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HETEROTIC PATTERNS IN RAPESEED (*BRASSICA NAPUS* L.): CROSSES BETWEEN SPRING-TYPE AND WINTER-TYPE GENOTYPES

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Abstract: Genetic improvement in a crop, viz. *Brassica* species, through plant breeding essentially requires the presence of adequate genetic diversity within the gene pool. Winter rapeseed is known to be genetically different from spring rapeseed and can therefore be used for extending genetic diversity in the progenies produced via crossing methods. Using line×tester analyses of two spring types of testers and six lines of winter-type rapeseed varieties, heterotic patterns of phenological traits, plant height and seed yield were estimated. Significant mean squares between parents and crosses revealed significant heterosis for all the traits. Line×tester mean squares, indicating the non-additive genetic effects, were significant only for plant height and seed yield. High narrow-sense heritability estimates for phenological traits underline the importance of additive genetic effects and thus the efficiency of selection for improving these traits. Based on the significant and positive expression of heterosis effects for phenological traits and plant height in the winter parents, it was concluded that the F1 progenies had earlier and shorter maturity than the winter parents. F1 progenies were also early flowering, early maturing and taller than the spring-type parents. Significant positive heterosis of the crosses for seed yield was observed in 75% and 42% of F1 progenies compared to the spring and winter parents, respectively, indicating a higher yield potential of the F1 hybrids than the spring and winter parents.

Key words: heritability, line×tester, phenological traits, seed yield.

Introduction

As the second most important source of oil production in the world, Brassica oilseeds have been significantly improved through common and modern breeding methods (Sabaghnia et al., 2010; Bennett et al., 2012; Ofori et al., 2012). Exploiting genetic variability of Brassica species is considered critical for genetic improvement of phenological traits, yield components and seed yield (Rahman, 2013; Gourrion et al., 2020). The limited geographic range of *Brassica* species

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along with intensive quality breeding resulted in a narrow genetic basis of this species (Seyis et al., 2005; Inamullah et al., 2006). Rapeseed cultivars are categorized as winter or spring types according to their vernalization requirements for flower initiation. Due to the restricted genetic basis of spring rapeseed, the winter type of rapeseed can be a suitable candidate for increasing yield potential and genetic variation of spring type in winter×spring type combinations (Qian et al., 2007; Kebede et al., 2010). Gourrion et al. (2020) investigated the possibility of increasing the yield of spring material by using winter germplasm. In many other reports, each spring type (Rameeh, 2010; Rahman et al., 2016) or winter type (Amiri-Oghan et al., 2009) was used separately in breeding programs. The line×tester analysis is a suitable method of evaluating a large number of genotypes as well as providing information on the relative importance of genetic parameters for determining the genetic basis of important plant traits (Singh and Chaudhury, 2001). Phenological and yield-associated traits were studied for heterosis and heritability estimates were made using this method (Shen et al., 2002; Wang et al., 2007). Several studies on spring cultivars of rapeseed have shown the important role of heterosis effects in high narrow-sense heritability estimates for phenological traits (Huang et al., 2010; Rameeh 2011). Likewise, studies with winter rapeseed cultivars (Diepenbrock, 2000; Amiri-Oghan et al., 2009; Sabaghnia et al., 2010; Rameeh, 2011) show that both additive and dominant gene effects play a significant role in the inheritance of phenological traits. With an appropriate level of heterosis, commercial development of F_1 hybrids would be justified (Nassimi et al., 2006; Bus et al., 2011). When breeding for high grain yield, the most promising crop type was both late maturing and early flowering (Dong et al., 2007; Radoev et al., 2008; Cuthbert et al., 2009). Heterosis is used commercially in rapeseed (*Brassica napus* L.) and its potential use has been demonstrated in turnip rape (*B. rapa* L.) and Indian mustard (*B. juncea* L.) for most agronomic traits (Teklwold and Becker, 2005; Zhang and Zhu, 2006). Significant negative mid-parent and better-parent heterosis was reported for days to 50% flowering and physiological maturity (Nassimi et al., 2006). In this study, mid-parent heterosis for days to 50% flowering and physiological maturity ranged from -0.04 to -2.78 and from -0.01 to -3.06, respectively, and its high parent heterosis for these traits also ranged from -0.92 to -2.78 and from -0.01 to -4.08, respectively. A significant correlation was found between the post-anthesis duration and some important agronomic traits, including the number of pods per plant, 1000-seed weight and oil yield (Marjanovic–Jeromela et al., 2007). For seed yield, an average high parent heterosis of 30% with a range of 20–50% was observed in spring rapeseed hybrids, while an average high parent heterosis of 50% with a range from 20 to 80% was reported for winter rapeseed hybrids, as reviewed by McVetty (1995).

Although line×tester analysis has been widely used in rapeseed breeding to assess genetic parameters for yield-associated traits, several studies have focused

on heterosis of winter or spring parents. Therefore, the objectives of this study were to compare heterosis of phenological traits, plant height and grain yield in winter and spring parents, and to identify narrow-sense heritability for these traits.

Material and Methods

Six winter rapeseed (*B. napus* L.) cultivars, including Zarfam, Licord, Talayeh, Okapi, Modena and Opera as lines were crossed with two rapeseed spring-type testers, including H308 and H401 in 2006–2007, based on the line×tester crossing method (Table 1).

Table 1. The origin and some characteristics of rapeseed genotypes.

Genotypes	Origin	Growth habit	Seed quality
1-H308	Iran	Spring	Double zero*
2-H401	Iran	Spring	Double zero
3-Zarfam	Iran	Winter	Double zero
4-Licord	Hungary	Winter	Double zero
5-Talayeh	Germany	Winter	Double zero
6-Okapi	France	Winter	Double zero
7-Modena	Denmark	Winter	Double zero
8-Opera	France	Winter	Double zero

*Double zero: the fatty acid content of the oil is less than 2% and the glucosinolates in the meal is less than 30 μM/g.

In winter 2007–2008, twelve F_1 progenies were grown together with their parents in a randomized complete block design with three replications at Biekol Agriculture Research Station, located in Neka, Iran (13º53′ E longitude and 43º36′ N latitude, 15 m above sea level). Each plot consisted of four rows, 5 m long and 40 cm apart. The distance between plants in each row was 5 cm, resulting in approximately 300 plants per plot, which was sufficient for the F_1 genetic analysis in each experiment. The soil which was classified as a deep loam soil (Typic Xerofluents, USDA classification) contained an average of 285 g clay kg^{-1}, 562 g silt kg^{-1}, 165 g sand kg^{-1}, and 21.4 g organic matter kg^{-1} at a pH of 7.1. Soil samples contained 40 kg ha^{-1} (mineral N in the upper 30-cm profile). Fertilizers were applied at the rates of 100: 50: 90 kg ha^{-1} of N: P: K, respectively. All plant protection practices were adopted to free the crop from insects. Seed yield (adjusted to kg ha^{-1}) was recorded based on the three middle rows of each plot. The data were recorded on ten randomly selected plants of each entry of each replication for days to flowering, days to complete flowering, duration of flowering and days to maturity and plant height.

Data for the genotypes were subjected to line×tester analysis (Singh and Chaudhury, 2001) to estimate general heritability and other genetic parameters.

The difference between the hybrid and the mean of each spring-type and winter-type parent was computed separately to estimate heterosis of phenological traits, plant height and seed yield over winter and spring parents. A least significant difference (LSD) test was used as the statistical test for heterosis.

Results and Discussion

Analysis of variance for line×tester design

There were significant differences among the crosses for all the traits except flowering duration, indicating the existence of genetic variability among the crosses (Table 2). The variance between the spring genotypes used as testers was greater than that of the winter genotypes, indicating some paternal effects on the studied traits. Line×tester variance was significant for plant height and seed yield, indicating the importance of non-additive genetic effects for these traits. High narrow-sense heritability estimates for phenological traits indicate the key role of additive genetic effects for these traits. Similarly, various studies on spring (Huang et al., 2010, Rameeh, 2011) and winter (Sabaghnia et al., 2010) cultivars of rapeseed have shown the important role of additive genetic effects for phenological traits.

Table 2. Line ×tester analysis of variance for phenological traits, plant height and seed yield of rapeseed genotypes.

S.O.V	df	M.S					
		Days to flowering	Days to complete flowering	Flowering period	Days to maturity	Plant height	Seed yield
Replication	2	76.32	2.52	105.87	0.62	4.58	1947658.84
Treatment	19	231.34**	203.69**	64.68**	134.22**	765.25**	808909.56**
Parents	7	565.05**	311.43**	48.00**	287.09**	1503.12**	704977.64
Parents vs crosses	1	127.21**	1488.40**	745.34**	251.67**	2822.40**	4422301.14**
Crosses	11	28.45**	18.33*	13.42	26.27**	108.69**	546557.91**
Lines	5	29.61**	13.89	2.94	23.47*	41.32	1748.27
Testers	1	138.81**	114.00**	112.81**	119.81**	452.82**	3144397.13**
Line × tester	5	5.23	3.64	4.03	10.36	107.23**	571799.71**
Error	38	7.05	8.41	12.69	8.77	24.83	109944.88
V_A		85.16	53.86	34.44	58.33	5.33	1713.43
V_P		91.60	60.68	44.25	67.64	59.22	171343.22
H^2_N		0.93	0.89	0.78	0.86	0.09	0.01

*, ** Significant at p=0.05 and 0.01, respectively. VA: Additive variance, VP: Phenotypic variance, H2N: Narrow-sense heritability.

Heterosis of spring and winter parents

Most of the cross combinations had higher seed yield than the spring-type parents (Table 3). Significant negative winter parent heterosis for days to flowering and days to complete flowering was observed in all the combinations (Table 4), but all combinations had significant positive results of spring parent heterosis for days to flowering. Out of 12 crosses, 5 crosses had significant positive spring parent heterosis for days to complete flowering (Table 5). Early flowering in Brassica can provide adequate time for the grain formation process and can certainly cause early maturity and higher yields, therefore, negative heterosis is desirable for flowering. The cross combinations including Opera×H308, Licord×H308, Talayeh×H308 and Modena×H308 with a mean value for days to flowering of 158, 157, 157 and 157, respectively (Table 3), also had high significant negative winter parent heterosis for days to flowering and they were found to be good combinations for improving this trait. The crosses including Opera×H308 and Talayeh×H308, with mean values of 180 and 182 days to complete flowering (Table 3), also had highly significant negative winter parent heterosis for this trait (Table 4).

Table 3. The mean performance of rapeseed genotypes for phenological traits, plant height and seed yield.

Genotypes		Days to flowering	Days to complete flowering	Flowering period	Days to maturity	Plant height (cm)	Seed yield (kg ha^{-1})
Testers	1-H308	142	177	35	218	130.00	3300.00
	2-H401	130	167	37	210	126.67	3033.33
Lines	3-Zarfam	157	190	33	225	169.97	4208.33
	4-Licord	166	193	27	236	184.63	3457.00
	5-Talayeh	163	194	31	233	173.23	3697.33
	6-Okapi	165	194	29	235	176.59	3349.18
	7-Modena	167	194	27	234	178.93	4334.20
	8-Opera	167	193	26	236	169.50	3105.76
Crosses	9-Zarfam × H401	149	174	25	221	154.50	4350.69
	10-Licord × H401	153	177	24	222	150.07	3470.10
	11-Talayeh ×H401	151	177	26	222	151.87	4050.10
	12-Okapi ×H401	153	177	24	222	157.17	3877.88
	13-Modena × H401	155	178	23	224	154.61	4566.87
	14-Opera × H401	152	174	22	223	144.30	4466.73
	15-Zarfam × H308	151	176	25	220	147.08	3250.20
	16-Licord ×H308	157	177	20	225	141.60	4233.77
	17-Talayeh × H308	157	182	25	227	160.27	3894.54
	18-Okapi ×H308	157	181	24	228	142.45	4183.33
	19-Modena × H308	157	180	23	227	145.87	4572.32
	20-Opera × H308	158	177	19	229	146.50	4461.21
	LSD (0.05)	4.38	4.79	5.88	4.89	8.22	547.15
	LSD (0.01)	5.85	6.39	7.85	6.53	10.98	730.98

The significant positive correlation between days to flowering and days to complete flowering (0.85**) indicates that the genotypes with high mean performance in days to flowering also had high mean performance in days to complete flowering (Table 6). Out of 12 crosses, 5 crosses had significant negative winter parent heterosis for the duration of flowering and all combinations had significant negative spring parent heterosis for this trait. Spring rapeseed genotypes mature earlier than winter rapeseed varieties, so significant positive spring parent heterosis was observed for days to flowering and days to maturity. Significant negative winter parent heterosis of days to flowering and days to maturity was observed for most of the combinations, suggesting that early flowering and early maturity were controlled by dominant spring-type genes of the parents. This finding is in agreement with Nassimi et al. (2006), who found significant negative mid-parent and better-parent heterosis for days to 50% flowering and physiological maturity. Most of late-maturity genotypes had low mean flowering duration; therefore, a significant negative correlation was found between these two traits. F_1 progenies of winter×spring varieties were taller than spring-type varieties but shorter than winter-type parents. Therefore, significant negative and positive effects of heterosis on plant height were observed in spring-type and winter-type parents, respectively.

Table 4. Heterosis over winter parents of rapeseed genotypes for phenological traits, plant height and seed yield.

Traits Genotypes	Days to flowering	Days to complete flowering	Flowering period	Days to maturity	Plant height	Seed yield
10-Zarfam × H401	-8.00**	-16.00**	-8.00**	-4.00	-15.47**	142.36
12-Licord × H401	-13.00**	-16.33**	-3.33	-14.00**	-34.57**	13.10
18-Talayeh ×H401	-12.00**	-17.00**	-5.00	-10.67**	-21.37**	352.77
16-Okapi ×H401	-11.67**	-16.67**	-5.00	-13.00**	-19.42**	528.70*
14-Modena × H401	-12.00**	-16.00**	-4.00	-10.00**	-24.32**	232.67
20-Opera × H401	-15.33**	-19.00**	-3.67	-13.00**	-25.20**	1360.98**
11-Zarfam × H308	-6.00**	-14.00**	-8.00**	-5.00*	-22.88**	-958.13**
13-Licord ×H308	-9.00**	-15.67**	-6.67*	-11.00**	-43.03**	776.77**
19-Talayeh × H308	-6.00**	-12.00**	-6.00*	-6.00*	-12.97**	197.21
17-Okapi ×H308	-7.67**	-12.67**	-5.00	-7.00**	-34.14**	834.15**
15-Modena × H308	-10.00**	-14.00**	-4.00	-7.00**	-33.07**	238.12
21-Opera × H308	-9.00**	-16.00**	-7.00*	-7.00**	-23.00**	1355.45**

*, ** Significant at p=0.05 and 0.01, respectively.

Among the gene pools of *Brassica* species, the winter type of *B. napus* proved to be genetically different from the spring types, while alleles of the winter type introduced into a spring type demonstrated great potential for increasing seed yield

in hybrid and open-pollinated cultivars (Huang et al., 2010; Kebede et al., 2010). Out of 12 crosses, 5 crosses had significant positive heterosis effects of grain yield on spring-type parents (Table 5).

Table 5. Heterosis over spring parents of rapeseed genotypes for phenological traits, plant height and seed yield.

Traits Genotypes	Days to flowering	Days to complete flowering	Duration of flowering	Days to maturity	Plant height	Seed yield
10-Zarfam × H401	7.00**	-2.67	-9.67**	3.00	24.50**	1050.69**
12-Licord × H401	11.00**	0.00	-11.00**	4.00	20.07**	170.10
18-Talayeh ×H401	9.00**	0.33	-8.67**	4.33	21.87**	750.10**
16-Okapi ×H401	11.00**	0.33	-10.67**	4.00	27.17**	577.88*
14-Modena × H401	13.00**	1.33	-11.67**	6.00**	24.61**	1266.87**
20-Opera × H401	10.00**	-2.67	-12.67**	5.00**	14.30**	1166.73**
11-Zarfam × H308	20.67**	9.00	-11.67**	10.00**	20.42**	216.87
13-Licord ×H308	26.67**	10.33**	-16.33**	15.00**	14.93**	1200.43**
19-Talayeh × H308	26.67**	15.00**	-11.67**	17.00**	33.60**	861.21**
17-Okapi ×H308	26.67**	14.00**	-12.67**	18.00**	15.78**	1150.00**
15-Modena × H308	26.67**	13.00**	-13.67**	17.00**	19.20**	1538.99**
21-Opera × H308	28.00**	10.00**	-18.00**	19.00**	19.83**	1427.88**

*, ** Significant at p=0.05 and 0.01, respectively.

Table 6. The correlation of phenological traits, plant height and seed yield for rapeseed genotypes.

Traits	Days to flowering	Days to complete flowering	Flowering period	Days to maturity	Plant height	Seed yield
Days to flowering	1					
Days to complete flowering	0.85**	1				
Duration of flowering	-0.37	0.16	1			
Days to maturity	0.97**	0.88**	-0.25	1		
Plant height	0.82**	0.89**	0.02	0.82**	1	
Seed yield	0.18	-0.12	-0.57**	0.08	-0.03	1

*, ** Significant at p=0.05 and 0.01, respectively.

The crosses Modena×H401, Opera×H401, and Modena×H308 gave 4566.87, 4466.73, and 4572.32 kg ha^{-1} of seed yield and significant positive winter and spring parent heterosis for this trait, respectively, and were found to be good combinations for improving seed yield. Similarly, for grain yield, an average high parent heterosis of 30% with a range of 20–50% was observed in spring rapeseed hybrids, while an average high parent heterosis of 50% was reported for winter rapeseed hybrids, ranging from 20 to 80%, as reviewed by McVetty (1995).

Conclusion

In conclusion, all the phenological traits were more heritable than the other studied traits, so the efficiency of selection will be high for these traits. Most of the crosses had significant negative heterosis effects for phenological traits and also significant positive heterosis for plant height in spring-type parents. Therefore, F1 progenies of winter×spring rapeseed varieties were characterized by early flowering, early maturity and great plant height as compared to spring-type parents. The general crossing between spring and winter types of rapeseed genotypes allows high yields of F1 progeny with medium maturity, which can be used for improving seed yield.

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HETEROTIČNI OBRASCI KOD ULJANE REPICE (*BRASSICA NAPUS* L.): UKRŠTANJA IZMEĐU GENOTIPOVA JAROG I OZIMOG TIPA

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Genetičko poboljšanje useva, kao što su *Brassica* vrste, putem oplemenjivanja biljaka, u suštini zahteva prisustvo adekvatne genetičke raznovrsnosti u okviru genskog pula. Poznato je da se ozima uljana repica genetički razlikuje od jare uljane repice i da se stoga može koristiti za proširenje genetičke raznovrsnosti kod potomstva dobijenog metodama ukrštanja. Koristeći linija×tester analize za dva jara tipa testera i šest linija ozimih sorti uljane repice, procenjeni su heterotični obrasci fenoloških osobina, visine biljke i prinosa semena. Značajni srednji kvadrati između roditelja i hibrida otkrili su značajan heterozis za sve osobine. Srednji kvadrati linija×tester, koji ukazuju na neaditivne genetske efekte, bili su značajni samo za visinu biljke i prinos semena. Procenjene visoke vrednosti heritabilnosti u užem smislu za fenološke osobine naglašavaju važnost aditivnih genskih efekata, a time i efikasnost selekcije za poboljšanje ovih osobina. Na osnovu značajnog i pozitivnog ispoljavanja heterotičkih efekata za fenološka svojstva i visinu biljke kod roditelja ozimog tipa, zaključeno je da su F1 potomci imali ranije i kraće sazrevanje od roditelja ozimog tipa. Potomci F1 su takođe ranije cvetali, ranije sazrevali i bili viši od roditelja jarog tipa. Značajan pozitivan heterozis hibrida za prinos semena uočen je kod 75% i 42% F1 potomaka u poređenju sa roditeljima jarog odnosno ozimog tipa, što ukazuje na veći potencijal prinosa F1 hibrida u odnosu na roditelje jarog i ozimog tipa.

Ključne reči: heritabilnost, linija×tester, fenološke osobine, prinos semena.

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A COMPARATIVE STUDY ON NARBON VETCH AND COMMON VETCH IN THE SEMI-ARID REGION OF SETIF (ALGERIA)

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Abstract: Algeria has a limited supply of fodder. Traditional methods of raising livestock rely on the exploitation of low-quality rangelands, fallow and natural pastures with forage crops receiving little attention. The population relies on imports to meet its protein needs. Indeed, fodder production is the primary lever for improving livestock nutrition and, as a result, the productivity of the livestock systems in question. It is also crucial to the sustainability of mixed systems that combine plant and animal production. The current study was conducted to evaluate the phenological stages, analyze the performances of fodder yield, grain yield and some of its components and determine the chemical composition of the species *Vicia narbonensis* L. in comparison with *Vicia sativa* L. in order to improve the fallow year in the cereal/fallow rotation and develop the marginal lands. Trials were conducted on the plots of FERHAT Abbas University Campus under the rainfed conditions in the semi-arid region of Setif during three growing seasons (2017–2020), using 10 ecotypes of narbon vetch and 2 ecotypes of common vetch (as control ecotypes) in a randomized complete block design with three replicates. Significant effects of the ecotype, the year and the ecotype x year interaction were found, as well as a great variability in the phenological stages, agronomic characteristics and chemical composition of the ecotypes studied. A significant positive relationship ($p<0.05$) was found between grain yield and dry matter yield and a significant negative relationship ($p<0.05$) was found between full flowering date and dry matter yield and grain yield. It seems that the early flowering ecotypes produce better yields than the late flowering ones in the semi-arid region of Setif.

Key words: fallow, chemical composition, livestock, production system, rainfed conditions, semi-arid region, *Vicia narbonensis* L., *Vicia sativa* L., yields.

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Introduction

Livestock husbandry in Algeria has always had a traditional character, based primarily on nomadism and the exploitation of natural resources (Carter, 1975). These poor food resources are mainly made up of by-products (bran, straw, and stubble), grazing on spontaneous fallow crops and only occasionally growing vetches, oats, and barley (CIHEAM, 2006). The most significant limitation to the expansion of cattle husbandry is a limited availability of fodder (Bourbouze, 1999). This lack of fodder has a significant effect on animal productivity and leads to a significant increase in the importation of dairy and meat products. This is because fodder production and cultivation in Algeria remain, in many ways, a minor activity of agricultural holdings (Bekhouche, 2011).

At the national level, nearly 39.5 million hectares of land are dedicated to fodder production, divided into natural grasslands, fodder crops, stubble, pastures, rangeland, and fallow land (MADR, 2006). In 2017, more than half of the UAA was devoted to field crops, particularly cereals, with fallow land occupying more than a third of the national UAA (37.3%) (Bessaoud et al., 2019). The fallow-cereal-livestock production system is common (Nedjraoui, 2001), with the cereal/fallow system accounting for more than 80% of the total UAA (Bessaoud et al., 2019).

