 ± 0.00	0.37 ± 0.01	1.76 ± 0.11
‘Meeker’-0.50m	2.37 ± 0.09	0.15 ± 0.01	0.32 ± 0.01	1.65 ± 0.00
‘Meeker’-0.30m	2.06 ± 0.19	0.14 ± 0.01	0.36 ± 0.02	1.87 ± 0.22
Samodiva-0.50m	2.62 ± 0.13	0.14 ± 0.03	0.30 ± 0.02	2.98 ± 0.01
Samodiva-0.30m	2.61 ± 0.01	0.17 ± 0.02	0.34 ± 0.03	3.08 ± 0.11
candidate cultivar ‘Magdalena’- 0.50m	2.56 ± 0.14	0.17 ± 0.01	0.34 ± 0.04	2.09 ± 0.00
candidate cultivar ‘Magdalena’- 0.30m	2.55 ± 0.34	0.20 ± 0.04	0.34 ± 0.03	1.76 ± 0.33
St. Dev	0.27	0.03	0.02	0.56
VC %	10.92	17.42	6.99	25.9
Minimum	1.98	0.11	0.30	1.65
Maximum	2.66	0.22	0.37	3.08
Proving the differences (P) among variants	n.s	n.s	n.s	n.s
Proving the differences (P) among genotypes	n.s	n.s	n.s	P<0.001

* n.s - non-significant

During the harvesting phenophase, a significant reduction of nitrogen was observed in the leaves of the raspberry cultivars, but they are in the optimal values for the plants. The element varies from 1.98% for ‘Willamette’ - 0.30 m to 2.66% for the same cultivar from the other variant (Table 11). Only in the case of the candidate cultivar ‘Magdalena’ an increase of the element was reported and it is respectively 2.56% in the first variant and 2.55% in the second variant. The phosphorus content is highest in both variants of the candidate cultivar. During the phenophase of fruit harvesting, the amounts of potassium are higher at short planting distances and are respectively at ‘Willamette’ - 0.37%, at ‘Meeker’ - 0.36%, Samodiva and candidate cultivar ‘Magdalena’-0.34%. Samodiva cultivar has the highest calcium content in both planting variants - 2.98 and 3.08%.

Table 12. Mineral composition of leaves of raspberry genotypes during fruit harvesting period in 2020.

Cultivars/indicator	N (%), $\bar{X} \pm S_x$	P (%), $\bar{X} \pm S_x$	K (%), $\bar{X} \pm S_x$	Ca (%), $\bar{X} \pm S_x$
‘Willamette’-0.50m	2.16 $\pm$ 0.08	0.06 $\pm$ 0.00	0.37 $\pm$ 0.02	2.08 $\pm$ 0.44
‘Willamette’-0.30m	1.97 $\pm$ 0.00	0.16 $\pm$ 0.02	0.35 $\pm$ 0.01	2.52 $\pm$ 0.01
‘Meeker’-0.50m	2.00 $\pm$ 0.03	0.13 $\pm$ 0.00	0.36 $\pm$ 0.02	1.99 $\pm$ 0.11
‘Meeker’-0.30m	2.08 $\pm$ 0.11	0.15 $\pm$ 0.02	0.35 $\pm$ 0.02	2.09 $\pm$ 0.01
‘Samodiva’-0.50m	2.77 $\pm$ 0.16	0.20 $\pm$ 0.04	0.34 $\pm$ 0.02	2.88 $\pm$ 0.13
‘Samodiva’-0.30m	2.59 $\pm$ 0.12	0.23 $\pm$ 0.04	0.32 $\pm$ 0.02	2.87 $\pm$ 0.12
candidate cultivar ‘Magdalena’-0.50m	2.02 $\pm$ 0.01	0.18 $\pm$ 0.06	0.34 $\pm$ 0.02	2.63 $\pm$ 0.12
candidate cultivar ‘Magdalena’-0.30m	1.95 $\pm$ 0.04	0.21 $\pm$ 0.03	0.35 $\pm$ 0.02	2.09 $\pm$ 0.01
St. Dev	0.31	0.06	0.01	0.37
VC %	14.24	33.13	4.28	15.64
Minimum	1.95	0.06	0.32	1.99
Maximum	2.77	0.23	0.37	2.88
Proving the differences (P) among variants	n.s	n.s	n.s	P<0.05
Proving the differences (P) among genotypes	P<0.01	P<0.05	n.s	P<0.05

* n.s - non-significant

After fruit harvesting, the nitrogen content in the leaves has not changed significantly and is in the range of 1.95 - 2.77% (Table 12). At larger planting distances, a higher amount of the element was reported. ‘Meeker’ cultivar is an exception. The highest phosphorus content is found in Samodiva (0.20% and 0.23%), and potassium ‘Willamette’ (0.50 m) - 0.37%, followed by ‘Meeker’ (0.50 m) - 0.36%. Samodiva (0.50 m and 0.30 m) has the highest content of calcium, respectively 2.88 and 2.87%, and ‘Meeker’ (0.50 m) has the lowest content of the element - 1.99%.

Table 13. Mineral composition of leaves of raspberry genotypes during the period of full blossoming of plants in 2020.

Cultivars/indicators	N (%), $\bar{X} \pm S_x$	P (%), $\bar{X} \pm S_x$	K (%), $\bar{X} \pm S_x$	Ca (%), $\bar{X} \pm S_x$
“Willamette”-0.50m	2.42 ± 0.01	0.25 ± 0.00	1.35 ± 0.15	2.62 ± 0.55
“Willamette”-0.30m	3.62 ± 0.06	0.24 ± 0.01	1.40 ± 0.20	2.07 ± 0.00
“Meeker”-0.50m	2.95 ± 0.01	0.35 ± 0.03	1.05 ± 0.05	2.08 ± 0.44
“Meeker”-0.30m	2.70 ± 0.18	0.44 ± 0.09	1.25 ± 0.15	1.54 ± 0.11
“Samodiva”-0.50m	3.49 ± 0.13	0.40 ± 0.03	1.25 ± 0.05	1.53 ± 0.11
“Samodiva”-0.30m	3.66 ± 0.00	0.46 ± 0.04	1.10 ± 0.00	2.41 ± 0.33
candidate cultivar ‘Magdalena’-0.50m	3.05 ± 0.18	0.40 ± 0.01	1.25 ± 0.05	1.53 ± 0.11
candidate cultivar ‘Magdalena’-0.30m	3.06 ± 0.05	0.43 ± 0.04	1.40 ± 0.10	1.42 ± 0.22
St. Dev	0.44	0.08	0.13	0.46
VC %	14.26	22.84	10.30	24.18
Minimum	2.42	0.24	1.05	1.42
Maximum	3.66	0.46	1.40	2.62
Proving the differences (P) among variants	P<0.01	n.s	n.s	n.s
Proving the differences (P) among genotypes	P<0.001	P<0.01	n.s	n.s

* n.s - non-significant

In the third year of the full blossoming phenophase experiment, the nitrogen content in the leaf samples was reported to be 2.42% for “Willamette” at a planting distance of 0.50 m to 3.66% for “Samodiva” 0.30 m (Table 13). Samodiva has the highest content of the element from the tested cultivars - 3.49% (0.50 m), 3.66% (0.30 m). In all variants of genotypes the element is in higher quantities compared to previous years of the same phenophase. The lowest phosphorus content is registered in ‘Willamette’ with almost the same values in both planting variants. In the other cultivars and candidate cultivar, phosphorus is higher at shorter planting distances and ranges from 0.43% in the candidate cultivar ‘Magdalena’(0.30 m) to 0.46% in ‘Samodiva’ (0.30 m). All variants of genotypes have the highest values of potassium compared to previous years of the same phenophase, which range from 1.05% to 1.40%. The highest content is registered in both variants of ‘Willamette’ (1.35%; 1.40%) and the second variant of the candidate cultivar (1.40%). ‘Willamette’ (0.50

m) - 2.62% and ‘Samodiva’ (0.30 m) - 2.41% have the highest calcium content.

Table 14. Mineral composition of leaves of raspberry genotypes during the harvesting period in 2019.

Cultivars/indicators	N (%), $\bar{X} \pm S_x$	P (%), $\bar{X} \pm S_x$	K (%), $\bar{X} \pm S_x$	Ca (%), $\bar{X} \pm S_x$
‘Willamette’-0.50m	2.97 $\pm$ 0.20	0.33 $\pm$ 0.02	0.85 $\pm$ 0.05	2.10 $\pm$ 0.00
‘Willamette’-0.30m	2.94 $\pm$ 0.02	0.15 $\pm$ 0.02	0.90 $\pm$ 0.00	2.64 $\pm$ 0.55
‘Meeker’-0.50m	2.75 $\pm$ 0.00	0.16 $\pm$ 0.03	0.60 $\pm$ 0.00	2.21 $\pm$ 0.11
‘Meeker’-0.30m	2.43 $\pm$ 0.00	0.16 $\pm$ 0.01	0.70 $\pm$ 0.10	1.66 $\pm$ 0.44
‘‘Samodiva’’-0.50m	2.72 $\pm$ 0.06	0.18 $\pm$ 0.02	0.85 $\pm$ 0.05	2.53 $\pm$ 0.00
‘‘Samodiva’’-0.30m	2.98 $\pm$ 0.04	0.18 $\pm$ 0.01	0.75 $\pm$ 0.05	3.64 $\pm$ 0.22
candidate cultivar ‘Magdalena’-0.50m	2.59 $\pm$ 0.06	0.16 $\pm$ 0.03	0.65 $\pm$ 0.05	2.09 $\pm$ 0.00
candidate cultivar ‘Magdalena’-0.30m	2.62 $\pm$ 0.07	0.16 $\pm$ 0.00	0.70 $\pm$ 0.00	1.87 $\pm$ 0.22
St. Dev	0.20	0.06	0.11	0.61
VC %	7.33	31.98	14.25	26.24
Minimum	2.43	0.15	0.60	1.66
Maximum	2.98	0.33	0.90	3.64
Proving the differences (P) among variants	n.s	P<0.01	n.s	n.s
Proving the differences (P) among genotypes	P<0.01	P<0.01	P<0.01	P<0.05

* n.s - non-significant

During the fruit harvesting period, the data show that the nitrogen content is higher in the variants of all cultivars and candidate cultivar compared to previous years of the same phenophase and has the highest value (2.98%) in the second variant of ‘Samodiva’ and 2.97% - 2.94%, respectively, in both variants of ‘Willamette’ (Table 14). Phosphorus content is highest in the first variant of ‘Willamette’ - 0.33% and this is the highest value reported during the three-year period of experiment. In the other cultivars the content of the element in the leaves is in very close values (from 0.15 to 0.18%). The quantities of potassium are in the range 0.60 - 0.90%, and again in both variants of ‘Willamette’ we report the highest values (0.85%; 0.90%), followed by both variants of ‘Samodiva’ (0.85%; 0.75%). The calcium content during fruit harvesting has increased more than three times

compared to the same phenophase in 2018. ‘Samodiva’ (0.30 m) has the highest content of the element - 3.64%, and ‘Meeker’ (0.30 m) with the lowest content - 1.66%.

Table 15. Mineral composition of leaves of raspberry genotypes during the harvesting period in 2020.

Cultivars/indicators	N (%), $\bar{X} \pm S_x$	P (%) $\bar{X} \pm S_x$	K (%) $\bar{X} \pm S_x$	Ca (%) $\bar{X} \pm S_x$
‘Willamette’-0.50m	2.37 $\pm$ 0.05	0.09 $\pm$ 0.01	0.70 $\pm$ 0.10	1.77 $\pm$ 0.11
‘Willamette’-0.30m	1.95 $\pm$ 0.05	0.09 $\pm$ 0.02	0.80 $\pm$ 0.00	2.43 $\pm$ 0.11
‘Meeker’-0.50m	2.00 $\pm$ 0.03	0.10 $\pm$ 0.01	0.65 $\pm$ 0.05	1.88 $\pm$ 0.00
‘Meeker’-0.30m	1.91 $\pm$ 0.10	0.12 $\pm$ 0.01	0.60 $\pm$ 0.00	2.21 $\pm$ 0.12
Самодива-0.50m	‘Samodiva’- 0.50m	0.11 $\pm$ 0.02	0.85 $\pm$ 0.05	2.65 $\pm$ 0.11
‘Samodiva’-0.30m	2.00 $\pm$ 0.04	0.12 $\pm$ 0.03	0.85 $\pm$ 0.05	3.32 $\pm$ 0.11
candidate cultivar ‘Magdalena’- 0.50m	1.98 $\pm$ 0.07	0.14 $\pm$ 0.01	0.75 $\pm$ 0.05	2.20 $\pm$ 0.11
candidate cultivar ‘Magdalena’- 0.30m	1.80 $\pm$ 0.19	0.12 $\pm$ 0.01	0.90 $\pm$ 0.00	2.19 $\pm$ 0.10
St. Dev	0.18	0.02	0.11	0.49
VC %	8.67	14.13	13.91	20.90
Minimum	1.80	0.09	0.60	1.77
Maximum	2.37	0.14	0.90	3.32
Proving the differences (P) among variants	P<0.01	n.s	n.s	P<0.001
Proving the differences (P) among genotypes	n.s	n.s	P<0.01	P<0.001

* n.s - non-significant

After fruit harvesting, the nitrogen content in the leaves is in the range of 1.80 - 2.37% (Table 15). At larger planting distances, a higher amount of the element was reported. The phosphorus content is in very close values (0.09 - 0.14%). The lowest is the content of the element in both variants of ‘Willamette’ - 0.09% and the highest in the first variant of candidate cultivar ‘Magdalena’ - 0.14%. The amount of potassium in the leaves after fruit harvesting is in the range of 0.60 to 0.90%. The highest value (0.90%) is registered in the candidate cultivar ‘Magdalena’ (0.30 m), followed by both variants of ‘Samodiva’ - 0.85%. The shorter planting distances of the plants have a higher element content. ‘Meeker’ cultivar is an exception. ‘Samodiva’ has the highest content of calcium in both variants of

planting with 2.65 and 3.32%. The shorter planting distances of the plants are characterized by a higher content of the element. An exception is the candidate cultivar 'Magdalena', but the difference between the options is minimal.