Cultivated fodder is usually located in the north of the country. They are predominantly composed of vetch and oats, whose hay is considered to be of poor quality (Abdelguerfi, 1987). The area under this crop accounts for around 70 to 80% of the total annual fodder consumed dry and varies from year to year, finally falling to less than 50% (Mebarkia and Abdelguerfi, 2007). The primary constraint to this weakness is its inability to adapt to all agro-ecological conditions in Algeria due to a lack of specific and varietal diversity (Açikgöz, 1982, 1988). In fact, only *Vicia sativa L.* and its Languedoc variety are grown in the various agro-ecological zones of Algeria (Açikgöz, 1982, 1988). Furthermore, this species is highly subject to abiotic stressors, pod cracking (Zulfiqar and Muhammad, 2006) and the lack of seed production (Mebarkia et al., 2003). Despite all efforts, Algerian fodder production remains considerably below the needs of cattle (Zeghida, 1987).

Many extremely interesting fodder species present throughout northeastern Algeria would have to be valorized (Issolah et al., 2001) in order to provide higher quality feed for livestock and improve soil fertility (Turk, 1997) and to be integrated into different production systems (Klein et al., 2014). Although they are of great importance, they have never taken their rightful place in Algeria.

Among all the species present in Algeria, fodder legumes alone account for 33 genera and 293 species (Issolah and Beloued, 2005). Annual forage legumes, such as those of the genus Vicia, are among the options to be considered for replacing fallow in cereal crop rotation (Issolah and Yahiaoui, 2008). Vetches (*Vicia sp.*) are common forage legumes in the rainfed, semi-arid agricultural systems of the

Mediterranean region (Turk, 1997). Vetches are more adaptable and promising as short-term fodder crop. One advantage of vetch is its versatility, which allows for a wide range of uses (Seyoum, 1994). Vetches may either be grazed as fresh forage (Haddad, 2006) or may be cut and conserved as silage or hay (Abdullah et al., 2010), which may be used as a protein supplement, while their grains are utilized as both protein and energy sources in the diets of ruminants and non-ruminants (Sadeghi et al., 2009) with less expensive costs compared to concentrates, particularly in developing countries (Seyoum, 1994). The contribution of vetch in crop-livestock production systems around the world is well recognized (Berhanu et al., 2003).

Within the genus Vicia, much attention has been given to narbon vetch (*Vicia narbonensis* L.), one of the species domesticated in the early centuries of agriculture (Bryant and Hughes, 2011). Furthermore, this species has been promoted in the eastern Mediterranean as a replacement for fallow in the traditional fallow-barley rotation (Maxted, 1995). Narbon vetch is a promising forage legume (Kroschel, 2001) that can grow without support (Bryant and Hughes, 2011) and is tolerant to drought and cold (Kroschel, 2001). It grows in winter (Davies et al., 1993) and does not lose its leaves after frost (El Moneim, 1989). Its high tolerance to pests and diseases is the key reason it is used in hot and dry areas instead of faba beans or common vetch (Mateo Box, 1961). *Vicia narbonensis* L. has a greater grain production potential as a livestock feed in non-tropical arid areas than common vetch (*Vicia sativa*), bitter vetch (*Vicia ervilia*), or woolly-pod vetch (*Vicia villosa* subspp. *dasycarpa*) (Larbi et al., 2010a). Its seeds have a high protein content (Abd-el Moneim, 1992), around 20–32%, (Eason et al., 1990; Thomson et al., 1990; Abd-el Moneim, 1992), which recommends it as a soybean substitute in animal rations (Larbi et al., 2010b; Huseyin, 2014; Renna et al., 2014). The species is also very important in crop rotation systems, either as pure stands or mixed with cereals to provide high quality livestock feed (Altınok, 2002; Altınok and Hakyemez, 2002; Iptaş and Karadağ, 2009; Nizam et al., 2011).

Besides, because of its high green biomass and its ability to fix a large amount of nitrogen into the soil, it is a valuable green manure crop (Özyazıcı and Manga, 2000; Avcıoğlu et al., 2009) in the modern trends such as sustainable agriculture and organic farming (Ćupina et al., 2004). Narbon vetch is also better than the other vetch species since it leaves enough time for planting second crops (Çakmakçi et al., 1999). Trials in Syria, Iraq, Cyprus, Turkey, France, and Australia have shown that *Vicia narbonensis* L. can produce high grain yields (1.5–5.1 t/ha) under dry Mediterranean winter rainfall conditions (250–550mm/annum) (Enneking and Maxted, 1995). Little work has been done on the species of the genus Vicia, particularly *Vicia narbonensis* L., in our country.

Therefore, the current study aimed to evaluate the phenological stages, analyze the performances of fodder yield, grain yield and some of its components and determine the chemical composition of two species of the genus Vicia,

including *Vicia narbonensis* L., (narbon vetch, 10 ecotypes) and *Vicia sativa* L. (common vetch, 2 control ecotypes) under the semi-arid conditions of Setif during three growing seasons (2017–2020).

Material and Methods

The trials were conducted on the plots of FERHAT Abbas University Campus Setif (36°12' N; 5°21' E) under rainfed conditions in the semi-arid region of Setif. In this region, the climate is continental with strong annual and daily thermal amplitudes, and the altitude is 1025 m. The average annual rainfall is around 450 mm (Seltzer, 1947) and 373.8 mm for the period between 2006 and 2017 (ONM, 2017). The soils of the experimental site belong to the group of steppe soils (Perrier and Soyer, 1970). The physicochemical composition indicates, for all the plots, a clay loam texture, a lumpy structure, with a basic water pH (7.81), a total calcium content of 17.7% and an organic matter content varying from 2.0 to 3.0%.

The trial was conducted during three agricultural seasons (2017–2020). The climatic conditions of the three seasons are presented in Table 1 (ONM, 2020). The three agricultural campaigns are characterized by:

- The good distribution of rainfall P (mm), with larger quantities during the first growing season (469.05 mm), followed by the third one (384.56 mm) against only 321.20 mm in the second growing season;
- The maximum temperatures (MaxT °C) recorded were homogeneous during the three growing seasons, as well as the minimum temperatures (MinT °C).

Plant material: The experiment was carried on two species of the genus Vicia represented by 10 ecotypes of *Vicia narbonensis* L. from different origins and 2 ecotypes of *Vicia sativa* L. from Algeria. The ecotypes of *Vicia sativa* L. were used as control ecotypes because this species is well known among Algerian farmers (Table 2). The sowing was carried out from the same batch of seeds, on January 4th, 2018 for the first year, on December 22nd, 2019 for the second year and on December 23rd, 2020 for the third year. All the ecotypes were sown manually and separately in a randomized complete block design with 3 replicates in a plot having a cereal (durum wheat) as a previous crop. Each elementary plot had 4 rows 4 m long, spaced 30 cm apart. In each of these plots, 336 seeds/plot (70 seeds/m^2) of vetch were sown.

Various cultural practices were carried out on this trial. Deep plowing (25 cm) was carried out using a disc plow just after the first autumn rains (September and October) followed by two cross passes of cover crops to reduce weed infestation and get a good seedbed. During the three trial campaigns, the plots were weeded manually as needed throughout the growing season and no fertilizers were used. The harvest was carried out from May 30th to June 19th for the first year; from June 11th to July 2nd for the second year and from June 17th to 28th for the third year.

Table 1. Climatic conditions in the semi-arid region of Setif.

	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Total	Mean
2006–2017													
P (mm)	33.74	30.27	31.95	27.88	39.68	43.26	41.68	52.42	27.19	33.01	12.72	373.80	
Max T°C	28.16	23.57	16.19	11.77	11.31	11.37	14.67	19.21	24.09	30.05	34.48		20.44
Min T°C	15.70	11.89	7.143	3.09	2.91	2.44	4.78	8.36	12.4	18.26	22.21		9.93
Mean T°C	21.39	17.20	11.23	7.118	6.64	6.69	9.54	13.65	18.15	23.85	28.27		14.88
2017–2018													
P (mm)	41.00	10.70	55.70	33.50	13.90	23.20	90.20	81.30	51.90	65.00	2.65	469.05	
Max T°C	28.30	22.26	14.80	9.38	11.81	9.60	13.46	17.72	21.33	27.10	35.40		19.15
Min T°C	13.50	9.63	3.60	1.19	1.05	0.60	4.20	4.70	9.47	20.00	17.30		7.72
Mean T°C	20.90	15.95	9.20	5.28	6.43	4.40	8.83	11.21	15.4	24.20	31.60		13.87
2018–2019													
P (mm)	25.00	63.70	25.70	10.80	77.10	15.10	24.00	43.80	12.40	10.90	12.70	321.20	
Max T°C	28.40	19.4	14.50	12.9	7.74	11.12	15.17	18.21	16.40	33.6	35.50		19.31
Min T°C	16.00	9.60	4.60	1.64	-0.50	-0.06	3.35	6.30	9.40	17.21	27.20		8.59
Mean T°C	22.20	14.5	9.55	7.27	3.62	5.53	9.26	12.26	12.9	28.5	31.70		14.19
2019–2020													
P (mm)	75.00	25.80	90.20	21.40	12.80	10.00	51.56	66.00	6.60	21.40	3.80	384.56	
Max T°C	27.90	22.10	12.40	12.80	11.10	16.80	14.80	19.30	26.60	29.60	33.80		20.58
Min T°C	15.40	10.30	4.20	3.70	0.30	1.90	4.30	8.00	11.80	14.50	24.60		8.95
Mean T°C	21.65	16.20	8.30	8.25	5.70	9.35	9.10	13.65	19.20	22.05	29.20		14.77

Observations and measurements: The phenological stages observed were: the date of the beginning of flowering (BF), evaluated by the number of days from the date of emergence to the appearance of the first inflorescence (Berrekia, 1985); the date of 50% flowering (50%F) and the date of full flowering (100%F), evaluated by the number of days from the date of emergence to the appearance of the maximum number of flowers. The morphological parameter was: plant height (PH), measured in centimeters from the base to the end of the main stem at the time of full flowering. At harvest, three randomly selected plants from each plot were dug up and separated to determine grain yield and its components. Agronomic parameters were as follows: dry matter yield (DMY), grain yield (GAY), hundred-

seed weight (HSW), number of pods per plant (NP/P), pod length (P-Leng) and number of seeds per pod (NS/P). Dry matter yield (DMY) was determined when 100% flowering was observed. Green matter was taken from each plot and weighed as soon as possible without losing weight. The dry matter yield was calculated after drying a fresh sample of 200 g of green matter in an oven at 80° C for 48 hours. Finally, grain samples of all vetch ecotypes produced during the growing season 2019–2020 were analyzed for dry matter (DM) content, ash, crude protein (CP) content and crude fiber (CF) according to the AOAC procedures (1990).

Table 2. Origins of the ecotypes of *Vicia narbonensis* L. and *Vicia sativa* L. studied.

Species	Ecotypes	Code	Origin
Vicia narbonensis L.	1	N-2380	Lebanon
	2	N-2383	Lebanon
	3	N-2390	Lebanon
	4	N-2392	Lebanon
	5	N-2393	Syria
	6	N-2461	Turkey
	7	N-2464	Turkey
	8	N-2466	Turkey
	9	N-2468	Lebanon
	10	N-2561	Syria
Vicia sativa L.	11	S-174	Algeria (Setif)
	12	S-BBA	Algeria (BBA)

Statistical analysis: The data collected was processed using the XLSTAT software (2014), according to an analysis of variance based on the means comparison of Fisher's LSD at the 5% level. The relationships between the different pairs of measured variables were described and analyzed by calculating the phenotypic correlations based on the genotypic averages.

Results and Discussion

For each species (*Vicia narbonensis* L. and *Vicia sativa* L.), the analysis of variance indicates significant effects of ecotype, year and ecotype x year interaction ($p< 0.05$), highlighting the great diversity in phenological evolution and productions for the different measured parameters (Table 3). The year effect was significant ($p<0.05$) for all the phenological variables analyzed. This indicates that the characteristics of the ecotypes of *Vicia narbonensis* L. and *Vicia sativa* L. tested in the semi-arid region of Setif were strongly affected by interannual variations.

Table 3. Variances of the measured parameters of the 12 vetch ecotypes over the three years (2017–2020).

Source of variation	Phenological stages (days)				
	DF	BF	50%F	100%F	PH (cm)
		Vicia narbonensis L.			
Total	89	787.2	804.2	830.5	377.2
Ecotype	9	71.39*	21.12*	15.60*	12.55*
Year	2	10463.7*	11232.9*	11664.7*	4946.5*
Ecotype*year	18	46.59*	10.12*	15.03*	16.33*
Overall average		52.50	61.90	68.13	34.66
SD		16.17	16.37	16.58	11.43
CV (%)		30.8	26.44	24.33	33.00
		Vicia sativa L.			
Total	17	1482.7	1491.1	1482.7	1006.8
Ecotype	1	2.00*	0.50*	2.00*	1.10*
Year	2	3678.5*	3708.5*	3678.5*	2474.1*
Ecotype*year	2	3.50*	3.50*	3.50*	1.67*
Overall average		57.17	67.67	73.83	37.97
SD		20.97	20.98	21.00	17.40
CV (%)		36.68	31.00	28.44	45.82

Source of variation	Production (kg/ha)			Yield components		
	DMY (kg/ha)	GAY (kg/ha)	HSW (g)	P-Leng (cm)	NP/P	NS/P
		Vicia narbonensis L.				
Total	1825740	3021589	12.8	9.5	26.9	6.7
Ecotype	157395*	192427*	8.29*	0.33*	1.79*	0.46*
Year	21781607*	35458888*	28.4*	127.5*	315.7*	88.43*
Ecotype*year	145654*	205704*	3.34*	0.28*	2.75*	0.28*
Overall average	1353.62	1271.22	23.0	4.85	5.43	3.41
SD	811.85	1058.29	2.52	1.79	3.13	1.52
CV (%)	60.00	83.24	10.95	36.90	57.644	44.60
		Vicia sativa L.				
Total	3278234	3956633	0.26	9.4	153.7	8.2
Ecotype	873842*	9113*	0.10*	0.19*	55.72*	0.02*
Year	7415429*	9552692*	0.01*	22.51*	343.2*	19.59*
Ecotype*year	113520*	3327*	0.04*	0.45*	6.09*	0.02*
Overall average	1603.89	1249.39	6.86	4.98	11.57	4.62
SD	1001.01	1103.74	0.41	1.68	6.81	1.59
CV (%)	62.40	88.34	5.98	33.73	58.86	34.41

*Significant at 5%; DF: Degree of freedom; SD: Standard deviation; CV: Coefficient of variation. The significance level is 5%.

Vicia narbonensis L. was very early for the beginning of flowering, 50% flowering and full flowering, at 31 to 77 days, 39 to 80 days and 44 to 86 days,

respectively. *Vicia sativa* L. was the latest at 32 to 82 days, 39 to 87 days and 45 to 92 days, respectively (Table 4). A significant ecotype × year interaction ($p<0.05$) indicates that the ecotypes were not stable for the parameters measured from one year to another. In terms of plant height between the two species, *Vicia sativa* L. presented the highest average values of 37.72 and 38.22 cm compared to *Vicia narbonensis* L. with 33.24 and 36.48 cm (Table 5).

Table 4. Phenological phases of vetch ecotypes in the three-year period.

Ecotype	Beginning of flowering % (days)			
	2017–18	2018–19	2019–20	Mean
		Vicia narbonensis L.		
N-2380	33.00^{B}	57.00^{I}	50.00^{I}	46.67
N-2383	33.00^{B}	72.00^{E}	57.00^{C}	54.00
N-2390	33.00^{B}	77.00^{B}	58.00^{B}	56.00
N-2392	33.00^{B}	69.00^{G}	65.00^{A}	55.67
N-2393	32.00^{c}	72.00^{E}	55.00^{E}	53.00
N-2461	31.00^{D}	73.00^{D}	58.00^{B}	54.00
N-2464	33.00^{B}	64.00^{H}	52.00^{G}	49.67
N-2466	32.00^{C}	71.00^{F}	54.00^{F}	52.33
N-2468	33.00^{B}	74.00^{C}	51.00^{H}	52.67
N-2561	34.00^{A}	69.00^{G}	50.00^{I}	51.00
Mean	32.70	69.80	55.00	52.50
		Vicia sativa L.		
S-174	32.00^{C}	82.00^{A}	56.00^{D}	56.67
S-BBA	33.00^{B}	82.00^{A}	58.00^{B}	57.67
Mean	32.50	82.00	57.00	57.17
Ecotype	50% flowering (days)			
	2017–18	2018–19	2019–20	Mean
		Vicia narbonensis L.		
N-2380	40.00^{A}	70.00^{J}	68.00^{H}	59.33
N-2383	40.00^{A}	76.00^{G}	69.00^{G}	61.67
N-2390	40.00^{A}	80.00^{C}	71.00^{E}	63.67
N-2392	40.00^{A}	79.00^{D}	75.00^{C}	64.67
N-2393	40.00^{A}	78.00^{E}	69.00^{G}	62.33
N-2461	39.00^{B}	75.00^{H}	71.00^{E}	61.67
N-2464	40.00^{A}	74.00^{I}	67.00^{I}	60.33
N-2466	39.00^{B}	75.00^{H}	69.00^{G}	61.00
N-2468	40.00^{A}	77.00^{F}	70.00^{F}	62.33
N-2561	40.00^{A}	74.00^{I}	72.00^{D}	62.00
Mean	39.80	75.80	70.10	61.90
		Vicia sativa L.		
S-174	39.00^{B}	85.00^{B}	78.00^{A}	67.33
S-BBA	40.00^{A}	87.00^{A}	77.00^{B}	68.00
Mean	39.50	86.00	77.50	67.67

$^{A, B, C, D, E, F, G, H, I, J}$ The groups of means according to the Fisher's test (LSD) at the 5% level.

Continuation of Table 4. Phenological phases of vetch ecotypes in the three-year period.

Ecotype	100% flowering (days)			
	2017–18	2018–19	2019–20	Mean
Vicia narbonensis L.				
N-2380	46.00^{A}	78.00^{I}	78.00^{D}	67.33
N-2383	46.00^{A}	81.00^{f}	79.00^{C}	68.67
N-2390	46.00^{A}	85.00^{e}	76.00^{F}	69.00
N-2392	46.00^{A}	83.00^{E}	83.00^{B}	70.67
N-2393	45.00^{B}	84.00^{D}	74.00^{G}	67.67
N-2461	44.00^{C}	80.00^{G}	77.00^{E}	67.00
N-2464	46.00^{A}	81.00^{F}	74.00^{G}	67.00
N-2466	45.00^{B}	79.00^{H}	76.00^{F}	66.67
N-2468	46.00^{A}	86.00^{B}	77.00^{E}	69.67
N-2561	45.00^{B}	79.00^{H}	79.00^{C}	67.67
Mean	45.50	81.60	77.30	68.13
Vicia sativa L.				
S-174	45.00^{B}	92.00^{A}	85.00^{A}	74.00
S-BBA	46.00^{A}	92.00^{A}	83.00^{B}	73.67
Mean	45.50	92.00	84.00	73.83

$^{A, B, C, D, E, F, G, H, I, J}$ The groups of means according to the Fisher's test (LSD) at the 5% level.

This great variability observed in the characters measured for the two vetches studied makes it possible to select the appropriate species for the enhancement of fallow land according to climatic conditions and production systems for the semi-arid region of Setif.

For dry matter yield and grain production, the analysis of variance shows highly significant differences between species and within the same species (Table 3).

The best dry matter yield were recorded for the *Vicia sativa* L. species with average values of 1604 kg/ha against 1355 kg/ha for *Vicia narbonensis* L. During the 2017–2018 growing season, the highest dry matter yield was recorded in *Vicia sativa* L. with 2406 kg/ha and 2271 kg/ha in *Vicia narbonensis* L. On the other hand, the lowest yields were obtained during the 2018–2019 growing season, with 335 kg/ha and 587 kg/ha, respectively (Table 5).

For grain yield, *Vicia narbonensis* L. was more efficient with 1271 kg/ha, followed by *V. sativa* L. with 1249 kg/ha (Table 5). It should be noted that the semi-arid region of Setif is characterized by very low temperatures, below 4°C, which most often harmonizes with the flowering of field crops (Baldy, 1974). These low temperatures affect the fertility of the vetches by reducing the number of

flowers and thus the grain yield. Ridge and Pye (1985) estimated that 68% of the variation in pea yield was due to extreme temperatures at the flowering stage in the Australian Mediterranean climate.

Table 5. Plant height, dry matter yield and grain yield in vetch ecotypes.

Ecotype	Plant height (cm)			
	2017–18	2018–19	2019–20	Mean
		Vicia narbonensis L.		
N-2380	41.72^{B}	18.89^{H}	43.78^{H}	34.80
N-2383	34.89^{I}	21.67^{A}	43.33^{I}	33.30
N-2390	36.56^{H}	20.44^{D}	45.00^{f}	34.00
N-2392	39.28^{E}	19.33^{G}	41.11^{K}	33.24
N-2393	39.11^{E}	19.33^{G}	48.33^{e}	35.59
N-2461	41.39^{C}	19.55F	44.66^{G}	35.20
N-2464	40.56^{D}	21.11^{B}	47.78^{D}	36.48
N-2466	40.67^{D}	20.22^{E}	41.89^{J}	34.26
N-2468	34.17^{J}	20.78^{C}	45.67^{E}	33.54
N-2561	43.11^{A}	20.44^{D}	45.00^{F}	36.18
Mean	39.15	20.18	44.66	34.66
		Vicia sativa L.		
S-174	37.72^{G}	16.89^{J}	58.56^{A}	37.72
S-BBA	38.21^{F}	18.44^{I}	58.00^{B}	38.22
Mean	37.97	17.67	58.28	37.97
Ecotype	Dry matter yield (kg/ha)			
	2017–18	2018–19	2019–20	Mean
		Vicia narbonensis L.		
N-2380	2410^{C}	546^{G}	1037^{G}	1331
N-2383	1783^{H}	754^{A}	1212^{F}	1250
N-2390	2239^{E}	588^{E}	1188^{F}	1338
N-2392	2597^{B}	519^{H}	1458^{D}	1525
N-2393	2261^{E}	621^{C}	925^{I}	1269
N-2461	1986^{G}	581^{F}	1459^{D}	1342
N-2464	2717^{A}	502^{I}	1647^{C}	1622
N-2466	2329^{D}	514^{H}	1003^{H}	1282
N-2468	2055^{F}	631^{B}	854^{J}	1180
N-2561	2330^{D}	609^{D}	1253^{E}	1397
Mean	2271	587	1204	1355
		Vicia sativa L.		
S-174	2075^{F}	269^{K}	1807^{B}	1384
S-BBA	2736^{A}	401^{J}	2335^{A}	1824
Mean	2406	335	2071	1604

$^{A, AB, B, C, D, DE, E, F, G, H, I, J, K}$ The groups of means according to the Fisher's test (LSD) at the 5% level.

Continuation of Table 5. Plant height, dry matter yield and grain yield in vetch ecotypes.

Ecotype	Grain yield (kg/ha)			
	2017–18	2018–19	2019–20	Mean
	Vicia narbonensis L.			
N-2380	2679^{D}	273^{D}	1014^{F}	1322
N-2383	2746^{C}	447^{A}	824^{H}	1339
N-2390	2850^{A}	298^{c}	1010^{F}	1386
N-2392	2267^{G}	303^{c}	1268^{C}	1279
N-2393	2345^{F}	210^{G}	1089^{E}	1215
N-2461	2317^{F}	250^{F}	1301^{B}	1289
N-2464	2591^{E}	253^{F}	1393^{A}	1412
N-2466	2809^{AB}	265^{E}	1128^{D}	1401
N-2468	2246^{G}	338^{B}	815^{H}	1133
N-2561	1508^{H}	194^{H}	1104DE	935
Mean	2436	283	1095	1271
	Vicia sativa L.			
S-174	2581^{E}	134^{J}	965^{G}	1226
S-BBA	2676^{D}	172^{I}	967^{G}	1272
Mean	2629	153	966	1249

$^{A, AB, B, C, D, DE, E, F, G, H, I, J, K}$ The groups of means according to the Fisher's test (LSD) at the 5% level.