From the leaf diagnostics on the dynamics of the values of nutrients we can summarize that all cultivars and candidate cultivar contain insufficient nitrogen - 2-3 times lower than allowed in the first year. In the second year, the content of the element, due to the applied fertilizing, is significantly higher in the variants of the four genotypes. In the case of 'Willamette' it reaches optimal values from the period of full blossoming - 3.37% of the first variant and in the case of 'Samodiva' of the same phenophase - 3.17% of the first and 3.16% of the second variant. The highest nitrogen content in the leaves was reported in 2020 in the phenophase full blossoming in 'Samodiva' (0.30 m) with 3.66%.

During the first two years, the phosphorus was lower than the reference values for the test crop. The phosphorus amount reaches the required optimal values in 2020. as the highest value was reported in 'Samodiva' (0.30 m) in the phenophase of full blossoming.

The potassium content in the leaves is low and below acceptable. The highest value of calcium was found in the third year of the experiment with 'Samodiva' (0.30 m) during the harvesting period and after fruit harvesting.

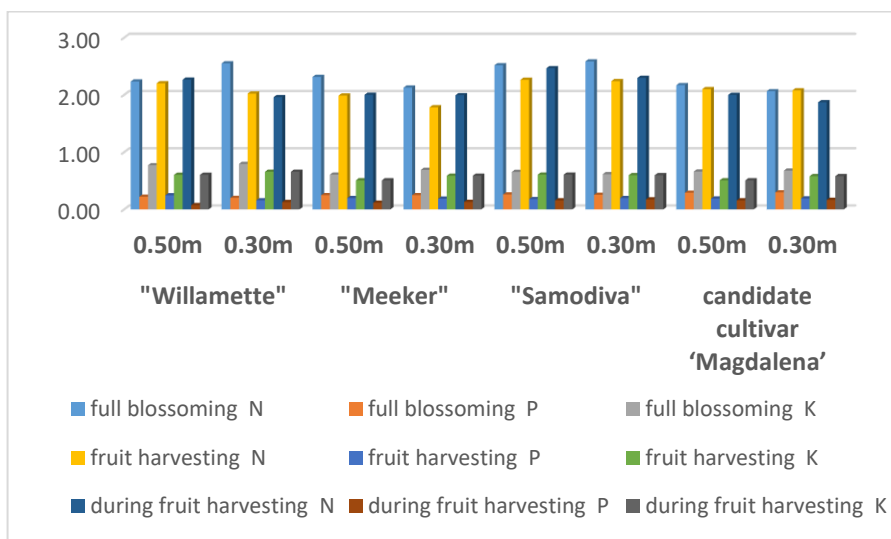


Fig. 10. Mineral composition in the leaves of raspberry genotypes on average for the period (2018-2020).

On average for the period 2018-2020 'Samodiva' (0.50 m) has the highest nitrogen content during the phenophases harvest and after fruit harvesting, and the second variant during blossoming. The highest amount of phosphorus was reported in the second variant of 'Samodiva' and candidate cultivar 'Magdalena' (0.17%), during blossoming and after fruit harvest. 'Willamette' (0.30 m) has the highest potassium content in the leaves during the three phenophases (Fig. 10).

7.2. Content of biologically active compounds

Carotenoids and chlorophylls (lipid-soluble pigments) are natural pigments responsible for the yellow, orange, red and green colours, widespread in various plant organs (leaves, flowers, stems). Chlorophyll is a group of green colour pigments present in plants. These pigments are able to capture light energy from sunlight and produce carbohydrates. Present in the leaf chloroplasts chlorophylls are responsible for the process of photosynthesis and green hues in plants.

The content of biologically active compounds in raspberry leaf samples was determined in 2019 and 2020.

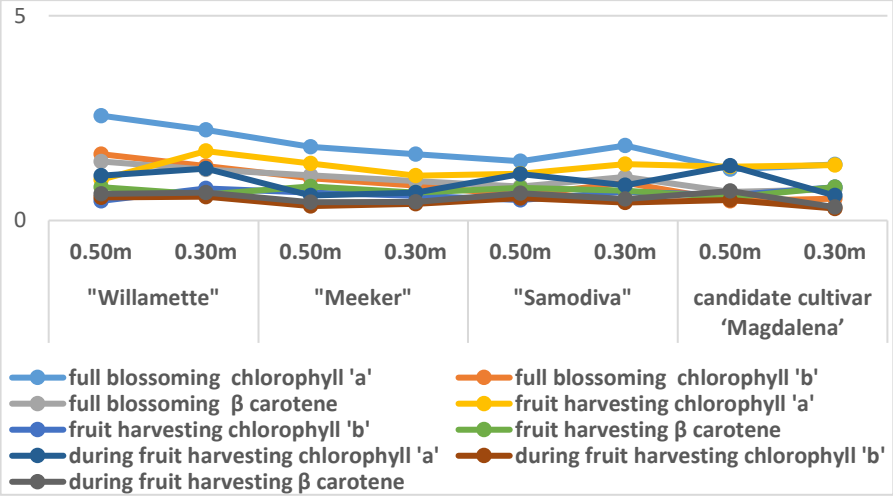


Fig. 11. Content of biologically active compounds in raspberry leaves for 2019.

In 2019 during the phenophase of full blossoming, the highest values of chlorophyll 'a', chlorophyll 'b' and β carotene were reported in both variants of 'Willamette' (Figure 11).

During the fruit harvesting period for the first year we report the highest content of chlorophyll a and b at the shorter planting distances of 'Willamette' and candidate cultivar 'Magdalena', and carotene in the second variant of Magdalena and the first variant of 'Meeker' (Figure 11).

In the third observed phenophase after fruit harvesting in 2019, the candidate cultivar 'Magdalena' has the highest content of chlorophyll a and carotene, and 'Willamette' 0.30 of chlorophyll b (Figure 11).

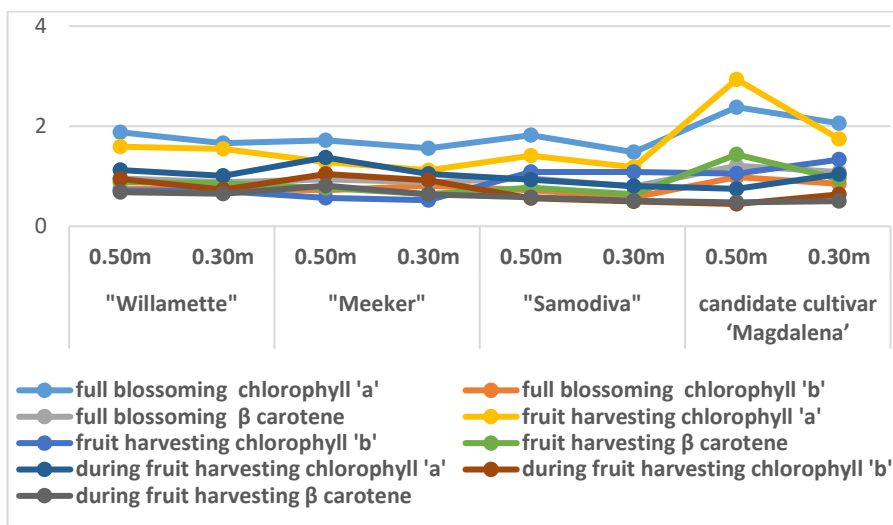


Fig. 12. Content of biologically active compounds in raspberry leaves for 2020.

In 2020 during the phenophase of full blossoming flowering the content of chlorophyll 'a', chlorophyll 'b' and β carotene in the leaves of the tested cultivars and candidate cultivar of raspberries is highest in both variants of candidate cultivar 'Magdalena' (Figure 12).

During the phenophase fruit harvesting, the values for chlorophyll "a" are highest again in both variants of the candidate cultivar 'Magdalena', reaching 2.94 mg/g in the first and 1.75 mg/g in the second. Regarding chlorophyll "b", the highest values were reported for candidate variety Magdalena from the second variant - 1.33 mg/g, followed by very close ones from 'Samodiva' (1.08, 1.09 mg/g) for both variants and candidate cultivar 'Magdalena' (1.05 mg/g) from the greater planting distance. The third indicator β carotene is again the highest in the candidate cultivar.

There are significant differences in the indicators after the fruit harvesting (Figure 12). During this phenophase we report the highest content of chlorophyll "a" in 'Meeker' (1.37 mg/g) and 'Willamette' (1.13 mg/g) of the first variant. Almost identical results were obtained for the other two indicators. For chlorophyll "b" at 'Meeker' (0.50 m)

it has a value - 1.04 mg/g, and at ‘Willamette’ (0.50 m) - 0.94 mg/g., Regarding β carotene at ‘Meeker’ (0.50 m) it has a value - 0.81 mg/g, and at ‘Willamette’ (0.50 m) the other high value was registered - 0.68 mg/l.

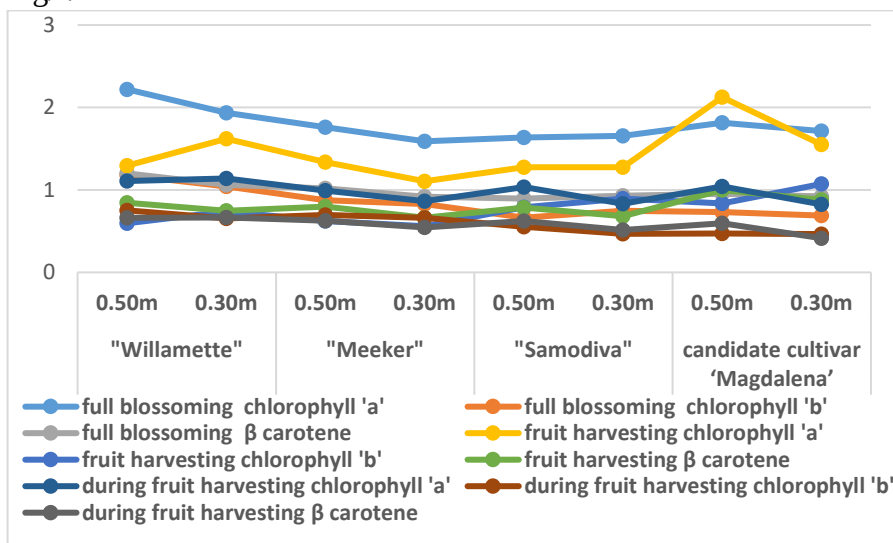


Fig. 13. Biologically active compounds in the leaves of raspberry genotypes for the period (2019-2020).

On average for the period 2019-2020 ‘Willamette’ (0.50 m) has the highest content of chlorophyll "a", "b" and carotene during blossoming phenophase. In the next phenophase of fruit harvesting, the studied indicators are the highest in both variants of the candidate cultivar ‘Magdalena’. In the third phenophase observed, after fruit harvesting, ‘Willamette’ of the two planting variants again showed the highest content of chlorophyll 'a', chlorophyll 'b' and β carotene (Figure 13).

8. Dependencies between vegetative and reproductive indicators in raspberry genotypes

For the quantitative characteristic of the dependences between the vegetative indicators (number of shoots, height and thickness of the shoots) and the reproductive indicator (yield) a linear regression

was applied.

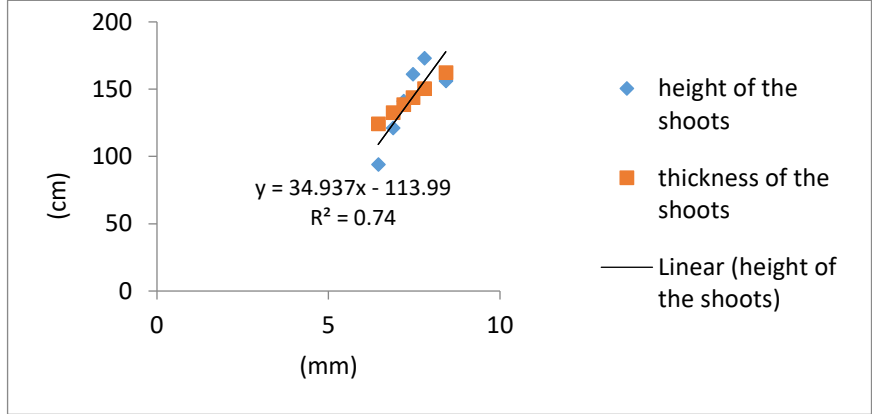


Fig. 14. Regression analysis between vegetative indicators of ‘Willamette’, with a variant of planting 0.50 m for 2019