Table 6. Hundred-seed weight and number of pods per plant in vetch ecotypes.

Ecotype	Hundred-seed weight (g)				Number of pods/plant			
	2017–18	2018–19	2019–20	Mean	2017–18	2018–19	2019–20	Mean
	Vicia narbonensis L.				*Vicia narbonensis* L.			
N-2380	23.67^{A}	23.00^{D}	25.27^{A}	23.98	9.00^{E}	2.11^{F}	5.00^{G}	5.37
N-2383	22.67^{C}	26.47^{A}	23.87^{G}	24.34	7.33^{G}	1.22^{J}	5.33^{F}	4.63
N-2390	22.67^{C}	24.83^{B}	24.20^{D}	23.90	10.33^{C}	2.22^{E}	5.00^{G}	5.85
N-2392	22.00^{E}	21.80^{F}	24.00^{EF}	22.60	7.33^{G}	1.78^{H}	7.00^{C}	5.37
N-2393	23.00^{B}	22.56^{E}	24.03^{E}	23.20	8.67^{F}	1.45^{I}	5.67^{E}	5.26
N-2461	22.00^{E}	21.23^{G}	23.93^{EFG}	22.39	6.67^{H}	2.11^{F}	7.00^{C}	5.26
N-2464	22.67^{C}	24.10^{C}	25.10^{B}	23.96	9.00^{E}	2.56^{C}	7.00^{C}	6.19
N-2466	21.67^{F}	21.73^{F}	24.33^{C}	22.58	10.00^{D}	1.78^{H}	6.00^{D}	5.93
N-2468	22.33^{D}	21.11^{GH}	22.63^{H}	22.02	8.67^{F}	2.00^{G}	5.00^{G}	5.22
N-2561	19.67^{G}	21.03^{H}	23.90F^{G}	21.53	7.33^{G}	2.66^{B}	5.67^{E}	5.22
Mean	22.24	22.79	24.13	23.05	8.43	1.99	5.87	5.43
	Vicia sativa L.				*Vicia sativa* L.			
S-174	6.94^{H}	6.82^{I}	7.03^{I}	6.93	20.00^{A}	3.67^{A}	16.33^{A}	13.33
S-BBA	6.78^{I}	6.83^{I}	6.73^{J}	6.78	15.00^{B}	2.44^{D}	12.00^{B}	9.81
Mean	6.86	6.83	6.88	6.86	17.50	3.06	14.17	11.58

$^{A, B, C, D, E, EF, EFG, F, FG, G, GH, H, I, J}$ The groups of means according to the Fisher's test (LSD) at the 5% level.

The highest 100-seed weight was recorded in *Vicia narbonensis* L. during the three growing seasons, while the lowest was recorded in *Vicia sativa* L. (Table 6). The difference in 100-seed weight may be due to the inherent variation in seed size complemented by environmental conditions. This agronomic trait is important for determining the seeding rate of vetch species (Gezahagn et al., 2013).

The ecotypes of *Vicia sativa* L. had the highest number of pods/plant, pod length and number of seeds/pod over the three growing seasons compared to the ecotypes of *Vicia narbonensis* L. (Tables 6 and 7). The number of pods is generally considered to be one of the most important yield elements for many grain legumes (Mikić et al., 2013). The increase and decrease in the number of pods per plant were probably due to the distribution of rainfall during the vegetative stage. Büyükburç and İptas (2001) also reported that the amount of rainfall had more effect on the number of pods per plant.

Table 7. Pod length and number of seeds per pod in vetch ecotypes.

Ecotype	Pod length (cm)				Number of seeds/pod			
	2017–18	2018–19	2019–20	Mean	2017–18	2018–19	2019–20	Mean
	Vicia narbonensis L.				*Vicia narbonensis* L.			
N-2380	6.22^{B}	2.77^{C}	6.34^{E}	5.11	4.67^{C}	2.07^{C}	4.67^{B}	3.80
N-2383	5.71^{F}	1.71^{G}	6.11^{H}	4.51	4.00^{E}	1.00^{L}	4.67^{B}	3.22
N-2390	5.78^{D}	2.86^{B}	6.33^{E}	4.99	4.00^{E}	1.33^{G}	4.33^{C}	3.22
N-2392	5.44^{K}	2.44^{D}	6.64^{A}	4.84	4.00^{E}	1.37^{F}	4.67^{B}	3.35
N-2393	5.63^{I}	1.80^{F}	6.45^{C}	4.63	4.67^{C}	1.11^{K}	4.67^{B}	3.48
N-2461	5.40^{L}	2.47^{D}	6.34^{E}	4.74	4.33^{D}	1.18^{I}	4.67^{B}	3.39
N-2464	5.65^{H}	2.80^{C}	6.45^{C}	4.97	4.33^{D}	1.15^{J}	4.33^{C}	3.27
N-2466	5.49^{J}	2.24^{E}	6.50^{B}	4.74	4.00^{E}	1.29^{H}	4.00^{D}	3.10
N-2468	5.69^{G}	2.86^{B}	6.42^{D}	4.99	5.00^{B}	1.81^{E}	4.33^{C}	3.71
N-2561	5.75^{E}	3.09^{A}	6.14^{G}	4.99	4.00^{E}	2.00^{D}	4.67^{B}	3.56
Mean	5.68	2.50	6.37	4.85	4.30	1.43	4.50	3.41
	Vicia sativa L.				*Vicia sativa* L.			
S-174	6.31^{A}	3.05^{A}	5.89^{I}	5.08	5.67^{A}	2.63^{A}	5.67^{A}	4.66
S-BBA	5.89^{C}	2.43^{D}	6.31^{F}	4.88	5.67^{A}	2.44^{B}	5.67^{A}	4.59
Mean	6.10	2.74	6.10	4.98	5.67	2.54	5.67	4.63

$^{A, B, C, D, E, F, G, H, I, J, K, L}$ The groups of means according to the Fisher's test (LSD) at the 5% level.

Significant differences were observed in seed quality (Table 8). *Vicia sativa* L. produced the seeds with the highest contents of crude protein (41.06%), dry matter (89.69%), crude fiber (11.79%) and ash (3.37%) compared to *Vicia narbonensis* L. which produced 33.18%, 89.18%, 11.01% and 3.31%, respectively (Table 9). Thanks to the ability of fodder legumes to exploit atmospheric nitrogen via symbiosis with rhizobia at the level of their nodules, the crude protein content of *Vicia sativa* L. and *Vicia narbonensis* L. grains was high, suggesting that these

species could be a complementary food source for animals in semi-arid areas. These two species, thanks to their richness in crude proteins, can be incorporated in the manufacture of concentrated feed intended for feeding ruminants. The trials conducted by Benyoussef (2019) and Kahlaoui et al. (2021) on adult sheep showed that soybean meal can be replaced by *Vicia narbonensis* L. in grains by 23% without negative effects on the performances.

Table 8. Analysis of variance of the chemical composition of *Vicia narbonensis* L. and *Vicia sativa* L.

Sources of variation	DF	Dry matter %	Ash (%DM)	Crude protein (%DM)	Crude fiber (%DM)
			Vicia narbonensis L.		
Total	29	0.208	0.116	3.757	10.749
Ecotype	9	0.18*	0.10*	3.67*	9.53*
Overall average		89.182	3.313	33.184	11.009
SD		0.278	0.204	1.096	1.949
CV (%)		0.312	6.163	3.303	17.706
			Vicia sativa L.		
Total	5	0.181	0.008	2.963	3.271
Ecotype	1	0.14*	0.003*	2.84*	0.09*
Overall average		89.693	3.370	41.062	11.788
SD		0.253	0.064	0.815	1.600
CV (%)		0.282	1.886	1.986	13.573

*Significant at 5%; SD: Standard deviation; CV: Coefficient of variation. The significance level is 5%.

Table 9. Mean values of the chemical composition of the grains of vetch ecotypes.

DF	Dry matter %	Ash (%DM)	Crude protein (%DM)	Crude fiber (%DM)
		Vicia narbonensis L.		
N-2380	89.11^{H}	3.70^{A}	30.89^{L}	10.97^{E}
N-2383	89.03^{I}	3.32^{F}	32.72^{J}	8.80^{I}
N-2390	89.16^{G}	3.28^{H}	33.73^{F}	9.78^{G}
N-2392	89.31^{E}	3.42^{C}	33.53^{G}	8.93^{H}
N-2393	89.30^{f}	3.31^{G}	34.30^{D}	9.79^{G}
N-2461	88.77^{K}	3.04^{L}	33.20^{H}	12.99^{B}
N-2464	88.89^{J}	3.18^{J}	31.92^{K}	13.05^{B}
N-2466	89.30^{f}	3.47^{B}	34.11^{E}	11.71^{D}
N-2468	89.59^{B}	3.25^{I}	34.36^{e}	10.34^{F}
N-2561	89.37^{D}	3.16^{K}	33.07^{I}	13.72^{A}
Mean	89.18	3.31	33.18	11.01
		Vicia sativa L.		
S-174	89.54^{e}	3.35^{E}	41.75^{A}	11.66^{D}
S-BBA	89.84^{A}	3.39^{D}	40.37^{B}	11.91^{C}
Mean	89.69	3.37	41.06	11.79

$^{A, B, C, D, E, F, G, H, I, J, K, L}$ The groups of means according to the Fisher's test (LSD) at the 5% level.

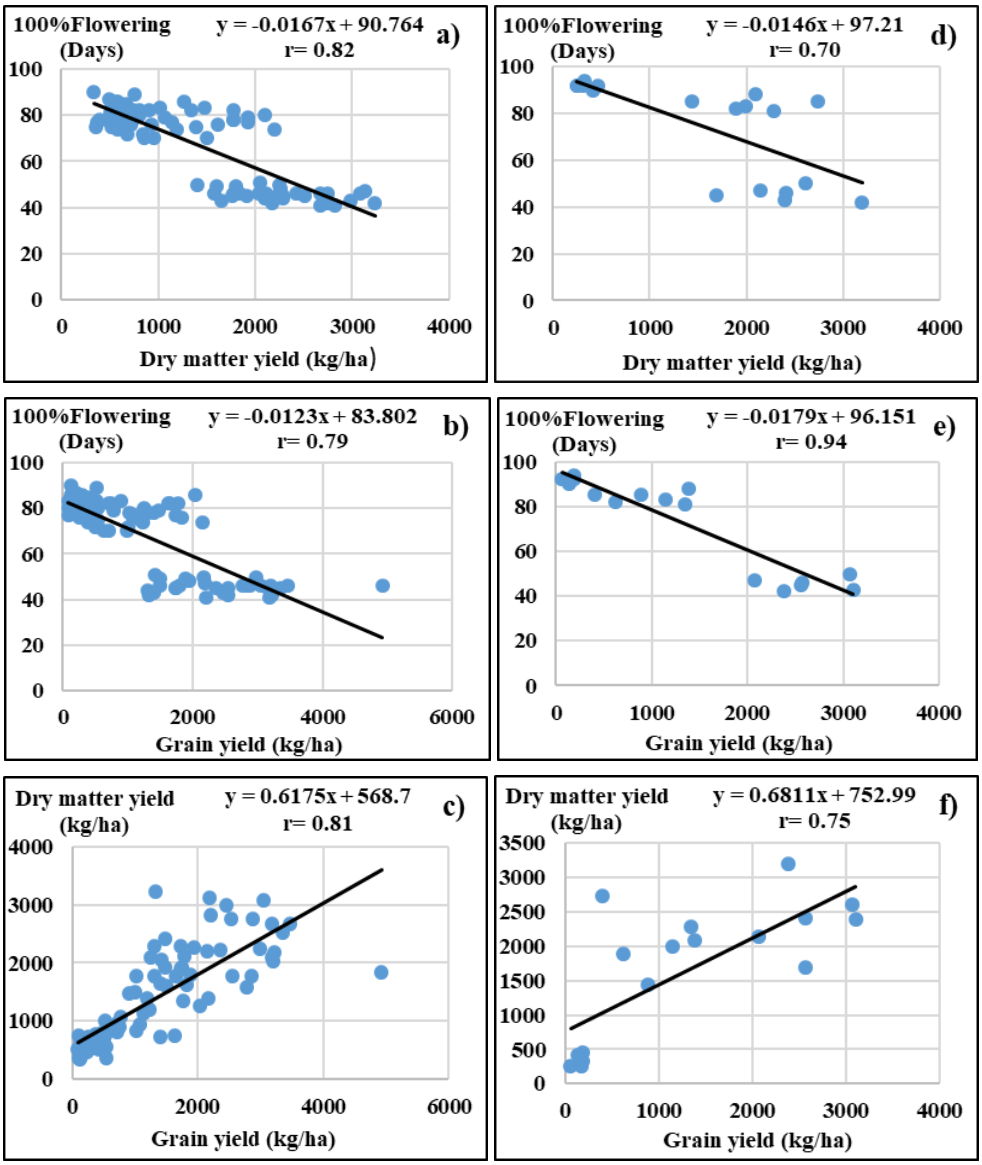

Figure 1. Relationships observed between a) dry matter yield and flowering date; b) grain yield and flowering date; c) grain yield and dry matter yield in *Vicia narbonensis* L. and between d) dry matter yield and flowering date; e) grain yield and flowering date; f) grain yield and dry matter yield in *Vicia sativa* L. over the three years of experimentation.

The study of the relationship between the date of flowering and the yields of dry matter and grains in the two species of vetch highlights some rather interesting information:

- A significant negative relationship ($p<0.05$) between dry matter yield and flowering date in the two species – *Vicia narbonensis* L. and *Vicia sativa* L. (Figure 1);

- Also a significant negative relationship ($p<0.05$) between grain yield and flowering date (Figure 1). It seems that the early ecotypes of the two species produced the best yields in dry matter and grain production. These ecotypes, which flower early, can escape from the drought at the end of the fodder legume cycle.

These negative relationships between grain and dry matter yields with the flowering stage suggest that the late ecotypes of these two species were strongly affected by heat and drought, unlike the early ecotypes.

- A significant positive relationship ($p<0.05$) between grain yield and dry matter yield in both species suggests that selection of ecotypes for dry matter resulted in good grain production (Figure 1).

This study emphasized the behavior of two species of the genus Vicia in altitude conditions characterized by water and thermal stress. The species *Vicia narbonensis* L. seems more cold tolerant than *Vicia sativa* L. The latter, characterized by its long and late flowering period, could allow it to escape the cold. These results corroborate those obtained by Keatinge et al. (1991) and Abd El Moneim (1992), who recommended the two species, *Vicia narbonensis* L. and *Vicia sativa* L., for semi-arid regions.

Conclusion

The results obtained in this study reveal that *Vicia narbonensis* L. has very interesting agronomic and qualitative characteristics. It has been shown to be more cold tolerant than *Vicia sativa* L.

It is possible to select the ecotype or ecotypes best suited to be included in the context of the fallow recovery land or in the development of marginal lands in semi-arid areas, depending on the climatic characteristics and the nutritional needs of the different production systems practicing a cereal/fallow rotation. This is due to the great variability of the parameters measured among the 10 ecotypes of *Vicia narbonensis* L., especially the different potential yields of dry matter and grains.

Compared to *Vicia sativa L.*, *Vicia narbonensis* L. is well adapted to seed production because it does not shatter at maturity so as not to soil the subsequent crop and has an erect habit allowing mechanical harvesting. Due to the high crude protein content in the grains, narbon vetch-based feeds can be utilized as an alternative source of protein instead of expensive and imported concentrates to meet the needs of national livestock.

It remains essential to evaluate and valorize this species and to encourage the production of seeds based on appropriate and adequate methodologies to alleviate the existing fodder shortage. The cultivation and production of *Vicia narbonensis* L. will probably be the key of the tomorrow's semi-arid agricultural system, making it profitable.

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UPOREDNO ISTRAŽIVANJE O NARBONSKOJ GRAHORICI I OBIČNOJ GRAHORICI U POLUSUŠNOM REGIONU SETIFA (ALŽIR)

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R e z i m e

Alžir ima ograničenu ponudu stočne hrane. Tradicionalne metode uzgoja stoke oslanjaju se na iskorišćavanje niskokvalitetnih pašnjaka, ugara i prirodnih pašnjaka, a krmnim kulturama se posvećuje malo pažnje. Stanovništvo se oslanja na uvoz da bi zadovoljilo svoje potrebe za proteinima. Zapravo, proizvodnja stočne hrane je primarna poluga za poboljšanje ishrane stoke i kao rezultat toga, produktivnost stočarskog sistema o kojima je reč. Takođe je ključno za dugoročnu održivost sistema kombinovane proizvodnje koji integrišu biljnu i životinjsku proizvodnju. Ova studija je sprovedena radi procene fenoloških faza, analize performansi prinosa stočne hrane, prinosa zrna i nekih njegovih komponenti, kao i određivanja hemijskog sastava vrste *Vicia narbonensis L.* u poređenju sa *Vicia sativa L.,* a sa ciljem poboljšanja ugarne godine u plodoredu žitarica/ugar i poboljšanja marginalnih zemljišta. Ispitivanja su sprovedena na parcelama Kampusa Univerziteta FERHAT Abbas u uslovima prirodnog vodnog režima u polusušnom regionu Setifa tokom tri vegetacione sezone (2017–2020), koristeći 10 ekotipova narbonske grahorice i 2 ekotipa obične grahorice (kontrola) u potpunom slučajnom blok dizajnu sa tri ponavljanja. Utvrđeni su značajni uticaji interakcije ekotipa, godine i interakcije između ekotipa i godine, kao i širok spektar varijabilnost u fenološkim fazama, agronomskim karakteristikama i hemijskom sastavu proučavanih ekotipova. Značajna pozitivna veza ($p<0,05$) utvrđena je između prinosa zrna i prinosa suve materije, a značajna negativna veza ($p<0,05$) između datuma punog cvetanja i prinosa suve materije i prinosa zrna. Čini se da ranostasni ekotipovi postižu bolje rezultate od kasnostasnih u polusušnom regionu Setifa.

Ključne reči: ugar, hemijski sastav, stočni fond, sistem proizvodnje, uslovi prirodnog vodnog režima, polusušni region, *Vicia narbonensis L.*, *Vicia sativa L.*, prinosi.

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GROWTH, YIELD AND VARIANCE COMPONENTS OF WATERMELON (*CITRULLUS LANATUS*) GROWN ON LIME ($CACO_3$)-AMENDED ACIDIC SOIL OF SOUTH-EASTERN NIGERIA

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Abstract: A field experiment was conducted to assess the growth and yield of watermelon *Citrullus lanatus* [(Thunb.) Matsum. & Nakai] under acidic soil conditions in Calabar, Cross River State, Nigeria. The experiment was a 3 × 3 factorial experiment laid in a randomized complete block design (RCBD) with three replications. The factors studied were varieties (Heracles F_1, Kaolack and Sugar Baby), lime rates (0 t ha^{-1}, 2.7 t ha^{-1} and 5.7 t ha^{-1}), and their interactions on watermelon growth and yield traits. Heracles F_1 and Kaolack outperformed Sugar Baby ($p \leq 0.05$) regarding growth and yield traits. Lime rates of 2.7 t ha^{-1} and 5.7 t ha^{-1} increased the initial soil pH range (4.6–4.9) by 21.74% (5.4–5.8) and 43.48% (6.4–6.7), respectively. These rates improved the soil pH to a range suitable for watermelon cultivation in the study area. Vine length, number of leaves, transverse and longitudinal sections of the fruits and sugar content of the fruits had ≥ 50% heritability, a useful index in the selection of choice growth and yield traits in watermelon. Overall, GAM was greater than GA for each of the traits except for the total number of seeds per fruit. Multi-location studies are recommended to give further insights to this pilot study.

Key words: brix, fruits, heritability, hybrid, lime, soil acidity, ultisol, watermelon.

Introduction

Watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai) is a warm-season crop and an important member of the cucurbit family, propagated primarily by seeds (van der Vossen et al., 2004). It is grown throughout India and other tropical countries, including Nigeria (Fehér, 1993). Watermelon is one of the most widely cultivated crops in the world (Huh et al., 2008). The crop, watermelon, refers to the fruit and plant of a vine-like (climber or trailer) herb (TFNet, 2016). The center of

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origin of watermelon has been traced to the Kalahari and Sahara deserts in Africa (Jarret et al., 1996). Its first harvest was documented in Egypt 5,000 years ago and subsequently expanded to other parts of the world (Strauss, 2015). In 2021, China was the leading global watermelon producer (60.8 MT), followed by Turkey (3.47 MT) and India (3.25 MT) (FAOSTAT, 2022). According to Balakrishnan et al. (2015), the fruit of watermelon has a water content of about 93%, which gives it the name 'watermelon'. The term 'melon' comes from the fact that the fruit is large and round and has a sweet, pulpy flesh. The scientific name of watermelon stems from Greek and Latin roots; the *'Citrullus'* is derived from the Greek word 'citrus' which refers to the fruit (Maynard, 2001). The Latin '*lanatus'* means woolly and refers to the small hairs on the stems and leaves of the plant, which has both nutritional and medicinal values (Gwana et al., 2014). The fruit's skin color can vary from white to shades of green and possibly marbled or striped. This genus contains a total of four species (Renner et al., 2014) and watermelon belongs to *Citrullus lanatus* because of its pink/red or yellow flesh and black seeds (Montesinos, 2006). It is important to differentiate between *Citrullus lanatus* (bigger fruits) and *Citrullus colocynthis* (L.) (smaller fruits), both commonly known as watermelons, which are excellent sources of high-quality proteins and edible oils used in cooking for human and cattle nutrition in some African and Middle Eastern American countries (Milovanović et al., 2009).

Watermelon is rich in vitamins A, B and C, amino acids and carotenoid lycopene (Alam et al., 2013; Maoto et al., 2019). Vitamin B is mostly responsible for generating energy in the body (Muhammad et al., 2014). Thus, consuming watermelon may increase energy levels. Vitamin C is an essential nutrient for humans because it helps in the synthesis of collagen and protects various tissues from oxidative stress (Akbari and Jelodar, 2013; Devaki and Raveendran, 2017). It also contains potassium, which helps control blood pressure and possibly prevent strokes (Adekunle et al., 2005). Lycopene, a red pigment from the carotenoid class present in only a few fruits and vegetables, is a potent oxygen radical collector and a highly effective antioxidant (Gerster, 1997). *Citrullus lanatus* prefers a warm, dry climate with an average daily temperature ranging from 22ºC to 30ºC (FAO, 2001). Watermelons can be grown on a wide range of soil types. Although sandy soils are preferred, the highest yields are usually obtained on well-drained sandy-loam soils and soil pH (in H_2O) should be about 5.8–6.2 for optimum yield (Watermelon Production Guideline, 2014). Watermelon has a smooth outer rind and a juicy, sweet, usually red, internal flesh. It can be used as a fresh salad, dessert, snack, and for decorative purposes (Perkins-Veazie et al., 2012). Fruity beverages may also be made from its juice.