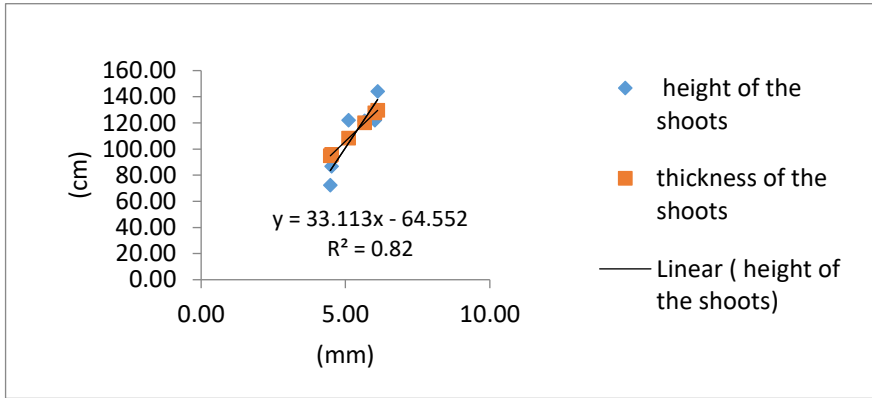


Fig. 15. Regression analysis of inter-vegetative indicators of ‘Willamette’, with a variant of planting 0.50 m in 2020.

After the regression analysis for ‘Willamette’ (0.50 m) a high dependence was found between the height and the thickness of the shoots in 2019 and 2020. The coefficient of determination R^2 is medium to high (0.74, 0.82).

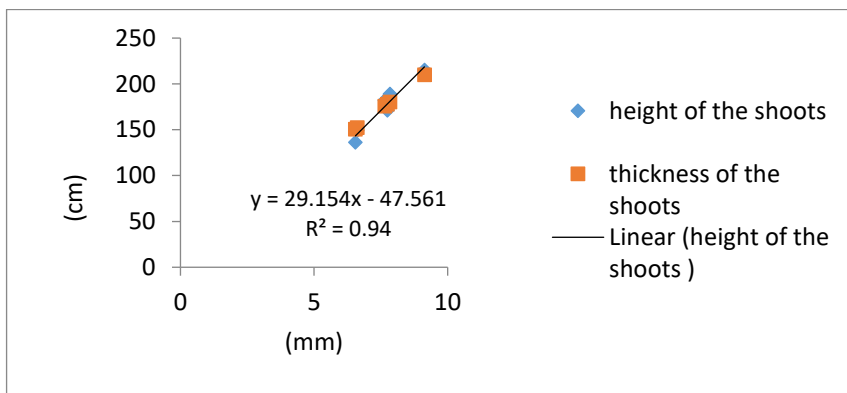


Fig. 16. Regression analysis of the inter-vegetative indicators of 'Meeker', with a variant of planting 0.50 m in 2019.

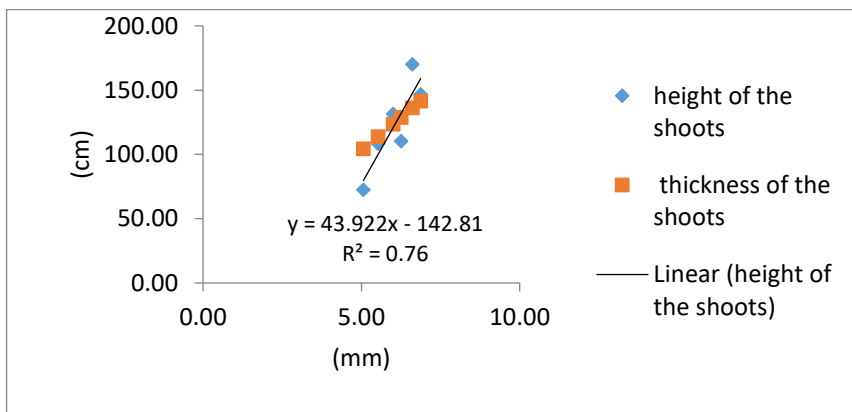


Fig. 17. Regression analysis of the inter-vegetative indicators of 'Meeker', with a variant of planting 0.50 m in 2020.

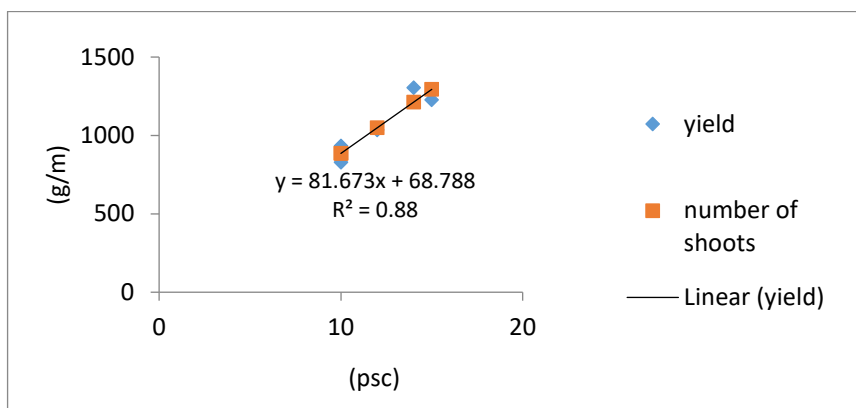


Fig. 18.Regression analysis of between vegetative and reproductive indicators of 'Meeker', with a variant of planting 0.50 m in 2020.

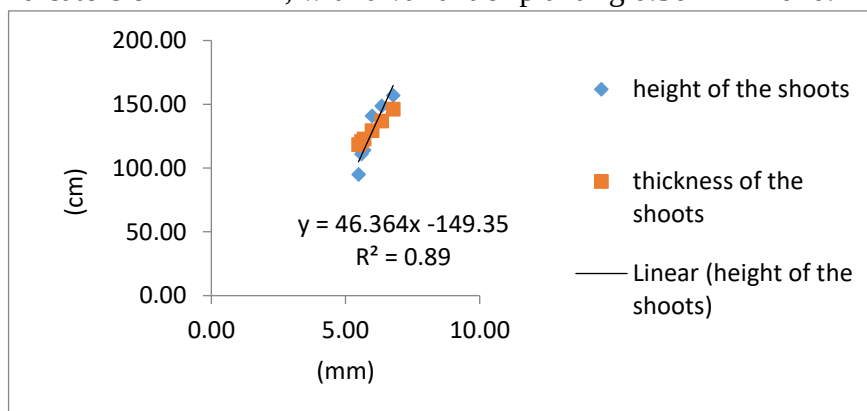


Fig. 19. Regression analysis of the inter-vegetative indicators of 'Meeker', with a plant planting variant of 0.30 m in 2020.

The linear regression analysis shows a high dependence between the vegetative indicators of height and thickness of the shoots in both variants of 'Meeker' in 2020 ($R^2 = 0.76$; $R^2 = 0.89$) and in the first variant of the cultivar in 2019 ($R^2 = 0.94$), as well as between the number of shoots and the yield at the variant of planting the plants 0.50 m of the variety for 2020 ($R^2 = 0.88$).

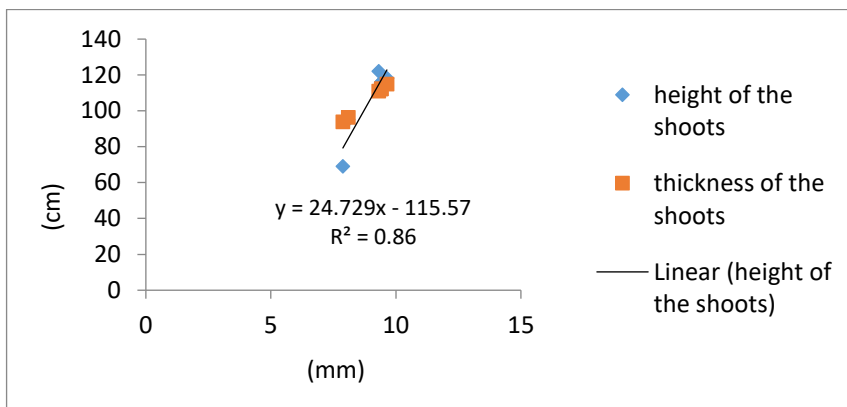


Fig. 20. Regression analysis between vegetative indicators of 'Samodiva', with a variant of planting 0.50 m in 2019.

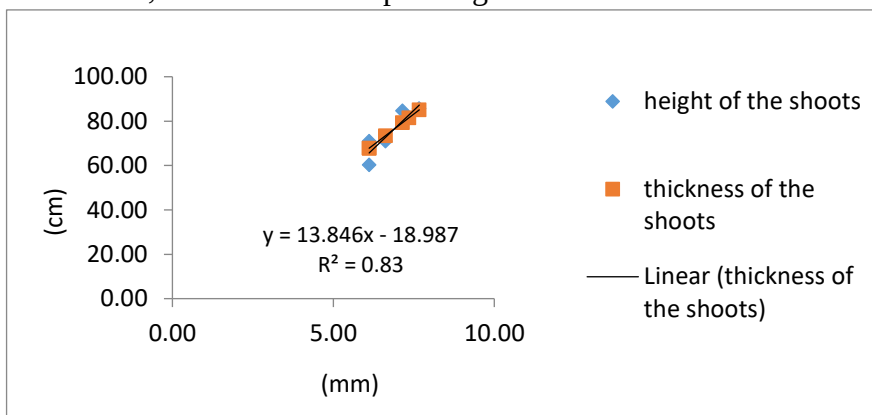


Fig. 21. Regression analysis of between vegetative indicators of 'Samodiva', with a variant of planting 0.50 m in 2020.

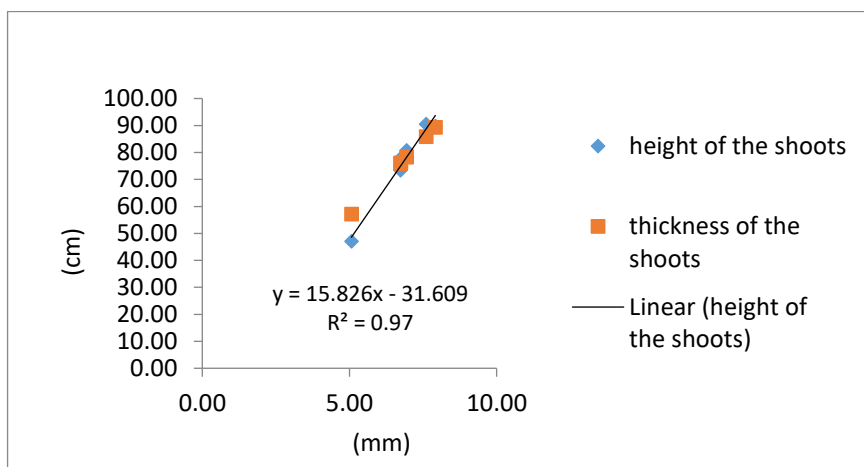


Fig. 22. Regression analysis between vegetative indicators of ‘Samodiva’, with a variant of planting 0.30 m in 2020.

In the case of ‘Samodiva’, the regression analysis shows a high positive dependence between the height and thickness of the shoots in the first variant of planting in 2019, as well as in both variants of planting in 2020. The coefficient of determination R^2 is high with values from 0.83 to 0.97.

Regression analysis of the interconnection between number, height, shoot thickness and yield in candidate cultivar ‘Magdalena’ over the three years of experiment showed that these indicators could not be determined accurately. The coefficient of determination R^2 has very low values ($R^2 < 0.70$).

VI. Conclusion

1. Candidate cultivar ‘Magdalena’ is the first to enter the vegetation and the earliest to enter the phenophase of fruit ripening.
2. The harvesting period in the first year is 22 days for ‘Willamette’ (0.30 m) and ‘Meeker’ (0.50 m). In the second and third year the longest harvest period is for the candidate cultivar ‘Magdalena’ (0.30 m), respectively 35 and 40 days.
3. With regard to the vegetative indicators for the period, the largest average number of shoots in the first year was reported in the candidate cultivar ‘Magdalena’ of the two variants, in the second again in Magdalena (0.50 m and 0.30 m) and (0.30 m) and in the third year at

‘Willamette’ and ‘Meeker’ from shorter planting distances. Average for the period, ‘Willamette’ (0.30 m) with 31.06 pcs. and candidate cultivar ‘Magdalena’ (0.50 m) with 28.06 pcs. form the largest number of shoots, and the least are registered for ‘Samodiva’ (0.50 m) with 11.89 pcs.