Watermelon fruits may weigh 1–100 kg or more (e.g., 'Carolina Cross' – Gusmini and Wehner, 2007), but most commercially available watermelons weigh between 3 and 13 kg (Wehner, 2008). The sugar content and the sweetness are the

critical factors in determining the quality of many watermelon varieties. Watermelon is known to be low in calories, but it is a highly nutritious and thirst-quenching fruit (Okonmah et al., 2011). Watermelon is a good source of carotenoids and lycopene, which have been shown to protect against a growing list of cancers (Cho et al., 2004). Over the past few decades, the presence of an appreciable amount of lycopene in watermelon has motivated farmers and fruit and vegetable producers to grow mainly red-fleshed watermelon varieties. A total of 1,200 cultivars of watermelon are produced worldwide (Helyes et al., 2009). Watermelon yield performance differs among cultivars due to variation in vine length, number of branches, number of male and female flowers, fruit number and weight (Mrema and Maerere, 2018). The critical periods in watermelon cultivation are planting, vegetative growth, flowering and fruiting.

Agriculture serves as the backbone of developing economies. Due to environmental factors such as soil salinization, erosion, and acidification, agricultural land is increasingly degraded and poorly suited for large-scale agricultural production (Jones et al., 2013; FAO and ITPS, 2015). Soil fertility is affected by soil acidity due to nutrient deficiencies (P, Ca and Mg) and the increased presence of certain nutrient elements such as soluble Al^{3+} and Mn^{2+} at phytotoxic levels. Liming is a longstanding and standard management practice used to maintain optimal soil pH for crop production (Goulding, 2015). The application of lime to acid soils could reduce Al^{3+} and Mn^{2+} toxicity, while improving pH, Ca, Mg and increasing P uptake in soils with high P fixation and plant root systems (Black, 1992). Liming has a positive effect on the yield of most arable crops. However, there are distinct differences between crops in yield responses to lime (Cifu et al., 2004) because crop varieties may have different tolerance for soil acidity.

The soils of the tropical rainforest of Nigeria are very acidic, deficient in micronutrients, and often result in low yields without any improvement or modification. Watermelons may be grown on a wide variety of soil types, although sandy soils are preferred. It has a long prostrate growth habit and therefore requires adequate spacing on well-drained sandy-loam soils that are rich in organic matter with good moisture retention (Lawal, 2000). Watermelons can tolerate a certain level of soil acidity (5.5 to 6.7), but the pH of the soil should not be less than 5.5 for good yields. Cultivation in heavy textured soils results in a slower crop development and cracked fruits (FAO, 2010).

During the early 2018 cropping season (March–April), 95% to 100% of immature fruits of all watermelon varieties planted split before maturity at the University of Calabar Teaching and Research Farm and yield loss was over 96%. Given that there are speculations on the influence of an interplay of weather conditions and soil pH on watermelon growth and yield, it was hypothesized that the application of lime and the cultivation of watermelon during the late growing

season (August–September) could influence (regulate) soil pH in favor of watermelon cultivation.

Thus, this study was designed to assess the growth and fruit yield of watermelon on the acidic soil of Calabar during the second cropping season, under the influence of lime. The objective of the study was to compare the changes in growth and yield characteristics of watermelon genotypes in response to the lime amendment on the acidic soils of Calabar. This was done by examining the growth and yield performances of watermelon genotypes on limed and non-limed acidic soils and estimating the extent of the inherent (genetic) and environmental-induced (soil pH-dependent) variations in the growth and yield responses of watermelon genotypes in Calabar.

Material and Methods

The experiment was performed at the Experimental Farm of the University of Calabar, Cross River State. Calabar is located in the south-eastern rainforest agroecological zone of Nigeria and lies between latitude 4.5–5.2^0 N, longitude 8.0–8.3^0 E and about 39 m above sea level. Cutting and scrubbing were done by a machete. Moderate stumping, where necessary, was manually done with spades, a cutlass and an axe. Neither the application of chemical herbicides nor bush burning was carried out during the process. The cuts and debris were left to decompose within two weeks and plowed into the soil during manual tillage. Representative soil samples were taken at random from a depth of up to 30 cm of topsoil. Three samples were taken from each of the three blocks using a soil auger (carefully cleaned of soil and possible debris between sampling). The nine soil samples were grouped (block-wise) into three composite samples for physical and chemical analyses of soil properties.

The lime type used for the study was agricultural lime with the chemical formula $CaCO_3$ and a neutralizing value of 100 (Bolan et al., 2003). It was acquired through the Cross River State Agricultural Development Program Office in Calabar. Three lime rates were used for the experiment: 0 t ha^{-1}, 2.7 t ha^{-1} and 5.7 t ha^{-1}. The watermelon seeds were sourced from Technisem® seed through the Cross River State Agricultural Development Seed Unit. The three watermelon varieties obtained were: Heracles F_1 hybrid, Kaolack and Sugar Baby.

The experimental design was a 3 × 3 (i.e., three watermelon genotypes and three levels of lime application) factorial design in a randomized complete block design (RCBD) with three blocks (replicates) on a 15 m × 45 m experimental plot. Each treatment plot measured 4 m × 4 m. Lime was applied in hollow holes (30 cm in diameter and 15 cm deep). After 7 days of lime application, three seeds each of the watermelon varieties were planted on September 11, 2018 at a spacing of 1 m x 1m. Thinning was done at 7 days after planting; leaving one plant per hill, resulting

in 16 stands per treatment plot and a plant population of 10,000 stands per hectare. The following agronomic data were collected from four labelled plants in the net plot (2 m × 2 m): vine length (cm), number of leaves per plant, days to 50% flowering, number of fruits per plant, cross-section of mature fruits (cm) (i.e., fruit width), longitudinal section (cm) (i.e., fruit length), fresh weight per fruit (kg), fresh fruit yield (t ha^{-1}), fruit rind thickness (cm), number of seeds from mature fruits, total soluble sugar content (Brix).

Data collected were analyzed with the GenStat package (16th edition) using the randomized complete block design (RCBD) with three replications. Means of significant F-tests for variety and lime rates were compared using a Fisher's protected least significant difference (LSD) at the 95% confidence level. The means of the interaction effects (variety x lime rate) were compared using the Duncan's New Multiple Range Test (DNMRT) at a 95% confidence level.

Plant Breeding Tools Version 1.4 was used for the estimation of genotypic, environmental and phenotypic variances (Table 1) based on the model below (Equation 1):

$$Y_{hijk} = M + L_h + R(L)_{k(h)} + G_j + GL_{hj} + e_{hijk} \quad (1)$$

where: Y_{hijk} = the measurement on plot *k* in environment *h*, block *i*, containing genotype *j*; M = the overall mean of all plots in all environments; L_h = the effect of environment *h*; $R(L)_{k(h)}$ = the effect of replicate *i* within environment *h*; G_j = the effect of genotype *j*; GL_{hj} = the interaction of genotype *j* with environment *h* and e_{hijk} = the plot residual.

Genetic advance (GA) (Allard, 1960) and genetic advance as a percentage of population means (GAM) were computed (Johnson et al., 1955).

Table 1. The variance component and the expected mean square analysis model.

Source of variation	df	MS	Expected MS
Lime rate (L)	l-1	MS_L	$\sigma_e^2 + r\sigma_{gl}^2 + g\sigma_{r(l)}^2 + rg\sigma_l^2$
Replicates within lime rate (R(L))	l(r-1)	$MS_{Rep(L)}$	$\sigma_e^2 + g\sigma_{r(l)}^2$
Genotype (variety) (G)	g-1	MS_G	$\sigma_e^2 + r\sigma_{gl}^2 + lr\sigma_g^2$
G x L interaction	(l-1) (g-1)	MS_{GL}	$\sigma_e^2 + r\sigma_{gl}^2$
Pooled error (E)	l(r-1) (g-1)	MS_E	σ_e^2

l = lime rate, g = genotype (variety), r = replicate, σ_g^2 = genotype variance, σ_l^2 = lime rate variance, σ_{gl}^2 = genotype × lime rate interaction variance, $\sigma_{r(l)}^2$ = replicate within lime rate variance, σ_e^2 = pooled error variance, df = degree of freedom, MS = mean square.

Results and Discussion

The chemical and physical properties of the soil in Table 1 show that the soil was highly acidic and needed to be limed for watermelon to thrive, as watermelon always does best in alkaline soils. The soil pH result (after lime application) at the end of the study (Figure 1) shows that the lime applied at different rates was able to increase the soil pH to 5.5 and 6.5. Lime applied at 2.7 t ha^{-1} increased soil pH from 4.6 to 5.6 and 5.7 t ha^{-1} increased soil pH to 6.6. The increase in soil pH obtained by applying lime before planting watermelon seeds significantly affected plant growth and yield attributes. Soil is a critical element of the life support system that provides several ecosystem goods and services such as carbon storage, water regulation, soil fertility and food production that impact human well-being (FAO and ITPS, 2015). In the natural environment, soil pH has an enormous influence on soil biogeochemical processes.

Table 2. Mean values of physical and chemical properties of the acidic soil used for the study.

Property	Unit	Mean value (n = 3)
pH (in H_2O)		4.60
Sand	g kg^{-1}	889.0
Silt	g kg^{-1}	26.7
Clay	g kg^{-1}	84.3
Texture		Loamy sand
Organic carbon	%	0.83
Total nitrogen	%	0.10
Available phosphorus	mg kg^{-1}	21.33
Exchangeable K^+	cmol (+) kg^{-1}	0.0023
Exchangeable Na^+	cmol (+) kg^{-1}	0.0035
Exchangeable Ca^{2+}	cmol (+) kg^{-1}	2.0000
Exchangeable Mg^{2+}	cmol (+) kg^{-1}	1.6700
Exchangeable Al^{3+}	cmol (+) kg^{-1}	0.0900
Exchangeable H^+	cmol (+) kg^{-1}	0.6700
Exchangeable acidity	cmol (+) kg^{-1}	0.7600
ECEC	cmol (+) kg^{-1}	4.4358
BS	%	82.87

ECEC = exchangeable cation exchange capacity, BS = base saturation, n = number of samples.

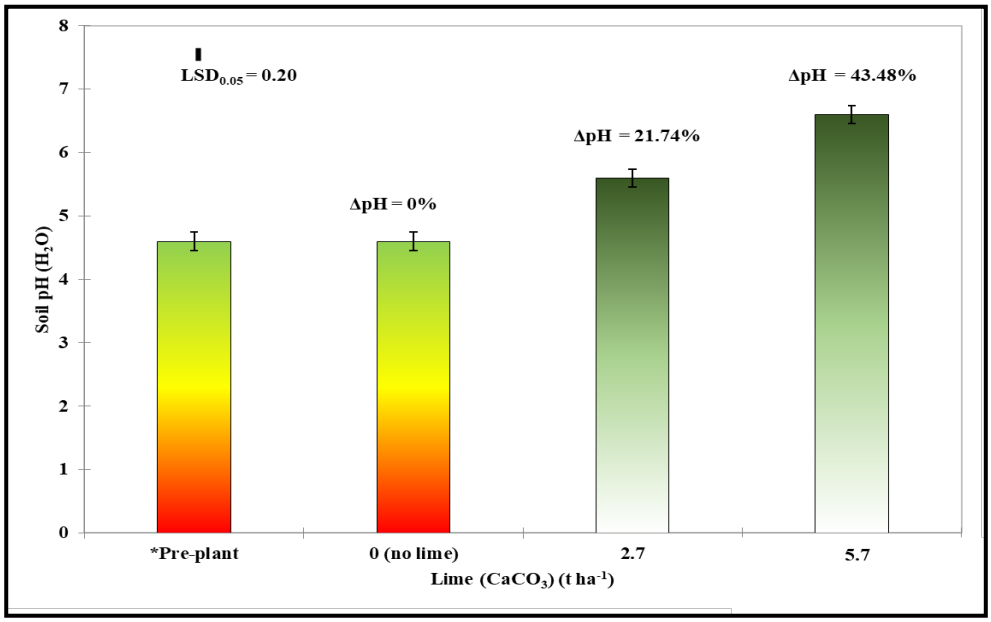

Figure 1. The soil pH (in H_2O) changes after lime application (*initial soil pH = 4.60).

The growth and yield performance of watermelon under lime-amended soil conditions are shown in Tables 3 and 4. When the interaction effect was significant, the result for the main factor (s) was not discussed. Heracles F_1 × 2.7 t ha^{-1} $CaCO_3$ produced the highest vine length (13.11 cm) and was significantly different ($p \leq 0.05$) from the other interaction effects at 21 DAP, except for Kaolack × 5.7 t ha^{-1}, Heracles F_1 × 5.7 t ha^{-1}, Kaolack × 2.7 t ha^{-1} and Heracles F_1 × 0 t ha^{-1} ($p > 0.05$). At 28 DAP, Kaolack × 5.7 t ha^{-1} produced the highest vine length (24.47 cm) but was not significantly different ($p > 0.05$) from Kaolack × 2.7 t ha^{-1} and Heracles F_1 at all lime rates. Sugar Baby × 0 t ha^{-1} produced the shortest vines at 28 DAP and was not significantly different ($p > 0.05$) from Sugar Baby × 5.7 t ha^{-1} and Sugar Baby × 2.7 t ha^{-1}. Kaolack × 5.7 t ha^{-1} (45.24 cm) still produced the longest vines at 35 DAP though was not significantly different ($p > 0.05$) from Kaolack × 2.7 t ha^{-1}, Sugar Baby × 5.7 t ha^{-1} and Heracles F_1 at all lime rates. There was no significant ($p > 0.05$) difference between Sugar Baby grown at all lime rates and Kaolack × 0 t ha^{-1}. At 42 DAP, a similar trend was observed where Kaolack × 5.7 t ha^{-1} (70.64 cm) produced the longest vines, which were not significantly different ($p > 0.05$) from Heracles F_1 at all lime rates, Kaolack × 0 t ha^{-1} and Kaolack × 2.7 t ha^{-1}, Sugar Baby × 2.7 t ha^{-1} and Sugar Baby × 5.7 t ha^{-1}. Sugar Baby grown on unamended acidic soil had the shortest vines at 42 DAP (Table 4).

Table 2. Single and interaction effects of lime and variety on vine length and number of leaves of watermelon.

Treatment	Vine length (cm)				Number of leaves			
	Days after planting				Days after planting			
	21	28	35	42	21	28	35	42
Variety								
Heracles F_1	11.63	22.31	41.05	65.61	5.13	8.43	11.70	20.00
Kaolack	10.21	20.39	37.53	61.27	4.74	8.11	10.82	18.91
Sugar Baby	7.15	15.51	28.66	50.09	4.17	6.96	8.02	15.00
$LSD_{0.05}$	*1.60*	*3.33*	*7.64*	*12.38*	*0.44*	*0.57*	*1.64*	*3.69*
Lime rate								
0 t ha^{-1}	8.30	16.43	30.23	51.84	4.32	7.46	9.09	15.70
2.7 t ha^{-1}	10.22	20.54	37.76	61.83	4.80	7.87	10.72	18.61
5.7 t ha^{-1}	10.47	21.23	39.25	63.30	4.93	8.17	10.72	19.59
$LSD_{0.05}$	*1.60*	*3.33*	*7.64*	*NS*	*0.44*	*NS*	*NS*	*NS*
Variety × lime rate								
Heracles F_1 × 0 t ha^{-1}	10.58 a	20.76 abc	40.89 ab	65.58 ab	5.06 ab	8.72 a	11.39 ab	20.00 abc
Kaolack × 0 t ha^{-1}	7.35 b	14.89 cd	26.12 cd	46.74 ab	4.00 c	6.94 c	8.28 c	13.89 bc
Sugar Baby × 0 t ha^{-1}	6.97 b	13.63 d	23.66 d	43.19 b	3.89 c	6.72 c	7.61 c	13.22 c
Heracles F_1 × 2.7 t ha^{-1}	13.11 a	24.44 a	43.26 ab	67.20 ab	5.28 a	8.56 a	12.33 a	20.67 ab
Kaolack × 2.7 t ha^{-1}	10.62 a	21.81 ab	41.22 ab	66.43 ab	4.89 ab	8.33 ab	12.17 a	20.67 ab
Sugar Baby × 2.7 t ha^{-1}	6.93 b	15.38 cd	28.81 bcd	51.87 ab	4.22 bc	6.72 c	7.67 c	14.50 bc
Heracles F_1 × 5.7 t ha^{-1}	11.21 a	21.72 ab	39.01 abc	64.06 ab	5.06 ab	8.00 ab	11.39 ab	19.33 abc
Kaolack × 5.7 t ha^{-1}	12.66 a	24.47 a	45.24 a	70.64 a	5.33 a	9.06 a	12.00 a	22.17 a
Sugar Baby × 5.7 t ha^{-1}	7.55 b	17.50 bcd	33.51 abcd	55.19 ab	4.39 bc	7.44 bc	8.78 bc	17.28 abc

Values within the same column with the same letter(s) of the alphabet were not significantly different using the Duncan's New Multiple Range Test at the 95% confidence limit. NS = not significant. *$LSD_{0.05}$* = Fisher's least significant difference at the 95% confidence limit.

There was no significant difference ($p > 0.05$) for variety × lime rate at 28, 35 and 42 DAP in terms of leaf counts (Table 4). The highest number of leaves at 21 DAP was recorded for Kaolack × 5.7 t ha^{-1} (5.33) and Heracles F_1 × 2.7 t ha^{-1} (5.28). These were not significantly different ($p > 0.05$) from Heracles F_1 × 0 t ha^{-1}, Heracles F_1 × 5.7 t ha^{-1} and Kaolack × 2.7 t ha^{-1}. The lowest number of leaves was produced by Sugar Baby × 0 t ha^{-1} (13.22) and Kaolack × 0 t ha^{-1} (13.89).

However, these were not significantly different ($p > 0.05$) from Sugar Baby × 2.7 t ha^{-1} and Sugar Baby × 5.7 t ha^{-1}. At 28 DAP, Heracles F_1 (0 t ha^{-1} and 2.7 t ha^{-1}) and Kaolack (5.7 t ha^{-1}) resulted in the greatest number of leaves.

There was no significant difference ($p > 0.05$) among watermelon genotypes in days to 50% flowering time, the number of fruits harvested per plant, the average number of fruits harvested per plant and the average weight of mature fruits. Generally, the weight of the fresh fruit followed the order: Kaolack >

Heracles F_1 > Sugar Baby. Heracles F_1 × 0 t ha^{-1} (58.74 t ha^{-1}) and Kaolack × 5.7 t ha^{-1} (56.20 t ha^{-1}) produced the heaviest fresh fruits. Sugar Baby × 5.7 t ha^{-1} (16.34 t ha^{-1}) produced fresh fruits with lighter weights that were not significantly different ($p > 0.05$) from Heracles F_1 × 5.7 t ha^{-1} (Table 4). Sugar Baby × 5.7 t ha^{-1} produced fruits with smaller transverse sections, whereas Kaolack × 5.7 t ha^{-1} (14.57 cm) led to fruits with wider transverse sections, but not significantly different ($p > 0.05$) from fruits produced of the other variety × lime interactions. Heracles F_1 × 0 t ha^{-1} (18.21 cm) and Heracles F_1 × 2.7 t ha^{-1} (17.93 cm) topped in the transverse section measurements of the fruits and were not significantly different ($p > 0.05$) from Heracles F_1 × 5.7 t ha^{-1}. Sugar Baby × 5.7 t ha^{-1} fruit cross-section was significantly smaller. No significant differences ($p > 0.05$) were observed between the genotypes, lime rates and interaction levels in terms of rind thickness and the total number of seeds per fruit.

Table 4. Single and interaction effects of lime and variety on selected watermelon yield traits at harvest.

Treatment	Days to 50% flowering	Number of fruits per plant	Fresh weight per fruit (kg)	Fresh fruit yield (t ha^{-1})	Fruit width (cm)	Fruit length (cm)	Rind thickness (cm)	Total number of seeds per fruit	Sugar content (Brix)
Variety									
Heracles F_1	42.67	3.33	1.76	32.22	13.56	17.58	0.86	299.83	6.22
Kaolack	42.00	4.66	1.60	38.66	14.22	14.84	0.80	349.28	8.28
Sugar Baby	46.33	3.66	1.43	27.39	12.04	12.65	0.71	315.22	6.71
$LSD_{0.05}$	*NS*	*NS*	*NS*	*3.00*	*0.96*	*0.96*	*NS*	*NS*	*0.59*
Lime rate									
0 t ha^{-1}	43.89	4.3	1.8	41.50	13.91	15.53	0.9	332.6	6.99
2.7 t ha^{-1}	43.22	3.7	1.5	27.16	13.48	15.53	0.9	299.8	7.88
5.7 t ha^{-1}	43.89	3.7	1.5	29.62	12.43	14.02	0.7	331.9	6.33
$LSD_{0.05}$	*NS*	*NS*	*NS*	*3.00*	*0.96*	*0.96*	*NS*	*NS*	*0.59*
Variety × lime rate									
Heracles F_1 × 0 t ha^{-1}	41.00 a	5.33a	2.17a	58.74 a	14.36 a	18.21 a	0.98a	329.83a	5.83 d
Kaolack × 0 t ha^{-1}	45.33 a	3.33a	1.53a	26.34 d	13.87 a	14.07 c	0.80a	334.00a	7.43 bc
Sugar Baby × 0 t ha^{-1}	45.33 a	4.33a	1.78a	39.42 b	13.50 a	14.30 c	0.80a	334.44a	7.70 bc
Heracles F_1 × 2.7 t ha^{-1}	42.67 a	2.33a	1.78a	21.59 def	13.23 a	17.93 a	1.03a	255.33a	6.98 c
Kaolack × 2.7 t ha^{-1}	40.00 a	4.33a	1.51a	33.46 c	14.23 a	15.13 bc	0.86a	298.83a	9.11 a
Sugar Baby × 2.7 t ha^{-1}	47.00 a	4.33a	1.18a	26.42 d	12.98 a	13.51 c	0.66a	345.33a	7.56 bc
Heracles F_1 × 5.7 t ha^{-1}	44.33 a	2.33a	1.33a	16.34 def	13.08 a	16.58 ab	0.58a	314.33a	5.83 d
Kaolack × 5.7 t ha^{-1}	40.67 a	6.33a	1.75a	56.20 a	14.57 a	15.32 bc	0.73a	415.00a	8.30 ab
Sugar Baby × 5.7 t ha^{-1}	46.67 a	2.33a	1.33a	16.34 f	9.65 b	10.15 d	0.68a	266.33a	4.87 d

Values within the same column with the same letter(s) of the alphabet were not significantly different using the Duncan's New Multiple Range Test at the 95% confidence Limit. NS = not significant. $LSD_{0.05}$ = Fisher's least significant difference at the 95% confidence limit.

The sugar content of Kaolack was significantly higher (8.28 Brix) compared to other genotypes. Kaolack × 2.7 t ha^{-1} produced fruits with higher sugar content (9.11 Brix), but not significantly different ($p > 0.05$) from Kaolack × 5.7 t ha^{-1}. Sugar contents at other levels of interactions were significantly different from Sugar Baby × 5.7 t ha^{-1} with the lowest sugar content, but not significantly different ($p > 0.05$) from Heracles F_1 × 0 t ha^{-1} and Heracles F_1 × 5.7 t ha^{-1}.