- The highest average height of the shoots is registered in the first year for ‘Willamette’ (0.30 m) and ‘Meeker’ in both variants. The following year for ‘Meeker’ (0.50 m and 0.30 m) and in the third year in both variants of ‘Willamette’ and ‘Meeker’. On average for the period, the highest shoots were reported for ‘Meeker’ in both variants and the lowest for Magdalena (0.30 m).
 - Regarding the average thickness of the shoots, the highest results were obtained in both variants at the beginning of the period in ‘Samodiva’ and ‘Meeker’, and in the second year in ‘Samodiva’ and at the end of the period in ‘Samodiva’ and candidate cultivar ‘Magdalena’. On average, for the period of the study in ‘Samodiva’, the thickest shoots in both variants were reported.
4. ‘Willamette’ (0.30 m) has the highest average fruit yield in the first year with 1767.5 g per 1 m. In the second year, the candidate cultivar ‘Magdalena’ in both variants (0.30 m - 2267.33 g/m, 0.50 m - 1843.5 g/m) and ‘Willamette’ (0.30 m) and at the end of the period ‘Willamette’ (0.30 m) - 2652.83 g/m in both variants of the candidate cultivar ‘Magdalena’. The highest average yield for the period was obtained for ‘Willamette’ (0.30m) - 2022.83 g/m and candidate cultivar ‘Magdalena’ (0.30m) - 1869.55 g/m. With the exception of ‘Samodiva’, in all other genotypes, higher yields were obtained from the variant with the shorter planting distance.
5. The highest average fruit weight was reported at the beginning of the period in ‘Meeker’ (0.50 m) in both variants of the candidate cultivar ‘Magdalena’, and in the next two years in ‘Meeker’ in both planting distances. On average for the period, the highest average fruit weight was registered in ‘Meeker’ in both variants, and the least in ‘Willamette’ and ‘Samodiva’ from the larger planting distance (0.50 m).

6. Fruits of 'Willamette' of the 0.50 m variant in the first two years have the highest content of ascorbic acid and anthocyanins, and in the third year in both variants of the cultivars.

Antioxidant activity is highest in 'Willamette' (0.30 m) and 'Samodiva' (0.50 m), and the lowest in the candidate cultivar 'Magdalena' in both plant planting densities.

7. The best tasting and sensory evaluations of fruits were obtained by 'Willamette' and candidate cultivar 'Magdalena' from the variant with shorter planting distances.

8. Regarding the colour parameters of the fruits, the highest brightness was reported in the candidate cultivar 'Magdalena' of both variants. The red colour tone has the highest value for 'Samodiva' (0.50 m) and 'Willamette' (0.50 m), and the yellow colour tone is the highest for all variants of the larger planting distances, with the best result reported. at the candidate cultivar 'Magdalena' (0.50 m).

9. The studied soil layers of 0-20 cm, 20-40 cm and 40-60 cm of the intra-row spacing show from strongly to moderately acid reaction in aqueous solution.

➤ The nitrogen content in the first year varies from medium (20-40 cm) during the period of fruit harvesting to very weak (40-60 cm) from the phenophase blossoming of plants. Over the next two years, the values range from good to very good.

➤ The amount of phosphorus is in the range from low to medium stock of soil depths.

➤ The content of potassium in the soil layers in the first year is low to medium. Over the next two years, a good stockpile was registered.

10. In the inter-row spacings of soil layers 0-20 cm, 20-40 cm and 40-60 cm, a strongly acid reaction in an aqueous solution has been established. The three soil depths have a very low supply of nitrogen, a low supply of phosphorus, a low supply of 0-20 cm and 20-40 cm and an average supply of potassium at 40-60 cm.

11. The content of nitrogen, phosphorus and potassium was determined in leaf samples during the phenophases of blossoming, fruit harvesting and after fruit harvesting.

- In the first year, nitrogen is in insufficient quantities, and in the second and third year during the phenophase of blossoming, in all cultivars and candidate cultivar optimal values are registered. High results were obtained during the fruit harvesting in the third year.
- The phosphorus content in the first year is in optimal values during the blossoming period in the candidate cultivar 'Magdalena' (0.50 m). In the second year the quantities of the element are low in all genotypes, while in the third year during the phenophase of blossoming they are in optimal values in the genotypes except 'Willamette'. When fruit harvesting, the element is in optimal value at 'Willamette' (0.50 m).
- The content of potassium is insufficient in the first two years and in the phenophases. In the third year, during the period of full blossoming of the plants, optimal values of the element were registered for all genotypes.

12. Correlation dependencies above $r < 0.7$ show the following regularities (with more than one repetition of the results) for the cultivars, the candidate cultivar and the variants by years:

- 'Willamette' (0.50 m) medium thickness with average height of shoots;
- 'Willamette' (0.30 m) average yield with average thickness of shoots;
- 'Meeker' (0.30 m) and 'Samodiva' (0.50 m; 0.30 m) medium thickness with average height of shoots;
- candidate cultivar 'Magdalena' (0.50 m) different results were obtained;
- candidate cultivar 'Magdalena' (0.30 m) medium thickness with average height of shoots.

13. The correlations over $r < 0.7$ between the nutrients nitrogen, phosphorus and potassium in the leaf samples and the reproductive indicators average yield and average fruit weight during the phenophases of blossoming and fruit harvesting in the genotypes report the following results with greater repeatability:

- during the phenophase of blossoming between the nutrients potassium and phosphorus;

- during the phenophase harvesting of fruits between phosphorus and the average weight of the fruit, nitrogen and potassium with the yield, potassium with the average weight of the fruit.
- Negative correlation dependences over $r < 0.7$ during the flowering phenophase were reported between nitrogen yield, phosphorus and potassium with average fruit weight.
- during the phenophase fruit harvesting negative correlations were registered between nitrogen with average fruit weight and phosphorus with nitrogen.

14. The performed correlation analyzes of the reproductive indicators, such as average yield and average fruit weight and the biologically active compounds chlorophyll "a", chlorophyll "b" and β carotene in the leaf samples determine the following dependences over $r < 0.7$:

- for all cultivars and candidate cultivar of the two variants, the relationship between β carotene and chlorophyll 'a' is taken into account; strong patterns are observed between chlorophyll "a" and average yield; chlorophyll 'a' and β carotene with average fruit weight; chlorophyll "a" with chlorophyll "b"; β carotene and chlorophyll 'b' and β carotene with medium yield.

VII. Contributions

Original characters

1. The harvesting periods of the genotypes are established, observing their increase during the period, with a difference of approximately twice in the first and the third year, with a predominance of the variant of the shorter planting distances of the plants.
2. The dynamics in the development of the vegetative indicators average number, average height and average thickness of the shoots by variants and years in raspberry cultivars and candidate cultivar is observed.
3. The values of the biologically active substances and the antioxidant activity of fruits of raspberry cultivars and candidate cultivar have been established.

4. The studied qualitative indicators and colour characteristics of fruits show the cultivar manifestations and the impact of the applied agricultural techniques.
5. The correlations between some of the indicators contribute to the determination of the genotypic manifestations in the cultivars and the candidate cultivar in the individual variants.
6. A high correlation was found in the genotypes 'Willamette', 'Meeker' and 'Samodiva' in the variant (0.50 m) between the indicators: average thickness with average height of shoots.
7. A correlation analysis was performed to monitor the relationships between reproductive parameters, average yield and average fetal weight with the elements nitrogen, phosphorus and potassium, as well as with the biologically active compounds chlorophyll "a", chlorophyll "b" and β carotene in leaf samples.

Affirmative character

1. A phenological calendar of some cultivars and candidate cultivar of raspberries from the world and Bulgarian selection has been made.

Scientific and applied character

1. The reproductive capabilities of the genotypes are determined, which show the potential of each of them during the period, in relation to the planting density of the plants. On average for the period, 'Willamette' from the 0.30 m variant has the highest yield - 2022.83 g/m².
2. The values of pH, nitrogen, phosphorus, potassium and humus in soil layers 0-20 cm, 20-40 cm, 40-60 cm were studied. Determining their dynamics in the content during the period of experiment contributes to establishing their influence on the vegetative and reproductive manifestations of the genotypes, as well as the degree of their assimilation by the plants.
3. The dynamics in the content of the nutrients nitrogen, phosphorus and potassium in leaf samples of the genotypes during the phenophases of blossoming, fruit harvesting and after fruit harvesting was monitored. It was found that potassium is not largely absorbed by plants, despite the good supply of soil with the element.

VIII. Recommendations for practice

1. On the basis of the results obtained for fruit yield, the scheme with denser planting of plants of 0.30 m can be recommended to farmers growing raspberries.

2. On the basis of the obtained fruit yields, the agricultural producers may be recommended to grow from the introduced 'Willamette', and from the Bulgarian ones - candidate cultivar 'Magdalena'.

3. The results show that in light gray forest soils, with a mechanical composition from heavy sandy-clayey to clayey, moderately eroded with low humus content, the absorption of the nutrient potassium by plants is insufficient, with some exceptions, compared to high its values in the soil.

IX. List of scientific publications on the dissertation

1. Atanasova, S., M. Georgieva, D. Georgiev, 2020. Reproductive potential of in vitro raspberry cultivars grown on poorly productive soils. Scientific Papers, Series B, Horticulture, Vol. LXIV, No.2, 35-38.
2. Atanasova, S., M. Georgieva, D. Georgiev, 2020. Vegetative changes in raspberry cultivars under various agrotechnic events. Journal of Balkan Ecology, vol.23, No 2, 139-144.
3. Atanasova, S., M. Georgieva, D. Georgiev, 2020. Correlation Dependences between Vegetative and Reproductive Characteristics of “Willamette” Cultivar, Journal of Mountain Agriculture on the Balkans, 23 (6), 219-234.

Поведение на *in vitro* малинови растения отглеждани при *in vivo* условия

Резюме

Научноизследователската работа по темата е проведена през периода 2018-2020г. в колекционно насаждение на Институт по планинско животновъдство и земеделие, гр.Троян. Обект на изследването са широко разпространените в световен мащаб сортове малини – ‘Willamette’ и ‘Meeker’, българския сорт Самодива и кандидат-сорт Магдалена. Посадъчният материал е произведен чрез клонално размножаване (*in vitro*). Опитът е заложен в два варианта с по шест повторения, всяко по един линеен метър от вътрередовото пространство.

- I var. - planting at 0.50 m in the intra-row area;

- II var. - planting at 0.30 m in the intra-row area;

Растенията са засадени в ями с размери 30/30/30 cm, с добавен в тях гранулиран пилешки тор от 0.200 kg. При двата варианта междуредовото разстояние е 3.00 m. Междуредията са естествено затревени, с прилагането на необходимите коситби на тревата, а вътрередовата площ се поддържа в черна угар посредством почвообработки. Посадъчният материал е произведен чрез клонално размножаване.

Целта на настоящия дисертационен труд е да се проследи поведението на сортове и кандидат - сорт малини произведени *in vitro* и отглеждани при *in vivo* условия при нормални и ултра гъсти разстояния на засаждане.

Резултатите от изследването ни позволяват да направим следните заключения, научни, научно-приложни приноси и препоръки за практиката.

1. Кандидат-сорт Магдалена първи встъпва във вегетация и най-рано навлиза във фенофаза узряване на плодовете.
2. Средно за периода най-голям брой издънки формира сорт ‘Willamette’ (0.30 m) – 31.06 бр.
3. Средно за периода най-високи издънки са отчетени при сорт ‘Meeker’ от двата варианта.
4. С най-голямо средно тегло на плодовете се отличава сорт ‘Meeker’ от двата варианта.
5. Средно за периода сорт ‘Willamette’ от варианта 0.30 m е с най-висок добив – 2022.83 g/m², следван от кандидат-сорт Магдалена (0.30 m) – 1869.55 g/m².
6. Плодовете на сорт ‘Willamette’ се отличават с най-високо съдържание на аскорбинова киселина и антиоксиданти.
7. Най-добри дегустационни и сензорни оценки на плодовете са получили ‘Willamette’ и кандидат-сорт Магдалена от варианта с по-малките разстояния на засаждане на растенията.

Behaviour of *in vitro* raspberry plants grown *in vivo* conditions

Summary

The research work on that topic was conducted in the period of 2018-2020 in a collection plantation of the Research Institute of Mountain Stockbreeding and Agriculture in Troyan. The objective of the study is worldwide spread raspberry cultivars, such as “Willamette” and “Meeker”, the Bulgarian cultivar “Samodiva” and the candidate cultivar ‘Magdalena’. Planting material were produced by clonal propagation (*in vitro*). The experiment was set in two variants with six replications, each of them measuring a linear meter in the intra-row spacing.

- I var. - planting at 0.50 m in the intra-row area;

- II var. - planting at 0.30 m in the intra-row area.

The plants were planted in pits measuring 0.30/0.30/0.30 m, with added granular chicken manure of 0.200 kg. In both variants the inter-row spacing was 3.00 m. Row-spacings are naturally grassed, with the application of the necessary mowing of the grass, and the intra-row area was maintained in black fallow by tillage. Planting material was produced by clonal propagation.

The objective of the present dissertation was to observe the behaviour of raspberry cultivars and candidate cultivar that had been produced *in vitro* and grown under *in vivo* conditions at normal and ultra dense planting distances.

The results of the research allow us to make conclusions, such as scientifically applied contributions and recommendations for the practice.

1. Candidate cultivar ‘Magdalena’ was the first that entered into vegetation and the earliest to enter the phenophase of fruit ripening.
2. On average for the period the largest number of shoots was formed by “Willamette” (0.30 m) with 31.06 pcs.
3. On average for the period, the highest shoots were reported for “Meeker” in both variants.
4. “Meeker” had the highest average fruit weight in both variants.
5. On average for the period, “Willamette” gave the highest yield (2022.83 g/m²) in the variant with 0.30 m planting distance, followed by the candidate cultivar ‘Magdalena’ (1869.55 g/m²) at 0.30 m.
6. Fruits of “Willamette” had the highest content of ascorbic acid and antioxidants.
7. The best tasting and sensory evaluations of the fruits were registered in “Willamette” and candidate cultivar ‘Magdalena’ from the variant with shorter planting distances.