Anikwe et al. (2016) reported that differences among watermelon varieties could be attributed to their inherent varietal characteristics as well as the soil condition and location of cultivation. The results obtained from the present study, where the different watermelon varieties showed significant differences in the growth trait conform with this report. Achigan-Dako (2008) has reported that there are differences in the growth and yield characteristics of watermelons. In terms of vine length and number of leaves, Heracles F_1 had the longest vines and more leaves followed by Kaolack and Sugar Baby. Achigan-Dako (2008), Uwah and Solomon (1999) also reported that the differences among watermelon varieties could be due to the genetic make-up of the watermelon varieties. This assertion aligns with the results of this study.

The application of lime to acid soils has been found to increase soil pH and therefore eliminates aluminium toxicity at pH > 5.5 by precipitating Al, making it unavailable for plant uptake (Meriño-Gergichevich et al., 2010). Hue and Mai (2002) have reported that adequate application of lime raises soil pH to 5.7 or higher, which is essential for normal watermelon growth. The results of the study showed that lime applied to the acid soil to raise the soil pH to 5.5 and 6.5 had a significant influence on the growth traits of the watermelon varieties compared to the area where no lime was applied. Lime applied at 5.7 t ha^{-1} raised the soil pH to 6.6, causing the vines to grow significantly longer and have a greater number of leaves, followed by lime applied at 2.7 t ha^{-1}. This finding is also in agreement with a report of Hue and Mai (2002) that the adequate application of lime, raising soil pH to 5.7 or higher, is essential for normal watermelon growth. Similar results were also reported by Hirpa et al. (2013) that phrenology and growth of common bean genotypes were significantly increased by the application of lime.

Tegen et al. (2021) have reported that there is a significant difference in the growth and yield characteristics of the different watermelon varieties, which also agrees with the results of this study, which showed a significant difference in fresh fruit weight (t ha^{-1}) among the watermelon varieties. Kaolack had the highest weight followed by Heracles F_1 and Sugar Baby.

Estimates of phenotypic (σ^2p), genotypic (σ^2g) and environmental (σ^2e) variances, phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), broad-sense heritability (H_b^2) and genetic advance are presented in Tables 5 and 6. The σ^2g ranged from 5.9×10^{-9} (fresh fruit yield) to 48.63 (vine length at 42 DAP), while the σ^2p ranged from 3.5×10^{-3} (rind thickness) to 332.52

(total number of seeds per fruit). GCV ranged from 1.2 × 10^{-4}% (fresh fruit yield) to 21.81% (vine length at 21 DAP). PCV ranged from 4.93% (total number of seeds per fruit) to 27.84% (fresh fruit yield). In terms of estimates for broad-sense heritability, the range was from 1.8 x 10^{-9}% (fresh fruit yield) to 90.45% (fruit length). Genetic advance (GA) ranged from 8.24 × 10^{-5} (fresh fruit yield) to 12.52 (vine length at 42 WAP). Genetic advance expressed as a percentage of the mean of the trait (GAM) ranged from 1.26 × 10^{-4}% (fresh fruit yield) to 99.44% (fresh weight per fruit). Overall, GAM was greater than GA for each of the traits except for the total number of seeds per fruit with 0.29% (GAM) and 0.93 (GA).

Table 5. The estimates of variance components and the heritability for selected growth traits in watermelons.

Estimate	Vine length (cm)				Number of leaves			
	Days after planting				Days after planting			
	21	28	35	42	21	28	35	42
Mean	9.66	19.40	35.75	58.99	4.68	7.83	10.18	17.97
Minimum	5.00	7.02	8.87	36.63	3.17	6.00	5.83	10.67
Maximum	14.98	27.37	50.53	88.90	6.33	9.17	15.67	27.50
CV (%)	29.89	25.81	28.62	23.34	16.79	12.53	25.15	25.15
σ^2_g	4.44	10.35	30.74	48.63	0.20	0.43	3.21	4.92
$\sigma^2_{r(l)}$	0.51	0.00	1.82 x 10^{-11}	0.00	0.25	0.00	0.81	0.00
σ^2_l	0.44	4.81	13.39	23.36	2.17 x 10^{-14}	0.00	0.13	2.10
σ^2_{gl}	1.31	1.39	7.77	0.00	0.04	0.39	0.42	1.13
σ^2_e	3.29	13.31	66.97	139.75	0.21	0.31	3.10	14.52
σ^2_{ph}	5.24	12.29	40.78	64.16	0.24	0.59	3.70	6.92
σ^2_g/σ^2_{ph}	0.85	0.84	0.75	0.76	0.84	0.72	0.87	0.71
$[\frac{\sigma^2_{gl}}{l}]/\sigma^2_{ph}$	0.08	0.04	0.06	0.00	0.06	0.22	0.04	0.05
$[\frac{\sigma^2_e}{lr}]/\sigma^2_{ph}$	0.07	0.12	0.18	0.24	0.10	0.06	0.09	0.23
GCV (%)	21.81	16.58	15.51	11.82	9.51	8.35	17.61	12.35
PCV (%)	23.70	18.07	17.86	13.58	10.37	9.82	18.89	14.63
H^2_b (%)	84.74	84.21	75.39	75.80	84.01	72.36	86.92	71.22
GA	4.00	6.09	9.93	12.52	0.84	1.16	3.44	3.86
GAM (%)	41.42	31.40	27.78	21.23	18.00	14.75	33.82	21.47

l = lime rate, g = genotype (variety), r = replicate, σ^2_g =, σ^2_l = genotype variance lime rate variance, σ^2_{gl} = genotype x lime rate interaction variance, $\sigma^2_{r(l)}$ = replicate within lime rate variance, σ^2_e = pooled error variance, σ^2_{ph} = $\sigma^2_g + \frac{\sigma^2_{gl}}{l} + \frac{\sigma^2_e}{lr}$ = phenotypic variance, GCV = genotypic coefficient of variability, PCV = phenotypic coefficient of variability, H^2_b = broad-sense heritability. GA = genetic advance at the 5% selection intensity, GAM = genetic advance as a percentage of the population mean.

Table 6. The estimates of variance components and the heritability for selected yield traits in watermelons.

Estimate	Days to 50% flowering	Number of fruits per plant	Fresh weight per fruit (kg)	Fresh fruit yield (t ha^{-1})	Fruit width (cm)	Fruit length (cm)	Rind thickness (cm)	Total number of seeds per fruit	Sugar content (Brix)
Mean	43.67	3.89	1.90	65.51	13.28	15.02	0.79	321.44	7.07
Minimum	38.00	1.00	1.05	12.00	6.65	7.15	0.45	242.00	4.50
Maximum	57.00	8.00	2.34	163.80	16.23	19.88	1.20	431.67	10.78
CV (%)	12.61	48.02	20.02	61.47	15.33	18.52	24.09	14.60	24.90
σ^2_g	2.42	0.14	5×10^{-4}	5.9×10^{-9}	0.75	5.50	8.3×10^{-4}	7.16	0.87
$\sigma^2_{r(l)}$	1.50	1.09	0.02	876.90	1.91	1.97	0.01	440.81	1.63
σ^2_l	0.00	0.16	0.00	2.6×10^{-9}	0.00	2.1×10^{-12}	9.7×10^{-9}	25.54	0.00
σ^2_{gl}	0.00	1.14	0.03	986.01	1.16	1.43	8.1×10^{-3}	731.64	0.78
σ^2_e	27.25	6.73×10^{-10}	0.00	34.68	0.93	0.93	6.7×10^{-10}	1.4×10^{-4}	0.35
σ^2_{ph}	5.44	0.52	0.01	332.52	1.24	6.08	3.5×10^{-3}	251.04	1.16
σ^2_g/σ^2_{ph}	0.44	0.27	0.04	1.8×10^{-11}	0.61	0.90	0.23	0.03	0.74
$[\frac{\sigma^2_{gl}}{l}]/\sigma^2_{ph}$	0.00	0.73	0.96	0.99	0.31	0.08	0.77	0.97	0.22
$[\frac{\sigma^2_e}{lr}]/\sigma^2_{ph}$	0.56	1.44×10^{-10}	5.7×10^{-9}	0.01	0.08	0.02	2.1×10^{-8}	6.4×10^{-8}	0.03
GCV (%)	3.56	9.61	1.34	1.2×10^{-4}	6.54	15.61	3.63	0.83	13.16
PCV (%)	5.34	18.53	6.58	27.84	8.40	16.41	7.51	4.93	15.26
H^2_b (%)	44.39	26.88	4.17	1.8×10^{-9}	60.60	90.45	23.42	2.85	74.38
GA	2.14	0.40	1.90	8.24×10^{-5}	1.39	4.60	0.75	0.93	1.67
GAM (%)	4.90	10.30	99.44	1.26×10^{-4}	10.46	30.64	95.10	0.29	23.57

l = lime rate, g = genotype (variety), r = replicate, σ^2_g = genotype variance, σ^2_l = lime rate variance, σ^2_{gl} = genotype x lime rate interaction variance, $\sigma^2_{r(l)}$ = replicate within lime rate variance, σ^2_e = pooled error variance, $\sigma^2_{ph} = \sigma^2_g + \frac{\sigma^2_{gl}}{l} + \frac{\sigma^2_e}{lr}$ = phenotypic variance, GCV = genotypic coefficient of variability, PCV = phenotypic coefficient of variability, H^2_b = broad-sense heritability, GA = genetic advance at the 5% selection intensity, GAM = genetic advance as a percentage of the population mean.

The different varieties also showed differences in the transverse section with Kaolack having a wider section and in the longitudinal section with Heracles F_1 with the longest section (Figure 2). According to Silva et al. (2018), liming resulted in higher fruit weight because calcium is the second most required element of the Crimson sweet watermelon crop. This is in line with the Kaolack variety when planted on limed soil at 5.7 t ha^{-1}. The significant interaction of liming × level factors from Silva et al. (2018) also conforms with the result here for the transverse section with Kaolack at 5.7 t ha^{-1}, which was wider than the other levels of interaction.

In the present study, the following traits had heritability values of >50%, namely, vine length, number of leaves, fruit transverse and longitudinal sections and fruit sugar content. The significant difference in performances among watermelon genotypes was due to their inherent genetic differences, phenotypic variations, and differences in soil pH levels influenced by soil amendment with agricultural lime. GCV and PCV estimates are normally categorized as low (< 10%), moderate (10–20%) and high (> 20%) as indicated by Deshmukh et al. (1992). In this study, the highest GCV and PCV values were recorded for vine length at 21 DAP (21.81%) and fresh fruit weight (27.84%). Simultaneously, moderate GCV and PCV values (i.e., where both estimates were 10–20%) were recorded for vine length (28–42 DAP), number of leaves (35–42 DAP), fruit length and Brix value. Our results are similar to those of Anburani (2018), where high PCV and moderate GCV were recorded for fruit diameter, flesh thickness, number of fruits per plant and yield per plant in thirty genotypes of watermelon of different origins. The present study showed that all PCVs were relatively higher than the corresponding GCVs. The differences between PCV and GCV indicated the level of the influence of the environment on the expression of these traits.

Figure 2. Harvested whole mature fruits of watermelon varieties.
R_0 = no lime; R_1 = 2.7 t ha^{-1} lime; R_2 = 5.6 t ha^{-1} lime; V_1 = Heracles F_1; V_2 = Kaolack; V_3 = Sugar Baby.

According to Singh (2001), estimates of heritability are classified as low (< 40%), medium (40–59%), moderately high (60–79%) and very high (≥ 80%). Low estimates of broad-sense heritability were reported for fresh weight per fruit (4.17%), number of fruits per plant (26.88%), fresh fruit yield (1.8 x 10^{-9}%), rind thickness (23.42%) and total number of seeds per fruit (2.85%). Medium heritability was reported for days to 50% flowering (44.39%), moderately high heritability estimates were recorded for vine length at 33–42 DAP (75.39–75.80%), number of leaves at 28 DAP (72.36%) and 42 DAP (71.22%), fruit width (60.60%) and Brix (74.38%). Very high heritability estimates were recorded for vine length at 21–28 DAP (84.21–84.74%), number of leaves at 21 DAP (84.01%) and 35 DAP (86.92%) and fruit length (90.45%). Traits with high heritability estimates result in an increased population response to selection in the desired direction (Acquaah, 2007).

Genetic advance is a measure of predetermined progress under an artificial selection program. According to Johnson et al. (1955), the value of GAM is categorized as low (< 10%), moderate (10–20%) and high (> 20%). In this study, a low GAM value was recorded for days to 50% flowering, fresh fruit yield, and total number of seeds per fruit, indicating that these traits are not governed by additive genes and selection for watermelon improvement is ineffective for these traits. High GAM estimates were recorded for vine length at 21–42 DAP (21.23–41.42%) and moderate to high GAM for number of leaves at 21–42 DAP (14.75–33.82%). Moderate GAM was also recorded for number of fruits per plant (10.30%), fruit width (10.46%). Fresh weight per fruit (99.44%), rind thickness (95.10%), fruit length (30.64%) and Brix (23.57%) showed high GAM estimates. The high heritability coupled with genetic advance indicates that additive gene action controls the expression of inheritance of these traits.

Overall, the estimates of heritability and GAM were moderate to high for vine length, number of leaves, fruit width (transverse section), fruit length (longitudinal section), rind thickness and Brix (sugar content). Apparently, these traits are critical to identify the potential for developing superior watermelon genotypes and/or improving the population through selection. In the present study, the marked difference in performances among watermelon genotypes was due to their inherent genetic differences and phenotypic variations, as well as differences in soil pH levels as influenced by soil amendment with agricultural lime. Heracles F_1 and Kaolack watermelon varieties outperformed Sugar Baby in terms of growth and yield traits. The application of 2.7 t ha^{-1} and 5.7 t ha^{-1} of $CaCO_3$ to the three watermelon varieties significantly influenced growth and yield traits: it effectively increased fresh fruit weight (t ha^{-1}), fruit transverse and longitudinal sections and sugar content. Heracles F_1 × 2.7 t ha^{-1}, Heracles F_1 × 5.7 t ha^{-1}, Kaolack × 2.7 t ha^{-1} and Kaolack × 5.7 t ha^{-1} showed the highest performance in both growth and yield traits.

Conclusion

The study reveals that watermelon varieties, Heracles F_1 and Kaolack, have great potential to thrive well in lime-amended acidic soils in Calabar, Cross River State. $CaCO_3$ at 2.7 t ha^{-1} and 5.7 t ha^{-1} proved suitable for watermelon production in Calabar as these rates could reduce soil acidity (pH = 4.6) by 21.74% and 43.48%, respectively. The findings of the present study highlight that watermelon production in Nigeria is no longer an exclusive agricultural enterprise of the northern states.

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VARIRANJE RASTA, PRINOSA I KOMPONENTI PRINOSA LUBENICE (*CITRULLUS LANATUS*) UZGAJANE NA KALCIFIKOVANOM ($CaCO_3$) KISELOM ZEMLJIŠTU JUGOISTOČNE NIGERIJE

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R e z i m e

Postavljen je poljski ogled kako bi se procenio rast i prinos lubenice *Citrullus lanatus* [(Thunb.) Matsum. & Nakai] u uslovima kiselih zemljišta u Kalabaru, država Kros River, Nigerija. Ogled je bio faktorijalni 3 × 3 postavljen u okviru potpuno slučajnog blok dizajna (PSBD) sa tri ponavljanja. Faktori koji su proučavani su bile sorte (Heracles F_1, Kaolack i Sugar Baby), količine kreča (0 t ha^{-1}, 2,7 t ha^{-1} i 5,7 t ha^{-1}), i njihove interakcije na osobine rasta i prinosa lubenice. Heracles F_1 i Kaolack su nadmašili Sugar Baby ($p \leq 0,05$) u pogledu osobina rasta i prinosa. Količine kreča od 2,7 t ha^{-1} i 5,7 t ha^{-1} povećale su početni raspon pH zemljišta (4,6–4,9) za 21,74% (5,4–5,8) odnosno 43,48% (6,4–6,7). Ove doze su poboljšale pH zemljišta do opsega pogodnog za gajenje lubenice u oblasti istraživanja. Dužina vreže, broj listova, poprečni i uzdužni presek plodova i sadržaj šećera u plodovima imali su ≥ 50% heritabilnosti, što je koristan indeks pri odabiru osobina rasta i prinosa kod lubenice. Uopšteno uzev, GAM je bio veći od GA za svaku od osobina, osim za ukupan broj semena po plodu. Preporučuje se istraživanje na više lokacija kako bi se dobio bolji uvid u ovu pilot studiju.

Ključne reči: briks, plodovi, heritabilnost, hibrid, kreč, kiselost zemljišta, ultisol, lubenica.

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MORPHOLOGY AND MOLECULAR CHARACTERIZATION OF *TYLENCHULUS SEMIPENETRANS* FROM CITRUS ORCHARDS IN NORTHERN IRAN

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Abstract: *Tylenchulus semipenetrans* Cobb, 1913 is among some of the most economically important plant-parasitic nematodes in the world. The nematode has been identified as the causal agent of slow decline. Most studies estimate the yield losses, due to *T. semipenetrans,* to range from 10% to 30%, depending on the level of infection, aggressiveness of the nematode population, soil characteristics, susceptibility of the rootstock, presence of other pathogens and grove management practices. In order to identify the citrus nematode in northern Iran, soil and root samples were collected from infected trees. The second-stage juveniles were isolated from the soils by the tray method. Eggs and females were extracted from roots by the centrifugal-flotation technique. Morphological observations and molecular evidence confirmed this population as *T. semipenetrans*. A phylogenetic tree of *T. semipenetrans* populations was reconstructed based on 28S rRNA gene sequences using RAxML. Morphologically, there is a slight difference between the studied population and the reported populations of *T. semipenetrans* from pomegranate and banana orchards in southern Iran. Phylogenetic analysis showed the close relationship of the *T. semipenetrans* population from northern Iran with other populations of this species. Based on molecular analysis, *Tylenchulus* was identified as a monophyletic group. The phylogenetic position and measurements of *T. semipenetrans* were provided.

Key words: *Citrus sinensis*, 28S rRNA, Iran, phylogeny, slow decline.

Introduction

Iran is the sixth-largest citrus fruit producer in the world, with an annual production of 4.1 million tons (FAO, 2016). Various citrus species are widely

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cultivated in northern and southern parts of Iran, such as sweet orange (*Citrus sinensis* L.), acid lime (*C. aurantifolia* (Christm.) Swingle), sour orange (*C. aurantium* L.), mandarin (*C. reticulata* Blanco), lemon (*C. limon* (L.) Osbeck), and grapefruit (*C. paradisi* Macfad). The main producing regions in Iran are Mazandaran, Fars, Hormozgan, Jiroft and Kahnouj (Espargham et al., 2020). Like many tropical and subtropical crops, citrus is attacked by numerous pests and pathogens. *Citrus* species are susceptible to a large number of destructive diseases caused by fungal, bacterial, and viral plant pathogens, which are continuously emerging and can severely hinder or completely destroy the entire production (Tennant et al., 2009).

Plant-parasitic nematodes are economically important pests that affect many horticultural crops produced in tropical and subtropical areas (Whitehead, 1998). The genus *Tylenchulus* contains four species in the world. Among them, *T. semipenetrans*, the causal agent of "slow decline" of citrus, has a worldwide distribution and causes significant crop losses in all citrus-growing regions of the world (Siddiqi, 1974; Duncan and Cohn, 1990). This nematode was first observed in orange tree in southern California by Cobb in 1912. Crop losses caused by *T. semipenetrans* are estimated to be in the range of 15% to 30% per year (Duncan, 2005). Symptoms of nematode attack often include reduced vigor, chlorosis, leaf fall, dieback, and reduced production and weakened fruit quality (Cohn, 1969). The citrus nematode has been previously reported from citrus orchards in Iran (e.g., Izadpanah and Safarian, 1968; Katcho and Allow, 1969; Abivardi, 1970; Minassian and Moadab, 1970; Sharafeh, 1972; Tanha Maafi and Kheiri, 1991; Tanha Maafi and Damadzadeh, 2008; Rashidifard et al., 2015). Izadpanah and Safarian (1968) first reported *T. semipenetrans* from citrus growing areas in the Ahvaz province. Tanha Maafi and Damadzadeh (2008) revealed that 89% of the soil and root samples of citrus orchards in Mazandaran were infested with citrus nematodes. Additionally, they evaluated the impact of two organophosphates and one carbamate nematicide on citrus nematode under both glasshouse and orchard conditions. Also, several studies were conducted to monitor the population dynamics of *T. semipenetrans* in southern (Sharafeh, 1972; Tanha Maafi and Kheiri, 1991) and northern (Tanha Maafi and Damadzadeh, 2008) parts of Iran. Tanha Maafi et al. (2012) analyzed the phylogenetic relationships within *Tylenchulus* using rRNA gene sequences. Recently, the molecular characterization, phylogenetic position, and seasonal dynamics of *T. semipenetrans* were elucidated in the southern part of Iran (Rashidifard et al., 2015).

The first and most important step in controlling slow decline is to diagnose the disease correctly. Traditionally, the identification of *Tylenchulus* species has been based on a few morphological characters of the male, the mature female, and the second-stage juveniles (Inserra et al., 1988a, b; 1994). However, morphological identification of the J2 of *T. semipenetrans* is difficult due to its small size and

requires taxonomic expertise. In addition, *Tylenchulus* J2 specimens can be easily misidentified with the J2 of some closely related genera (e.g., *Trophotylenchulus* and *Meloidogyne* spp.). Therefore, an accurate and reliable identification procedure for monitoring and diagnostic purposes to distinguish *Tylenchulus* species becomes a very important task. In the past decade, molecular techniques have been developed and applied to identify plant-parasitic nematodes (e.g., Blok et al., 2002; Adam et al., 2007; Park et al., 2009; Liu et al., 2011; Tanha Maafi et al., 2012; Yan et al., 2013; Lin et al., 2016). Recent studies have shown that molecular techniques are more sensitive and accurate tools for the identification of *T. semipenetrans* (Liu et al., 2011).

So far there has been no comprehensive study on molecular identification or diagnosis of *Tylenchulus* species in the northern part of Iran. Therefore, the aims of the present study were to identify the species of citrus nematode in northern Iran by morphological and molecular methods and to explain the phylogenetic position of the population of *T. semipenetrans* with closely related populations in GenBank.

Material and Methods

Specimens and collections

The samples were collected in the Guilan province. Five trees were selected from each orchard and three samples were collected from each tree. Each soil sample was taken at a depth of 5–30 cm. The samples from each tree were completely mixed and a representative sample of 500 g was prepared. The soil and root samples were transferred to the Nematology laboratory of the University of Guilan and stored at 4°C. The juvenile nematodes were extracted from the soil samples by the centrifugal-flotation technique and the tray method, whereas a centrifugation method (Jenkins, 1964) was used to recover mature females and eggs from the roots. In the present study, *T. semipenetrans* was identified using the descriptions provided by Goodey (1963) and Crozzoli et al. (1998).

DNA extraction, PCR and sequencing

DNA was extracted using the method of Subbotin et al. (2006). Polymerase chain reactions (PCRs) were carried out in a 25-µl reaction mixture, containing 4 µl of master mix, 9 µl of molecular-grade water, 1 µl of each primer and 10 µl of genomic DNA template. The primer pairs D2A (5”-ACAAGTACCGTGAGGGAAAGTTG–3”) and D3B (5”-TCGGAAGGAACCAGCTACTA–3”) were used to amplify ~750-bp fragment of the 28S region (Subbotin et al., 2006). The PCR program consisted of an initial denaturing step at 94°C for 3 min, 37 amplification cycles (94°C for 45s, 56°C for

45s, 72˚C for 60s), and a final step at 72˚C for 6 min. The size of the amplification products was determined using a 1% agarose gel. The PCR product was purified and sequenced in both directions. The sequencing was performed by Bioneer company (South Korea) (http://eng.bioneer.com). The newly obtained sequence data was deposited into the GenBank database (Table 1).

Table 1. The accession numbers of the sequences used in the phylogenetic analysis. The sequence in bold was sequenced in the present study.

Species	GenBank accession number	Origin	Reference
Caloosia longicaudata	GU989627	United States	(Van den Berg et al., 2011)
Coslenchus costatus	DQ328719	Germany	(Subbotin et al., 2006)
Criconema mutabile	AY780954	Venezuela	(Subbotin et al., 2006)
Criconema sp.	AY780952	Italy	(Subbotin et al., 2006)
Criconema sp.	AY780953	Venezuela	(Subbotin et al., 2006)
Criconemoides brevistylus	JQ231185	South Africa	(Van den Berg et al., 2012)
Criconemoides informis	AY780970	Venezuela	(Subbotin et al., 2005)
Criconemoides obtusicaudatus	JQ231186	South Africa	(Van den Berg et al., 2012)
Criconemoides obtusicaudatus	JQ231187	South Africa	(Van den Berg et al., 2012)
Hemicaloosia vagisclera	JQ246423	United States	(Inserra et al., 2013)
Hemicriconemoides alexis	AY780959	Greece	(Subbotin et al., 2005)
Hemicriconemoides gaddi	KC520470	China	(Yang et al., 2013)
Hemicriconemoides ortonwilliamsi	AY780948	Italy	(Subbotin et al., 2005)
Hemicycliophora lutosa	GQ406240	South Africa	(Van den Berg et al., 2010)
Hemicycliophora lutosa	GQ406241	South Africa	(Van den Berg et al., 2010)
Meloidoderita kirjanovae	DQ768428	Italy	(Vovlas et al., 2006)
Ogma civellae	AY780955	Venezuela	(Subbotin et al., 2005)
Paratylenchus aquaticus	KF242240	United States	(Van den Berg et al., 2014)
Paratylenchus aquaticus	KF242239	United States	(Van den Berg et al., 2014)
Paratylenchus bukowinensis	AY780943	Italy	(Subbotin et al., 2005)
Paratylenchus dianthus	KF242229	South Africa	(Van den Berg et al., 2014)
Paratylenchus hamatus	KF242219	United States	(Van den Berg et al., 2014)
Paratylenchus nanus	AY780946	Germany	(Subbotin et al., 2005)
Paratylenchus sp.	AY780944	Italy	(Subbotin et al., 2005)
Paratylenchus sp.	AY780945	United States	(Subbotin et al., 2005)
Paratylenchus straeleni	KF242236	United States	(Van den Berg et al., 2014)
Psilenchus sp.	DQ328716	United States	(Subbotin et al., 2006)
Sphaeronema alni	JQ771954	Czech Republic	(Codejkova and Cermak, 2013)

Continuation of Table 1. The accession numbers of the sequences used in the phylogenetic analysis. The sequence in bold was sequenced in the present study.

Trophotylenchulus floridensis	JN112254	United States	(Tanha Maafi et al. 2012)
Trophotylenchulus floridensis	JN112253	United States	(Tanha Maafi et al., 2012)
Tylenchulus furcus	JN112257	South Africa	(Tanha Maafi et al., 2012)
Tylenchulus furcus	JN112258	South Africa	(Tanha Maafi et al., 2012)
Tylenchulus graminis	JN112259	United States	(Tanha Maafi et al., 2012)
Tylenchulus graminis	JN112260	United States	(Tanha Maafi et al., 2012)
Tylenchulus musicola	JN112247	Iran	(Tanha Maafi et al., 2012)
Tylenchulus musicola	JN112248	Iran	(Tanha Maafi et al., 2012)
Tylenchulus palustris	JN112255	United States	(Tanha Maafi et al., 2012)
Tylenchulus semipenetrans	AY780972	Egypt	(Subbotin et al., 2005)
Tylenchulus semipenetrans	JN112249	United States	(Tanha Maafi et al., 2012)
Tylenchulus semipenetrans	JN112250	United States	(Tanha Maafi et al., 2012)
Tylenchulus semipenetrans	JN112251	South Africa	(Tanha Maafi et al., 2012)
Tylenchulus semipenetrans	JN112252	United States	(Tanha Maafi et al., 2012)
Tylenchulus semipenetrans	FJ969710	Korea	(Park et al., 2009)
Tylenchulus semipenetrans	FJ969711	Korea	(Park et al., 2009)
Tylenchulus semipenetrans	FJ969712	Korea	(Park et al., 2009)
Tylenchulus semipenetrans	FJ969713	Korea	(Park et al., 2009)
Tylenchulus semipenetrans	FJ969714	Korea	(Park et al., 2009)
Tylenchulus semipenetrans	FJ969715	Korea	(Park et al., 2009)
Tylenchulus semipenetrans	KJ577615	Iran	(Rashidifard et al., 2015)
Tylenchulus semipenetrans	KM598333	Iran	(Rashidifard et al., 2015)
Tylenchulus semipenetrans	KM598334	Iran	(Rashidifard et al., 2015)
Tylenchulus semipenetrans	KM598335	Iran	(Rashidifard et al., 2015)
Tylenchulus semipenetrans	**?**	**Iran**	**Present study**
Xenocriconemella macrodora	AY780960	Italy	(Subbotin et al., 2005)

Phylogenetic analysis

All sequences were aligned in the MAFFT v.7 online servers (http://mafft.cbrc.jp/ alignment/server/; Katoh et al., 2019) and concatenated for phylogenetic analysis, with *Coslenchus costatus* and *Psilenchus* sp. added as outgroups. Maximum likelihood (ML) analysis was performed with RAxML (Stamatakis, 2006) as implemented in raxmlGUl 1.3 (Silvestro and Michalak, 2012), using the ML + rapid bootstrap setting and the GTRGAMMA substitution model with 1000 bootstrap replicates. Bootstrap support values above 50% are given in Figure 2.

Results and Discussion

Morphological characteristics

In adult females (Figure 1, Table 2), the body was 349–406 µm long, proximally elongated and irregular, distal half swollen. The thickness of the cuticle in the middle of the body was 5–9 µm. The stylet was 12–20 µm long with rounded knobs. The dorsal esophageal gland Orifice (DEGO) was 4–8 µm below the stylet knobs. The size of the Post-Vulval Section Cavity (PVSC) reached 3–4 µm. The length of the the post-vulval sac was 14–28 µm. The width of the post-vulva section (PVSW) was 11–18 micrometers. The basal bulb was oval, 14–24 µm long and 14–25 µm wide. The excretory pore was located at 69–78% of body length. The reproductive system was monodelphic. Eggs were ovoid, with sizes ranging from 33 to 67 µm. The tail was curved towards the abdomen.

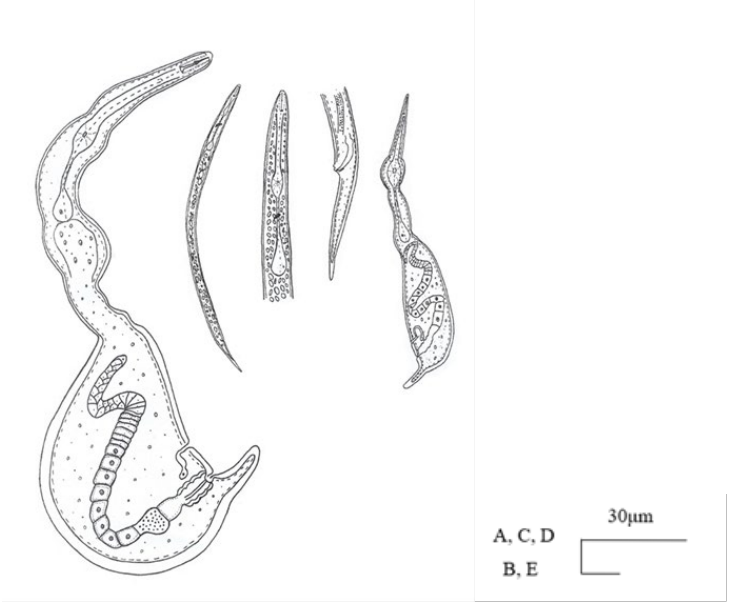

Figure 1. *Tylenchulus semipentrans*, A: Entire body of female; B: Entire body of second-stage juvenile; C: Anterior body portion of juvenile; D: Posterior body portion of male; E: Young female; (Scale bars: A–D: 30µm.)

In males (Figure 1, Table 3), the body was "J" shaped, 326–399 µm long. The esophagus and the stylet were weakly developed. The stylet was 7–9 µm long with rounded knobs. Excretory pores were located in 20–23% of the body length. The gubernaculum was simple and 4.2–5 µm long.

In second-stage juveniles (Figure 1, Table 3), bodies were 302–333 µm long. The stylet was 12–16 µm long with rounded knobs. The excretory pore was located at 47–54% of the body length and 118–148 µm to the genital primordium. The deirid was not visible. The genital primordium had 3 cells and was 60–67% of the distance from the anterior end.

De Man ratios obtained were: female: a = 3.7 ± 1.1; male: a = 29.6 ± 1.4, b = 4.2 ± 0.6, c = 8.7 ± 0.5; second-stage juvenile: a = 22 ± 0.5, b = 2.9 ± 9.2, c = 6.4 ± 0.8.

Table 2. Measurements of females of *Tylenchulus semipenetrans* Cobb, 1913 collected from the Guilan province. All measurements are in µm.

Province	Chabahar (Tanha Maafi et al., 2012)	Shahdad (Rashidifard et al., 2015)	Present study
Number	12 ♀♀	6 ♀♀	5 ♀♀
L	287 ± 32.6 (240–370)	273.9± 21.6 (245–295)	267 ± 11 (254–292)
a	4.8 ± 1.4 (3.7–8.2)	3.8 ± 1.2 (2.7–5.2)	3.7 ± 1.1 (2.8–4.9)
Stylet	10 ± 1.2 (8–12)	14.3 ± 6.1 (10–22)	13.8 ± 5.3 (12–20)
DEGO	4.0 ± 0.8 (3–5)	5 ± 1.9 (4–8)	4.5 ± 1.7 (4–8)
Anterior end to median bulb	46 ± 7.7 (35–55)	27 ± 2.9 (25–31)	26 ± 2.2 (25–29)
Median bulb length	15.3 ± 2.4 (12–19)	18 ± 4.7 (15–24)	19 ± 3.4 (16–22)
Median bulb width		14.4 ± 3.7 (11–19)	13.3 ± 2.6 (12–19)
Pharynx length	94 ± 15.2 (66–112)	131.6 ± 3.2 (129–135)	130.4 ± 3.3 (128–134)
Basal bulb length	21.5 ± 3.4 (15–27)	20 ± 2.9 (13–25)	20.1 ± 2.4 (14–24)
Basal bulb width	12.6 ± 2.0 (10–16)	18 ± 3.9 (13–26)	19 ± 2.3 (14–25)
Anterior end to excretory pore	222 ± 37.7 (150–280)	205.2 ± 30.3 (162–230)	210 ± 22.5 (173–228)
Excretory pore from anterior end as % of body length	77.4 ± 8.8 (61.2–93.3)	72±5.7 (66.5–78)	71 ± 5.1 (69–78)
Vulva-excretory pore distance	14.1 ± 3.3 (10–20)	17.2 ± 3.4 (13–22)	17.2 ± 3.4 (13–22)
Post-vulva section width (PVSW)	11.0 ± 1.4 (9–14)	13 ± 3.1 (10–18)	14 ± 2.2 (11–18)
Post-vulva section length (PVSL)	27 ± 6.0 (17–36)	19.4 ± 5.9 (13–31)	18.3 ± 4.2 (14–28)
Post-vulva section cavity (PVSC)	6.6 ± 0.7 (6–8)	4.2 ± 0.9 (4–5)	3.9 ± 0.8 (3–4)
Swollen posterior body length	-	178 ± 21.2 (154–211)	167 ± 18.2 (160–209)
Swollen posterior body as % of total body length	45.6–54.0	57.5 ± 3.7 (55–63)	53.2 ± 3.1 (50–60)
Body width at vulva	23 ± 3.8 (15–29)	47 ± 8.6 (37–65)	42 ± 1.9 (38–62)
Body width at mid-body	64 ± 16.8 (30–80)	79.5 ± 13.5 (55–97)	73 ± 8.8 (60–94)
Cuticle thickness at mid-body	4.2 ± 0.8 (3–5)	5.4 ± 1.2 (4–9)	5.2 ± 1.3 (5–9)

Remarks: The measured characters were generally similar to reported values for *T. semipenetrans* from Iran (Rashidifard et al., 2015, Tanha Maafi et al., 2012) (Tables 2 and 3). The differences between the studied traits and the population

reported from the south of Iran were as follows. The values of stylet length, DEGO, median bulb length, and body width were lower in the Chabahar population.

Table 3. Measurements of second-stage juveniles (J2) and males of *Tylenchulus semipenetrans* Cobb, 1913 collected from the Guilan province.

Province	Chabahar (Tanha Maafi et al., 2012)		Shahdad (Rashidifard et al., 2015)		Present study	
Number	31 J2	5 ♂♂	5 J2	6 ♂♂	6 J2	4 ♂♂
Body length	306 ± 13.8 (278–334)	310 ± 19.2 (286–326)	323.6 ± 13.7 (309–345)	328.1 ± 21.8 (305–355)	322 ± 10.6 (302–333)	372 ± 17.9 (326–399)
a	30.1 ± 1.6 (27.7–33.4)	27.2 ± 3.8 (23.8–32.5)	27.3 ± 2.3 (20.9–27.1)	32.1 ± 2 (29.5–33.6)	22 ± 0.5 (21–22.2)	29.6 ± 1.4 (27.1–30)
b	3.5 ± 0.2 (3.2–4.0)	3.5 ± 0.2 (3.3–3.7)	3.3 ± 0.3 (3.1–4)	4.4 ± 0.8 (3.3–5.8)	2.9 ± 9.2 (2.8–3)	4.2 ± 0.6 (4.2–4.6)
c	-	8.1 ± 0.1 (8.0–8.2)	7.5 ± 1.3 (6–9.2)	7.3 ± 0.6 (6.4–7.8)	6.4 ± 0.8 (6–6.7)	8.7 ± 0.5 (8.1–8.8)
Stylet	11.1 ± 0.6 (10–12)	8.5 ± 0.6 (8–9)	13.4 ± 2.9 (12–19)	8.8 ± 2.1 (7–11)	14 ± 2.1 (12–16)	8 ± 0.8 (7–9)
Anterior end to median bulb	43.6 ± 2.5 (38–48)	36.0 ± 1.4 (35–37)	43 ± 6.8 (37–54)	36.5 ± 7.4 (27–46)	47 ± 1.9 (45–55)	38 ± 1.2 (33–45)
Pharynx length	87 ± 4.3 (78–100)	89 ± 1.5 (87–90)	96.1 ± 7.4 (85–105)	73.1 ± 5.2 (68–82)	108 ± 3.4 (98–112)	88 ± 6.4 (70–90)
Anterior end to hemizonid	65 ± 4.3 (57–71)	57 ± 6.7 (50–65)	69.6 ± 4.8 (66–77)	73.7 ± 4 (69–78)	82 ± 1.3 (80–88)	80 ± 4.3 (77–83)
Anterior end to excretory pore	169 ± 8.4 (148–184)	174 ± 4.0 (170–178)	169.6 ± 5.4 (163–177)	171.5 ± 10.6 (163–182)	84 ± 0.8 (80–86)	77 ± 2.1 (75–79)
Anterior end to nerve ring	-	-	75.2 ± 8.5 (66–88)	49.7 ± 12 (32–63)	75 ± 1.5 (68–77)	65 ± 1.8 (59–69)
Excretory pore to genital primordium	28.5 ± 6.8 (15–40)	-	46.7 ± 13.5 (37–70)	-	127 ± 10.3 (118–148)	-
Anterior end to genital primordium	198 ± 9.9 (179–217)	-	216.3 ± 16.1 (200–243)	-	199 ± 9.8 (185–212)	-
Genital primordium to posterior end	109.2 ± 7.6 (90–124)	-	134.6 ± 9.9 (124–151)	-	125 ± 1.9 (122–130)	-
Maximum body diameter	10.1 ± 0.3 (10–11)	11.5 ± 1.0 (10–12)	13.7 ± 1 (13–15)	10.9 ± 1.3 (10–13)	14.5 ± 0.5 (14–15)	12.5 ± 0.6 (12–13.5)
Excretory pore from anterior end as % of body length	55.3 ± 2.0 (49.5–58.4)	54.9 ± 3.4 (52.1–58.7)	52.4 ± 1.4 (50–54)	17.7 ± 6.2 (13–28)	50 ± 3.1 (47–54)	22.2 ± 1.5 (20–23)
Genital primordium (%)	64.7 ± 2.0 (61–70)	-	66.7 ± 2.3 (65–70)		65 ± 1.9 (60–67)	-
Spicules	-	15.7 ± 2.1 (14–18)		20 ± 1.1 (19–22)	-	21.3 ± 0.5 (21–22)
Gubernaculum	-	4.5 ± 0.7 (4–5)		4 ± 0.3 (3–4)	-	4.4 ± 0.3 (4.2–5)
Tail	-	38.3 ± 2.4 (35–40)	43.9 ± 7.8 (33–54)	44.8 ± 5.8 (39–53)	50.3 ± 2.2 (45–55)	42.5 ± 2.3 (40–45)

Molecular phylogenetic analysis

The present study confirmed the occurrence of *T. semipenetrans* in the main citrus growing areas of the Guilan province. The best ML tree (lnL = -9636.915433) obtained by RAxML is shown in Figure 2. Of the 775 nucleotide characters of the matrix, 372 were parsimony informative. Phylogenetic analysis revealed the existence of five major clades including:

I) *Caloosia longicaudata* (Loos, 1948), *Hemicaloosia vagisclera* (Inserra et al., 2013), *Criconemoides* spp., *Hemicycliophora lutosa* (Loof and Heyns, 1969), *Criconema* spp., *Ogma civellae* (Steiner, 1949; Raski and Luc, 1987), *Hemicriconemoides* spp., *Xenocriconemella macrodora* (Taylor, 1936; De Grisse and Loof, 1965);

II) *Paratylenchus* spp;

III) *Trophotylenchulus floridensis* (Raski, 1957), and T*ylenchulus* spp;

IV) *Sphaeronema alni* (Turkina and Chizhov, 1986);

V) *Meloidoderita kirjanovae* (Pogosjan, 1966).

As in previous studies, *Tylenchulus* was found to be a monophyletic group and all species were included within the strongly supported clade (83%) (e.g., Subbotin et al., 2005; Tanha Maafi et al., 2012). Our analysis supports the taxonomic status of *Trophotylenchulus* Raski, 1957 as a separate taxon from *Tylenchulus*. This result is similar to the analysis of Tanha Maafi et al. (2012) based on molecular data. *Trophotylenchulus* was also united with *Tylenchulus* as a sister group, confirming another recent study (Rashidifard et al., 2015). Our sequence of *T. semipenetrans* was clustered with other *T. semipenetrans* sequences from GenBank with maximum support (99%), which is in agreement with other previous studies (e.g., Tanha Maafi et al., 2012; Rashidifard et al., 2015). Further phylogenetic studies are needed in order to provide a clearer idea of the generic relationships within *Tylenchuloidea.*

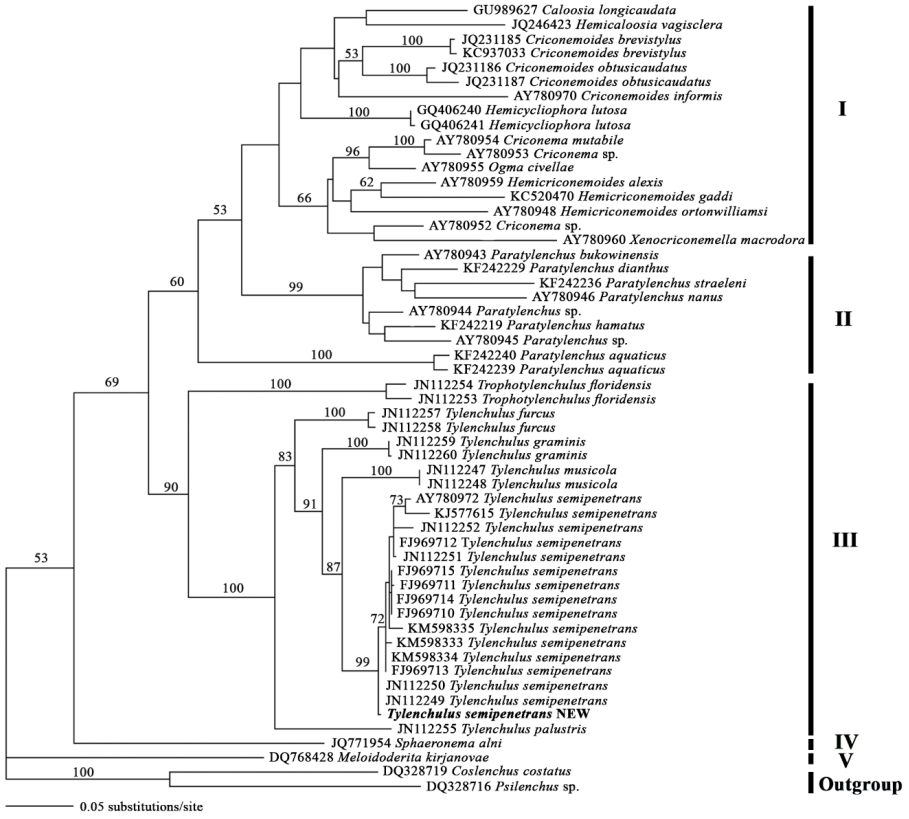

Figure 2. The phylogram of the best ML trees (lnL = -9636.915433) revealed by RAxML from an analysis of the 28S rDNA region.
The sequence in bold is new from Iran.

Conclusion

Morphologically, there is a slight difference between the studied population and the reported populations of *T. semipenetrans* from pomegranate and banana orchards in southern Iran. These characters include stylet length, DEGO, median bulb length and body width. The minimum morphological differences were observed in the Shahdad population of pomegranates.

Phylogenetic analysis using 28S rDNA showed the close relationship of the *T. semipenetrans* population from northern Iran with other populations of this species. The most similarity was observed in the JN112249 and JN112250 populations. This result indicates that *Tylenchulus* forms a monophyletic group.

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MORFOLOGIJA I MOLEKULARNA KARAKTERIZACIJA *TYLENCHULUS SEMIPENETRANS* IZ VOĆNJAKA AGRUMA U SEVERNOM IRANU

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R e z i m e

Tylenchulus semipenetrans Cobb, 1913 spada među neke od ekonomski najvažnijih biljnih parazitskih nematoda na svetu. Nematoda je identifikovana kao uzročnik sporog propadanja citrusa. Većina studija procenjuje da se gubici prinosa, usled prisustva *T. semipenetrans* kreću od 10% do 30%, u zavisnosti od nivoa zaraze, agresivnosti populacije nematoda, karakteristika zemljišta, osetljivosti podloge, prisustva drugih patogena i proizvodne prakse upravljanja voćnjakom. Da bi se identifikovala citrusna nematoda u severnom Iranu, sa zaraženih stabala su prikupljeni uzorci zemljišta i korena. Juvenili drugog stadijuma izolovani su iz zemljišta metodom sita. Jaja i ženke su izdvojene iz korena centrifugalno-flotacionom tehnikom. Morfološkim i molekularnim analizama potvrđeno je prisustvo populacije *T. semipenetrans*. Filogenetsko stablo populacija *T. semipenetrans* je rekonstruisano na osnovu sekvenci gena 28S rRNK korišćenjem RAxML. Morfološki, postoji mala razlika između proučavane populacije i već opisanih populacija *T. semipenetrans* iz voćnjaka nara i banana iz južnog Irana. Filogenetska analiza je pokazala blisku vezu populacije *T. semipenetrans* iz severnog Irana sa drugim populacijama ove vrste. Na osnovu molekularne analize, *Tylenchulus* je identifikovan kao monofiletička grupa. Pružene su informacije o filogenetskom položaju i sličnosti populacija *T. semipenetrans*.

Ključne reči: *Citrus sinensis*, 28S rRNA, Iran, filogenija, sporo propadanje citrusa.

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ESTHETIC ASSESSMENT OF THE ORNAMENTAL FORMS OF NORTHERN WHITE CEDAR (*THUJA OCCIDENTALIS* L.) AND THEIR USE IN GARDEN AND PARK COMPOSITIONS

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Abstract: Northern white cedar (*Thuja occidentalis* L.) is represented in the world by about 200 ornamental forms, 43 of which were discovered and described in landscaping and nurseries of Lviv. The first plantations in Lviv date back to the 1920s. They were introduced by the professor at the Medical Institute T. Vilchynskyi, who brought material for cuttings from a nursery in Kurnik (Poland). The decorative qualities of these cultivars were determined on the basis of characteristic features and evaluated in points: the shape and density of the crown, the color of the needles, the nature of branching, and the features of seed-bearing. These decorative qualities formed the basis for the construction of various elements of garden and park compositions with the participation of tapeworms, alleys, green walls, hedges, boskets, topiaries, and rock gardens. The highest number of points (12) was obtained by 46.4% of ornamental forms, 11 – 16.3%, 10 – 9.3%, and 8 – 11.6%. Cultivars with a distinct regular crown shape received high evaluation points. The options for the spatial arrangement of individuals were proposed and the distance between seating positions was recommended. Taking into account the high plasticity of cultivars and the ability to form crowns, variants of clipped hedges and topiaries were recommended. Several models have been proposed based on the distribution of ornamental plant groups based on their symmetry, silhouettes, alignments, contrasts, colors, density, and sizes. Cultivars were selected for each variant, and their group arrangement was proposed.

Key word: northern white cedar (*Thuja occidentalis* L.), ornamental form, landscaping element, composition, esthetic assessment.

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Introduction

Due to the amateur efforts of Prof. T. Vilchynsky, northern white cedar, originating from the Atlantic coast of North America, was introduced in the mild humid climate of Lviv in the 1930s in one columnar and two pyramidal forms (Kharachko and Skolskyi, 2017). A number of pyramidal and columnar forms were introduced during this period. In the post-war period, A. Shcherbyna, an associate professor at the Ivan Franko State University of Lviv, discovered 5 ornamental forms in the landscaping of the city. In the seventies, a large number of cultivars of northern white cedar were introduced by R. Karmazin at the Ivan Franko State University. In the 1970s and 1980s, more than 10 ornamental forms were grown in urban nurseries. Kycheryavyi et al. (2021) found 43 ornamental forms, the vast majority of which are not used in urban landscaping. In our opinion, the main reason for this is the poor awareness among gardeners of the peculiarities of using a rich assortment to create garden and park compositions (Kycheryavyi et al., 2018; Kycheryavyi et al., 2021). *T. occidentalis* attracts the interest of many scientists in the fields of ecology, forestry, and medicine (Landsberg, 1981; Klausnitzer, 1987; Czernecki and Jabłońska, 2016). In particular, the chemical components of *T. occidentalis* have been of interest for decades due to the essential oil, coumarins, flavonoids, tannins, and proanthocyanidins it contains. Pharmacology includes antioxidant, anti-inflammatory, antibacterial, antifungal, antitumor, antiviral, gastrointestinal protective, radioprotective, antipyretic, and regulatory effects on lipid metabolism. *T. occidentalis* is widely used in landscape design. The features of the developmental stage, ornamental values, and varieties of garden hedges used as integral components of landscape architecture and urban design in Kosovo were investigated (Balaj, 2020). It was concluded that the studied varieties of *Thuja sp*. and *Leylandii* species should be included in the structure of the hedge planting for good urban landscape design. Another positive role of the plant was found in the absorption of dust particles from the air. The authors report the degree of absorption of dust particles from the air by some tree species. The presence of wax on the leaves was significant for the adhesion of particulate matter. All species captured dust, with their overall capture efficiency ranked from highest to lowest as follows: *Thuja occidentalis* > *Hedera helix* > *Phyllostachys nigra*. All types of green barriers contributed to the improvement of air quality by capturing dust particles, regardless of their location within the barrier (Redondo-Bermúdez et al., 2021).

The physiological features of growth are of considerable interest in different parts of the world. The study (Bouslimi et al., 2022) examined regional, local, inter-tree, and radial variations in ring density and width in *Thuja occidentalis* in the boreal forest of Quebec. The average ring density of trees growing at Abitibi-Témiscamingue was 356 kg m^{-3}, with slight variation between earlywood and latewood (167 kg m^{-3}), revealing relatively homogeneous wood. Regional and local

variations in growth and wood density parameters of *T. occidentalis* indicate a complex response to environmental factors.

This research reports the results of studies of six old growth stands of northern white cedar (*Thuja occidentalis*) in the Big Reed Reserve in northern Maine, USA, aimed at reconstructing the frequency and severity of past natural disturbances. Overall, 63% of cedar trees contained internal rot, and the likelihood of decay increased with the diameter. Evidence of growth thinning reveals irregular pulses of canopy disturbance with low to moderate severity (Fraver et al., 2020). A study by Johnson et al. (2022) compared seed biomass, morphology, seed set, and similarity between two natural populations and three additional seed sources of *Thuja occidentalis* L. in Chicago, Illinois. Differences between the two adjacent sites indicate that Trout Park (Site 1) is disproportionately affected by environmental factors (e.g., road salt and altered hydrology) compared to Chicago Elementary School (Site 2), potentially affecting the growth and reproduction of *T. occidentalis* in these urban populations. A large dataset of 842 *T. occidentalis* was randomly collected from favorable (cliff-top plateau) and unfavorable (cliff-side) habitats to determine whether trunk streaks were directly or indirectly related to age, growth rate, and habitat (Matthes et al., 2002). The earlywood tracheids of older trees had the most enormous pits, indicating that these trees are more sensitive to cavitation-related scarcity of water than younger trees and supporting the hypothesis that tracheid cavitation is involved in trunk streak formation (Caruntu et al., 2020). Rooting percentage was significantly influenced by the cultivar and type of cuttings, while the root length was significantly influenced by the type of cuttings. A higher average percentage of rooted cuttings was recorded in the cultivar 'Smaragd' compared to the cultivar 'Columna' (Karlović et al., 2019).

Thuja occidentalis was introduced from North America. Northern white cedar, belonging to the *Cupressacae* family, is a well-known medicinal and ornamental plant. *Thuja occidentalis*, commonly known as American Arbor vitae or white cedar, is indigenous to eastern North America. It has medicinal properties. The plant was first recognized as a cure by native Indians in Canada during a 16^{th}-century voyage and was found to prove effective in the treatment of weakness from scurvy (Adhikary, 2020). In the countries of North America, the species is found in the wild.

Research on the development of other types of ornamental plants is conducted in the presented region. The peculiarities of under tree microclimate of *Juniperus L.* shrubs are presented. The studied plants were registered within the municipal area of the city of Lviv and its green belt. Temperature and humidity conditions of the under-tree space in summer and winter periods were studied in detail. A comparison of the indices of microclimate under the shrubs and the adjacent open area was carried out (Shuplat and Popovich, 2016).

Morphological features of shrubby stunted and stunted species and forms of junipers make it possible to form a sub-tent phytoclimate in the urbogenic

conditions of the city of Lviv with increased air dryness and temperatures, providing favorable temperature and humidity regimes of air and soil for plants, contributing to the spatial development of bushes, increasing their vitality and decorativeness. The obtained microclimatic data on cloudy and sunny days are characterized by a greater difference in the values of air and soil temperature indicators than on cloudy days (Shuplat and Popovich, 2016).

Material and Methods

The aim was to identify the available cultivars of northern white cedar (*Thuja occidentalis* L.) available in the landscaping and ornamental nurseries of Lviv, determine their shape and color diversity, and evaluate their esthetics.

A route-visual method was used for identifying ornamental forms in landscaping and nurseries (Belochkina, 2006; Kycheryavyi and Kycheryavyi, 2019). The characteristic features of the plants were recorded: the shape and density of the crown, the nature of branching, the color of the needles, and the peculiarities of seed production. A scoring system based on existing methodological approaches was used to evaluate the decorative characteristics of the plants (Shlapak et al., 2014).

The research was conducted during 2015–2022 in the territory of the Lviv region in Ukraine. Figure 1 shows the layout of the studied objects.

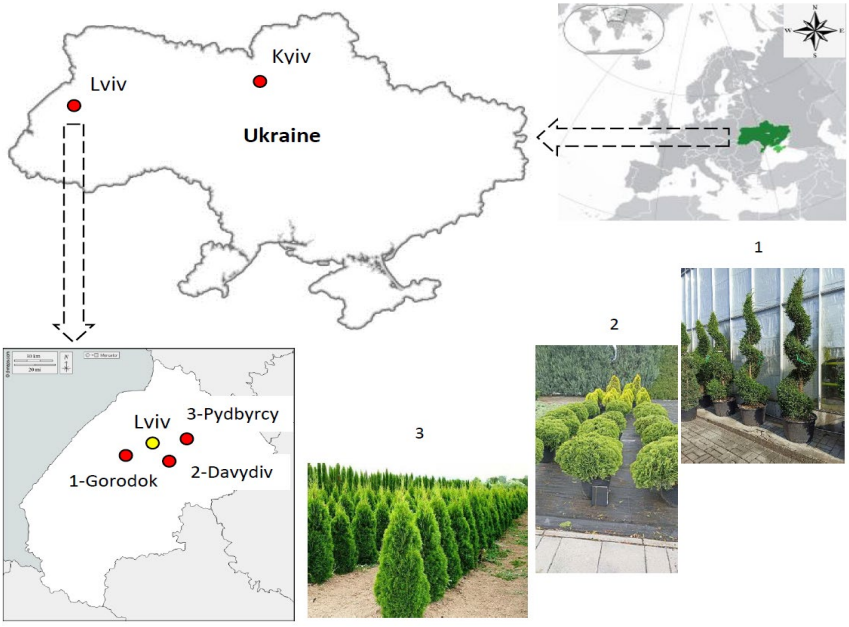

Figure 1. The location scheme of the studied objects within the Lviv region.

Results and Discussion

The wide distribution of ornamental forms of northern white cedar in modern landscaping and nurseries in Lviv is a confirmation of its high esthetics. In general, the decorative qualities of plants are revealed during their development and depend on age, season, location in compositions, and landscaping elements. In this regard, we decided to determine the decorative qualities of its cultivars before designing landscape elements with ornamental forms of northern white cedar. A three-point scale for esthetic evaluation of plants was developed. It takes into account the most characteristic features: crown shape, needle color, branching pattern, seed production, and crown density (Table 1). Forty-three cultivars of northern white cedar were tested by this method (Table 2).

Table 1. The esthetic evaluation of northern white cedar cultivars (point scale).

Crown shape								
Columnar weeping		Pyramidal	Ovoid		Spherical	Pulvinate		Weeping
3		2	2		3	2		3
Needle color								
Dark green year-round	Light green year-round	Dark green during the growing season. Color changes in winter	Light green during the growing season. Color changes in winter	Bluish green	Gray-green	Brownish-green	Golden-yellow	White-striped
3	3	2	2	3	2	1	3	3
Branching pattern								
Thin			Medium			Thick		
3			2			1		
Crown density								
Dense			Medium density			Loose		
3			2			1		
Seed production, points								
5		4	3		2		1	
1		1	2		2		3	

Table 2. Summarized data on the esthetic evaluation of the ornamental forms of northern white cedar.

No.	Ornamental form	Biomorph / Height, m	Crown shape, point	Needle color, point	Branching pattern, point	Density, point	Total
1.	*'Albospicata'*	B / 5.0	P, 2	Gr., 3	2	2	9
2.	*'Aureavarigiata'*	T / 5.0	P, 2	G, 3	2	2	9
3.	*'Aurea'*	T / 10.0	P, 2	G, 3	2	2	9
4.	*'Amberglow'*	B / 2.0	G, 3	G, 3	3	3	12
5.	*'Aureospicata'*	B / 5.0	G, 2	G, 3	2	2	9
6.	*'Aniek'*	B / 2.0	O, 3	Y, 3	3	3	12
7.	*'Bodmeri'*	T / 5.0	P, 2	Gr., 3	1	1	8
8.	*'Brabant'*	T / 5.0	O, 3	Gr., 3	3	3	12
9.	*'Columna'*	T / 15.0	Col., 3	Gr., 3	3	3	12
10.	*'Danica'*	B / 1.5	G, 3	Gr., 3	3	3	12
11.	*'Douglasii Pyramidalis'*	T / 10.0	P, 3	Gr., 2	3	3	11
12.	*'Ellwagneriana'*	B / 5.0	P, 2	Gr., 2	2	2	8
13.	*'Ellwagneriana Aurea'*	B / 5.0	P, 2	G, 3,	2	1	8
14.	*'Ericoides'*	B / 3.0	P, 2	Gr., 1	2	3	8
15.	*'Fastigiata'*	T / 20.0	Col., 3	Gr., 3	2	3	11
16.	*'Filiformis'*	B / 3.0	P, 3	Gr., 2.	2	2	9
17.	*'Globosa'*	B / 2.5	G, 3	Gr., 2	3	3	11
18.	*'Globosa Nana'*	B / 0.6	G, 3	Gr., 3	3	3	12
19.	*'Golden Anne'*	B / 2.0	P, 3	G, 2	3	3	11
20.	*'Golden globe'*	B / 2.0	G, 3	G, 3	3	3	12
21.	*'Hoseri'*	B / 2.0	Pul., 3	Gr., 3	2	3	11
22.	*'Hoveyi'*	B / 2.0	Pul., 2	Gr., 2	3	3	10

Continuation of Table 2. Summarized data on the esthetic evaluation of the ornamental forms of northern white cedar.

23.	*'Holmstrup'*	T / 10.0	Col., 3	Gr., 3	2	2	10
24.	*'Jantar'*	T / 5.0	Con., 3	G, 3	3	3	12
25.	*'Little Gem'*	B / 2.0	Pul., 3	Gr., 2	2	2	9
26.	*'Magic Moment'*	B / 2.0	Col., 3	Gr., 2	3	2	10
27.	*'Malonyana'*	B / 15.0	Col., 3	Gr., 3	3	2	11
28.	*'Mr. Bowling'*	B / 2.0	G, 3	Gr., 3	3	3	12
29.	*'Myriam'*	B / 2.0	G, 3	Gr., 3	3	3	12
30.	*'Ohlendorfii'*	B / 10.5	O, 2	Gr., 3	2	2	9
31.	*'Pyramidalis'*	T / 20.0	P, 3	Gr, 3	3	3	12
32.	*'Pendula'*	T / 5.0	P, 3	G, 3	3	3	12
33.	*'Rheingold'*	B / 5.0	O, 3	G, 3	3	3	12
34.	*'Smaragd'*	T / 10.0	Col., 3	Gr., 3	3	3	12
35.	*'Spiralis'*	T / 10.0	Col., 3	Gr., 3	3	3	12
36.	*'Sunkist'*	T / 5.0	Col., 3	G, 3	3	3	12
37.	*'Umbraculifera'*	B / 1.5	Pul., 3	Gr., 2	3	3	11
38.	*'Variegata'*	T / 10.0	P, 3	G, 3	3	3	12
39.	*'Wagnerianna'*	B / 5.0	O, 2	Gr., 2	2	2	8
40.	*'Wareana'*	B / 5.0	P, 3	Gr., 2	2	2	9
41.	*'Wareana Lutescens'*	B / 5.0	P, 3	G, 3	2	3	11
42.	*'Woodwardii'*	B / 3.0	G, 3	Gr., 3	3	3	12
43.	*'Yellow Ribbon'*	T / 3.0	Con., 3	G, 3	3	3	12

Remarks: T – tree, B – bush, Col. – columnar, Con. – conical, P – pyramidal, G – globular, O – obovate, Pul. – pulvinate, G – golden, Gr. – green, Y – yellow

The highest number of points (12) was given to 46.4% of the ornamental forms, 11 points to 16.3%, 10 points to 9.3%, and 8 points to 11.6%. Plants with a distinct regular crown shape, primarily columnar and spherical, received a high number of points. They can be widely used in hedges and container plantings, due to their good plasticity. In general, the vast majority (88.4%) of cultivars were characterized by a high decorative index. This indicator is related to the demand and professional selection made by the business. After all, the plant market is supplied with ornamental plants that meet the esthetic tastes of customers.

Design of elements and compositions

The existing compositional solutions of various landscaping elements using analyzed ornamental forms of northern white cedar indicate the lack of standard algorithms developed in forestation (schemes for mixing species). In any case, in modern landscaping, control over these decisions is given to gardening practitioners, who create rather primitive compositions at their own discretion. Because practitioners are unaware of the basic laws and principles of composition, and the richness of forms in dendroflora, they impoverish their psycho-emotional perception when creating plantings. The variants of application of gardening elements in garden and park compositions are suggested.

Solitary

Several possible variants of cultivars of northern white cedar were investigated as solitaries, including both trees and shrubs (Tables 3, 4). We took into account the wind resistance of the plants as well as their high esthetic and sanitary-hygienic properties.

Table 3. Woody forms of northern white cedar as solitaries.

Compositional element	Forms		
	Columnar	Pyramidal golden yellow	Pyramidal
Center solitary	9,15,23,24,27,34,35,36	11	-
Foreground solitary (lawn solitary)	7,26	31	41

In parks, in open spaces, we recommend planting solitary trees with tall columnar (*'Fastigiata', 'Columna', 'Spiralis'*) and pyramidal (*'Pyramidalis', 'Douglasii Pyramidalis'*) shapes in the middle of the lawn or against the background of the forest edge opposite the viewpoint, with a distance of 3–4 m from it, where the tree is a transition from the massif to the lawn. We recommend

introducing golden-yellow forms to contrast with the dark-leaved trees (*'Aurea', 'Aureospicata', 'Ellwagneriana Aurea', 'Wareana Lutescens'*).

We suggest planting the foreground solitaries separately in a place convenient for viewing. We recommend planting them in city squares and small gardens. Low (up to 5 m) columnar, conical, and pyramidal forms of trees and shrubs should be used for planting. The optimal distance from the viewpoint to the exposed solitaries should be two to five times the height of the tree.

Table 4. Shrub forms of northern white cedar as solitaries.

Compositional element	Columnar	Pyramidal	Pyramidal golden yellow	Oval-ovoid	Spherical	Pulvinate	Weeping	Heather-like
Foreground solitary of the group	33	13	2, 3, 4, 13, 33, 39, 43	6	10, 17, 19, 21, 22, 28, 29, 42	25	16	14
Solitary in the branching of the garden path	26, 33	13, 39	13,4	40	17, 25, 42	-	16	14
Solitary near an architectural object	26	13	-	-	17, 21, 22	-	16	14

We also suggest using shrub forms as solitaries, for accentuating the foreground of a group, branching paths, or decorating an architectural construction.

Alley

Columnar and pyramidal forms, having a vertical impact on the open space, can be used to form compositional axes, accentuate the perimeter of the garden, and create a play of light and shadow.

Alleys with a simple rhythm, when the same shape is repeated at the same distance (*'Fastigiata'*, *'Columna'*, *'Malonyana'*, etc.), are recommended for the entrance areas of parks, squares, and gardens (Table 5).

Table 5. Tree and shrub ornamental forms of northern white cedar in alleys with a simple and complex rhythm.

Compositional element	Possible options
Alley with a simple rhythm (1-1-1…)	9-9-9; 11-11-11; 15-15-15; 27-27-27. 36-36-36; 40-40-40;
Alley with a complex rhythm (1-2-1-2…)	3-17-9; 32-20-32; 35-18-35. 36-29-36; 23-22-23
Alley with a complex rhythm (1-2-2-1-2-2…)	3-10-10-9; 11-12-12-11; 27-28-28-27. 34-30-30-34

The play of light and shadow on the alley with a complex rhythm is represented by: *'Fastigiata' – 'Hoseri' – 'Fastigiata'* or *'Columna' – 'Globosa' – 'Globosa' – 'Columna'*, etc. Taking into account the possibility of crown growth and competition for light, we recommend planting at a distance of 2.5–3.5 m.

Green wall

Columnar, pyramidal and oval-ovoid shapes (height from 3.0 m to 3.5 m and higher) play a protective role (wind, dust, noise, snow), and also form a fence for various objects – gardens and squares, children's, sports, and household playgrounds, internal boundaries of gardens and allotments. Such walls can be used for visual isolation of unplastered walls and walls with unattractive views.

For green walls, we recommend columnar, pyramidal, and oval-ovoid shapes (Table 6).

Table 6. Woody forms of northern white cedar recommended for green walls.

Compositional element	Shapes			
	Columnar	Pyramidal	Oval-ovoid	Pyramidal golden-yellow
Perimeter for a large area (park)	9; 15; 23; 27	11; 32	40	1; 39
Perimeter for a small area (garden, square)	35; 33; 36;	13; 14; 20; 40	8	14;
Visual fencing	9; 15; 23; 27	11; 32	8	2; 3; 4; 14;

Walls using forms with golden yellow coloring are original. However, mixing green and golden-yellow colors should be avoided. The distance in the row for columnar forms is 0.5 m, for pyramidal – 1.0 m.

Hedges

We recommend using borders, low and medium hedges to frame parterres, flower beds, front gardens, lawns, and paths. Low and medium-height trees and shrubs are suggested (Table 7).

We recommend planting the plants in trenches 50–60 cm deep in a single row (at a distance of 25–35 cm) or in several rows in a checkerboard pattern (spacing in the row 30–40 cm, between rows 20–35 cm).

The topiary work should be carried out at least 4 times during the summer on straight trapezoidal and semi-oval profiles. Despite the simplicity of the rectangular profile, we do not recommend it. Firstly, it forms rigid contours in landscape parks that are not characteristic of a free layout. Secondly, in such hedges, the down part receives less light, which leads to the death of small aerial branches and exposure of the trunks.

Table 7. Forms of northern white cedar recommended for trimmed hedges.

Landscaping elements	Shapes					
	Columnar	Pyramidal	Ovoid	Spherical	Pulvinate	Golden-yellow
Border	23	-	6;12; 16	10; 17; 19	25	5
Low hedge	26	-	6; 12; 30	28; 42; 29	28	20
Medium hedge	35; 36	1; 3	6; 12	-	16	43

Bosquets

Medium-sized hedges in the form of a closed square, rectangle, circle, or other regular shape that delimit a place of rest or exercise, children's playgrounds and sports areas are called bosquets. We recommend upright tree and shrub forms with a dense crown for their creation. The height of the bosquet is 1–2 m. Tending of bosquets (topiary work) is the same as for ordinary hedges (at least 4 times during the summer).

Topiary

Topiaries are a variety of trimmed figures as a tribute to the distant past (this name, according to legend, comes from the name of the ancient Roman gardener Topiarius, a contemporary of Emperor Hadrian [117–138]) (Table 8).

Table 8. Forms of northern white cedar recommended for different types of topiaries.

Compositional elements	Columnar	Pyramidal	Oval-ovoid	Spherical	Pulvinate
Sphere	-	-	-	5; 6; 10; 17; 18; 19; 20; 21; 29; 33	38
Cube	9; 15; 23; 24; 26;34; 35; 36	-	-	-//-	-//-
Cone	-//-	-	40	-//-	-
Pyramid	-//-	32; 39; 41	-	-	-
Drop	35	-	-	18	
Sphere on the stem	9; 15; 23; 24; 26;34; 35; 36	-	-	-	-
Spiral	-//-	-	-	-	-
Bon-bom	-//-	-	-	-	-
Digit	34	-	22; 33	-	38
Letter	34		22; 33	-	38
Animal	-	-	-	17; 18; 19; 20	-

Topiaries are widespread in contemporary landscape gardening art. We have analyzed the modern trimmed decorative forms and proposed plants with good plastic qualities for topiaries.

For topiaries of various decorative and artistic forms, we recommend plants with dense crowns and thin branches pressed against the axial trunk.

Rocky gardens

Ornamental forms of northern white cedar are well represented in rocky gardens today, including spherical and pulvinate forms.

Based on the prospects for this type of landscape gardening, we have proposed ornamental forms suited to the sizes of the gardens (Table 9).

Table 9. Ornamental forms of northern white cedar recommended for rocky gardens.

Rocky garden category	Dimensions, length, m	Recommended ornamental forms
Very small	Up to 5	10, 18, 25
Small	5–15	5, 6, 10, 12, 17, 21, 22, 24, 25
Medium	15–30	5, 6, 10, 12, 14, 16, 17, 18, 19, 20, 21, 24, 25, 28, 29, 31, 39, 43
Big	30–60	1–43
Large	More than 60	1–43

Taking into consideration the scale of the composition, we have selected large-sized plants for small and medium-sized rocky gardens, whereas all 43 cultivars may be used in large and very large gardens. It is recommended to arrange them according to the suggestions of the models.

Conclusion

The high potential of ornamental forms of northern white cedar (88.4% of 43 identified in landscaping and nurseries) makes it possible to increase the estheticization level of green spaces.

Proposals for garden and park elements for decorative compositions (group, solitary, alley, green wall, hedge, bosquet, topiary, rockslide) allow the rational use of planting material, improve esthetics, and increase the durability of plants.

The maximum points (12) were given to 46.4% of the ornamental forms, 11 – 16.3%, 10 – 9.3%, and 8 – 11.6%.

To increase the esthetic value of green spaces with ornamental forms of northern white cedar, it is recommended to use the developed models of compositional grouping based on symmetry, contrast, color, silhouette, crown size, and density.

We have developed possible variants for the use of ornamental forms of northern white cedar as solitaries – 12, alleys with a simple and complex rhythm – 20, green walls – 20, trimmed hedges – 19, topiary – 27, rocky gardens – 43.

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ESTETSKA OCENA UKRASNIH OBLIKA SEVERNOG BELOG KEDRA (*THUJA OCCIDENTALIS* L.) I NJIHOVA UPOTREBA U VRTNIM I PARKOVSKIM KOMPOZICIJAMA

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R e z i m e

Severni beli kedar (*Thuja occidentalis L.*) je u svetu zastupljen sa oko 200 ukrasnih oblika, od kojih su 43 otkrivena i opisana u pejzažnom uređenju i rasadnicima Lavova. Prve plantaže u Lavovu datiraju iz dvadesetih godina 20. veka. Uveo ih je profesor Medicinskog instituta T. Vilčinski, koji je doneo reznice iz rasadnika u Kurniku (Poljska). Dekorativne osobine ovih sorti utvrđivane su na osnovu karakterističnih osobina i ocenjivane bodovima: oblik i gustina krune, boja iglica, način grananja i osobine plodonošenja. Ove dekorativne osobine bile su osnova za izgradnju različitih elemenata vrtnih i parkovskih kompozicija pomoću traka, aleja, zelenih zidova, živih ograda, šumarka, topijara, kamenih vrtova. Najveći broj poena (12) dobilo je 46,4% ornamentalnih formi, 11 – 16,3%, 10 – 9,3% i 8 – 11,6%. Visoke ocene su dobile sorte sa izraženim pravilnim oblikom krune. Predložene su opcije prostornog rasporeda pojedinačnih oblika i preporučena je udaljenost između mesta za sedenje. Uzimajući u obzir visoku plastičnost sorti i sposobnost oblikovanja krune, preporučene su varijante ošišanih živih ograda i topijara. Predloženo je nekoliko modela na osnovu rasporeda grupa ukrasnih biljaka na osnovu njihove simetrije, silueta, usklađenosti, kontrasta, boja, gustine i veličine. Za svaku varijantu odabrane su sorte i predložen je njihov grupni raspored.

Ključne reči: severni beli kedar (*Thuja occidentalis* L.), ukrasni oblik, element pejzaža, kompozicija, estetska ocena.

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SERBIAN BLACK TRUFFLE *TUBER AESTIVUM*: MICROBIOTA AND EFFECTS OF DIFFERENT FREEZING REGIMES ON VOLATILE AROMA COMPOUNDS DURING STORAGE

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Abstract: The use of truffles in food is based mainly on the addition of artificial flavors, aiming to achieve an intense aroma in the products. As truffle is a natural product with nutritional and functional properties, it is important to find an optimal method for truffle storage. As the microbiota contribute to truffle aroma, the bacterial and yeast compositions in the rhizosphere and fruiting body of the truffle and the impact of different freezing methods on the volatile profile of the truffle *Tuber aestivum* during 90 days of the storage were determined. Bacteria and yeasts isolates were identified using 16s rRNA and 18s rRNA. The effect of freezing truffles at -20°C and -80°C with and without previous dipping in liquid N_2 on the volatile compounds was observed using GC/MS. The results demonstrated that the isolated bacteria belonged to the phylum *Proteobacteria*, *Firmicutes* and *Actinobacteria*, and the identified species mainly belonged to *Firmicutes,* genus *Bacillus* sp. Isolated yeasts were identified as *Cryptococcus* sp., *Debaromyces hanseinii*, *Candida fermentati* and *Rhodotorula mucilaginosa*. The GC/MS analysis revealed that frozen truffle samples were richer in the compounds 2-butanone, 2-methyl-butanal, methanethiol and 2-butanol after freezing or during storage. The content of DMS, acetaldehyde, 3-octanone, ethanol, and 2-methyl-1-propanol significantly decreased immediately after freezing. Overall, the gained results indicated that freezing of truffles as a preservation method had profound effects on the volatile compounds, while previous dipping in liquid N_2 showed no significant impact on the volatile profile of truffle *Tuber aestivum*.

Key word: aroma compounds, bacteria, fungi, preservation, yeast.

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Introduction

Truffles are ectomycorrhizal fungi, which belong to the order *Pezizales*, with representatives within *Ascomycota*, *Basidiomycota* and *Zygomycota* (Bonito et al., 2013). Many species and genera are attractive for human consumption, but more attention has been paid to *Tuber* species, especially *Tuber aestivum* Vittad. (Perlińska-Lenart et al., 2020). This truffle species exhibits a much wider distribution than all the others and has been found in almost all European countries, as well as in North Africa (Morocco) and China (Lin et al., 2019; Randazzo et al., 2002; Stobbe et al., 2013; Zambonelli, 2012). Since they develop their fruiting bodies underground (Splivallo et al., 2011), mycorrhizae are established within the roots of *Gymnospermi* and *Angiospermi*, and usually form irregular round fruiting bodies with fleshy consistency or ascocarps (Zambonelli, 2012).

Bacterial communities isolated from truffle ascocarps, their potential participation in nutrition of fruiting bodies, protection from parasitic microorganisms present in the soil, or decomposition of the ascocarps themselves have been the subject of numerous studies. During different stages of development, truffles form symbiotic interactions with bacteria (Archaea and Eubacteria) (Antony-Babu et al., 2014; Barbieri et al., 2007; Gryndler et al., 2013), fungi (yeasts and filamentous fungi) (Buzzini et al., 2005), and viruses (Stielow and Menzel, 2010). Previous studies on the bacterial community of truffles indicate that the surface (peridium) and inner tissues (gleba) are colonized by complex bacterial communities composed of a few hundreds of species, such as *Pseudomonas*, *Bacteroides* and Gram positive bacteria, which dominate in the culturable bacterial communities that can reach up to 10^7–10^8 cells/gram of truffle (Barbieri et al., 2007; Sbrana et al., 2002; Vahdatzadeh et al., 2015). Furthermore, truffle fruiting bodies harbor a diverse microbial community of bacteria, with culture-dependent and culture-independent methods showing that truffles are colonized by *Proteobacteria*, *Bacteroidetes*, *Firmicutes*, and *Actinobacteria* (Perlińska-Lenart et al., 2020; Splivallo et al., 2015; Vahdatzadeh et al., 2019). Moreover, yeasts (Buzzini et al., 2005) and filamentous fungi (Pacioni et al., 2007) also represent the part of the truffle microbiota.

The quality of truffles is determined by their specific aromas, which could be different among truffle species and are also responsible for their high economic value all over the world. Today, more than 200 volatile compounds have been isolated and identified from truffle fruiting bodies. Sampling techniques such as direct solvent extraction (DSE), static headspace (SHS), purge and trap (PT), dynamic headspace (DHS), HS-solid phase microextraction (HP-SME) and gas chromatography coupled with mass spectrometry (GC/MS), gas chromatography coupled with flame ionization detector (GC-FID), etc. were used. Most of the truffle volatile compounds were identified as fatty acids, terpenoids, aromatic

compounds, and sulphur-containing compounds (Mustafa et al., 2020). Moreover, studies have shown that many aspects had an influence on the volatile profile of truffles, such as the geographic origin of truffles, different storage conditions, and microbiota (Buzzini et al., 2005; Vahdatzadeh et al., 2019). Recent research on the truffle microbiota indicates that different bacteria are involved in the distinct aroma of truffles through the production of aromatic volatile compounds (Splivallo et al., 2015). Furthermore, correlations between the changes in truffle volatile profiles and specific bacterial classes were obtained during *T. aestivum* storage (Vahdatzadeh et al., 2019). Nonetheless, some studies showed that yeasts isolated from the ascocarps of truffle species can produce volatile organic compounds, indicating that the unique aromas of truffles might also be the result of the yeast community composition (Splivallo et al., 2011).

Freezing is one of the most important methods for retaining food quality during long-term storage. Freezing truffles at a temperature of -20°C and -80°C was among the methods that led to a decrease in enzymatic reactions, preserving the aromatic substances of truffles (Culleré et al., 2013). Saltarelli et al. (2008) found that truffles retained their biochemical and microbiological characteristics during a short storage period at 4°C. A study by Al-Ruqaie (2006) revealed that the freezing process of two truffle species, *Terfezia claveryi* and *Terfezia hakizi*, could be more effective than drying, and concluded that the best preservation method in terms of truffle quality was blanching in a 4% NaCl solution and storing at -18°C. Furthermore, Jaworska and Bernás (2009) proposed a maximum storage period of 4 months for frozen, unblanched mushrooms. Moreover, Culleré et al. (2013) determined differences in the volatile profiles of fresh and frozen truffles.

The study of the bacterial and yeast microbiota and volatile profile of truffles, as well as an appropriate preservation method that preserves aromatic compounds during truffle storage, has been a great challenge in recent years. Therefore, the aim of this study was to determine the bacterial and yeast composition in the rhizosphere and fruiting body of the Serbian truffle, as well as the impact of freezing at different low temperatures with and without liquid N_2 on the volatile profile of the truffle *T. aestivum* by means of HS combined with GC-MS/FID during 90 days of the storage.

Material and Methods

Sample collection

Truffles were collected manually, with the help of a trained dog in northern Serbia, in the area of Srem, in broad-leaved Bojčin forest (44°73'N and 20°15'E), where linden (*Tilia tomentosa*) and oak (*Quercus robur*) predominate. The analyzed samples were black summer truffles (*T. aestivum*), collected randomly

(10 samples) in early August of 2018. The post-harvested truffles were wrapped in a highly absorbent blotting paper and transported in refrigerated conditions in insulated boxes with ice packs to the laboratory, where they were analyzed upon arrival, while the rest of the samples were frozen in appropriate conditions.

Isolation of different microorganisms from truffles

Fresh and undamaged ascocarps of *T. aestivum* (i.e., no signs of insect larval galleries, dry damage, or injuries from animals or harvesting equipment) were carefully washed with sterile water, the surface of the ascocarps was removed under sterile conditions, and the gleba and ascocarp surface were submerged in sterile saline (0.86% NaCl). For bacterial isolation, the samples of gleba and ascocarp surface were homogenized with vortex and appropriate dilutions were plated on Tryptone Soya Agar (TSA, Merck, Germany) and cetrimid agar for isolation of bacteria and *Pseudomonas*, respectively. Plates were incubated at 30°C for 48 h (Perlińska-Lenart et al., 2020). Single colonies were sub-cultured on appropriate agar to obtain pure cultures, which were stored at -80°C in medium with 20% of glycerol.

From the same material, YEPG agar was used to isolate yeasts (Zacchi et al., 2003). The plates were incubated at 25°C for 48 h. Then, distinct single colonies were picked out and sub-cultured a few times onto fresh YME agar plates to obtain pure cultures. The isolated yeasts were stored at -80°C in a suitable medium with 20% of glycerol.

DNA isolation and identification of microorganisms

Total DNA of bacterial isolates was isolated by using a commercial kit for DNA isolation (Quick-DNA Fungal/Bacterial Kit, Zymo Research) according to the manufacturer's instructions. For DNA isolation of yeast isolates, a commercial kit (DNA Isolation Kit for Cells and Tissues, Merck, Darmstadt*)* was used, according to the manufacturer's instructions. Identification of selected bacterial isolates was performed by sequencing the 16s rRNA gene using specific primers P1 16S and P2 16S, whereas identification of yeast isolates was performed using specific primers for 18s rRNA (NS1 and NS2), which are listed in Table 1. Taq DNA polymerase (Kapa Biosystems Inc., Boston, USA) was used to amplify the 16s rRNA and 18s rRNA genes using a PCR system thermal cycler (GeneAmp PCR System 2700, Applied Biosystems, Foster City, CA, USA). A 1% agarose gel at a constant voltage of 1–10 V/cm was used to verify the PCR products. PCR products were purified by using a Thermo Scientific PCR Purification Kit (Thermo Scientific, Lithuania) according to the manufacturer's instructions. The purified PCR products were sequenced by the Macrogen Sequencing Service (Macrogen

Europe, Amsterdam, Netherlands). The BLAST algorithm was used for analyzing of nucleotide sequences (Altschul et al., 1997) http://www.ncbi.nlm.nih.gov/BLAST.

Table 1. Sequences of primers.

Name	Nucleotide sequence	Reference
P1 16S	5' -GAGAGTTTGATCCTGGC-3'	Jovčić et al., 2009
P2 16S	5' -AGGAGGTGATCCAGCCG-3'	Jovčić et al., 2009
NS1	5'- GTAGTCATATGCTTGTCTC-3'	White et al., 1990
NS2	5'- GGCTGCTGGCACCAGACTTGC-3'	White et al., 1990

Freezing of truffle samples

Volatile compounds were detected in fresh samples, frozen at -20°C (Deep Freezer Samsung, Germany) and -80°C (Thermoscientic, HERA freeze, Germany), and in samples immersed in liquid nitrogen and stored at -20°C and -80°C. Aromatic compounds were determined immediately after freezing and after 30, 60, and 90 days of storage.

Truffle preparation for GC/MS

The fresh samples were ground in a laboratory mill and transferred to 20-ml HS vials. Frozen samples at -20 and -80°C were transferred to a cold room and analyzed. Samples were heated at 100°C for 20 min using the following program: incubation temperature: 100°C; incubation time: 1200 s; syringe temperature: 110°C. For the analysis of volatiles, 2000 μL of the generated vapor was extracted from the vial and injected directly into the gas chromatograph using a heated gas-tight syringe (Nikolić et al., 2018, 2019).

Gas chromatography-flame ionization detector (GC-FID) and gas chromatography-mass spectrometry (GC/MS) analyses

Gas chromatography (GC) and gas chromatography/mass spectrometry (GC-MS) analyses were performed using an Agilent 7890A GC equipped with an inert 5975C XL EI/CI mass spectrometer detector (MSD) and a flame ionization detector (FID) connected to the makeup through a 2-way capillary flow technology splitter. HP-Innowax fused silica capillary column (30 m × 0.32 mm × 0.25 μm). For the HS analyses, 2000 μL of the generated vapor was extracted from the vial and injected directly into the gas chromatograph using a heated gas-tight syringe (110°C). The column temperature program began at 35°C (5 min), then increased to 65°C at 3°C min^{-1}, and finally reached 225°C at a rate of 10°C min^{-1} and lasted for 4 min. Helium

was used as the carrier gas at 3.0 mL/min (constant flow mode). The sample was analyzed in the split less mode with a split ratio of 3:1. The injector temperature was 250 °C and the detector temperature was 300 °C. MS data were acquired in EI mode with a scan range of 40–550 *m/z*, a source temperature of 230 °C, and a quadrupole temperature of 150 °C, and the solvent delay was 3 minutes.

The components were identified based on their retention index and comparison with reference spectra (Wiley and NIST databases) as well as by the retention time locking (RTL) method and the RTL Adams database. The retention indices were determined experimentally using the standard method of Van Den Dool and Kratz (1963), involving retention times of *n*-alkanes injected after the sample under the same chromatographic conditions. The relative abundance of the *n*-alkanes was calculated from the signal intensities of the homologues in the GC-FID traces.

Results and Discussion

Identification of bacteria and yeasts

The results of identification of isolates from truffles, based on 16s rRNA sequencing, are presented in Table 2.

Table 2. Identified bacteria from truffles.

Isolates from ascocarps	% of abundance	Isolates from gleba	% of abundance
Bacillus sp.	56.3	*Arthrobacter* sp.	3.1
Microbacterium sp.	3.1	*Pseudomonas* sp.	9.3
Pseudomonas sp.	3.1	*Bacillus* sp.	6.2
Staphylococcus sp.	9.3	*Enterococcus* sp.	3.1
		Brevibacterium sp.	6.2

Totally 32 bacteria were isolated from truffle, out of which 23 isolates from ascocarps and 9 from gleba. The predominant genus of the selected isolates was *Bacillus* spp. (20 isolates), out of which 18 isolates were isolated from the ascocarp and 2 from the gleba. Other selected isolates were classified into 6 genera: *Pseudomonas* sp. (5 isolates), *Staphylococcus* sp. (2 isolates), *Brevibacterium* sp. (2 isolates), *Microbacterium* sp. (1 isolate), *Arthrobacter* sp. (1 isolate), and *Enterococcus* sp. (1 isolate).

In a previous study (Barbieri et al., 2005), the bacterial isolates of *T. borchii* were affiliated to the γ-*Proteobacteria* class, whereas some isolates belonged to the Bacteroidetes group and Gram positive bacteria, mostly *Bacilliaceae*. Furthermore,

sequence analysis of the bacterial isolates of *T. magnatum* identified *Proteobacteria* comprising α, β and γ subdivision, the *Bacteroidetes* group and the *Acinetobacter* and *Firmicutes* phyla (Barbieri et al., 2007). The difference in the obtained results could be explained by the study of Splivallo et al. (2015), where changes in the microbiota of truffles were noticed during storage. The bacteria isolated on the first day of storage mostly belong to the class of α-*Proteobacteria* and *β-Proteobacteria,* while after 6 days of storage, an increase in abundance of the *Firmicutes* was observed. The genus *Bacillus* belongs to the phylum *Firmicutes*, which was the dominant bacterial microbiota in our results. Moreover, differences were observed between truffle species in some bacterial microbiota, such as *Betaproteobacteria*, *Gammaproteobacteria*, and *Bacteroidetes,* which were more abundant in *T. borchii* than in *T. melanosporum* and *T. magnatum* (Vahdatzadeh et al., 2015). Moreover, a specific comparison of the gleba and peridium compartments during maturation showed that some phyla, such as *Firmicutes*, increased significantly in the gleba of fully mature ascocarps, while remaining stable in the peridium (Antony-Babu et al., 2014).

The present bacterial population could be related to the production of volatile compounds released by the interaction of truffles and bacteria. The absence or rare occurrence of microorganisms that form a specific aroma indicates that the aromatic compounds could be synthesized by the truffle itself or during its sexual phase (Splivallo et al., 2015).

Yeasts were isolated from the surface of the ascocarp and from the gleba and 4 out of 7 isolates were selected after microscopic examination. The yeast isolates were identified as *Cryptococcus* sp., *Debaromyces hanseinii*, *Candida fermentati* and *Rhodotorula mucilaginosa* based on 18s rRNA. Previous studies have revealed that the yeast microbiota consist of five species: *Cryptococcus albidus*, *Cryptococcus humicola*, *Rhodotorula mucilaginosa*, *Debaryomyces hansenii*, and *Saccharomyces paradoxus* (Zacchi et al., 2003). The yeasts *Candida saitoana*, *Deb. hansenii*, *Cryptococcus*, *Rhodotorula* and *Trichinosporun* were also isolated from *T. melanosporum* and *T. magnatum* (Buzzini et al., 2005). Interestingly, *Cryptococcus* sp., *R. mucilaginosa*, *D. hansenii*, and *Saccharomyces* sp. were also isolated from *T. melanosporum*, *T. magnatum*, or *T. aestivum* and might therefore be common to distinct truffle species (Vahdatzadeh et al., 2015). The obtained results indicate that the yeast microbiota might vary between different truffle species, as well as within the part of the tissue.

Volatile compounds in fresh and frozen samples of *T. aestivum*

The volatile organic compounds of fresh and frozen truffles during storage are shown in Tables 3, 4, 5, 6 and 7. The results of the volatile organic compounds presented represent the percentage of each compound in relation to the total content of volatile compounds detected. A total of 57 volatile compounds were detected, of

which 19 were selected as significant volatile compounds. The selected compounds detected in the truffle samples were sulphur-containing (3), aldehydes (4), ketones (4), alcohols (6) and furans (2).

The presence of sulphur compounds was determined in all truffle samples, regardless of the regime of freezing, with methanethiol and dimethyl sulphide (DMS) accounting for a significant share in relation to other sulphur compounds. Previous studies have shown that these compounds are essential components contributing to the aroma of black truffles in Europe and Asia (Chen et al., 2019; Culleré et al., 2010). The content of DMS decreased significantly on day 0 of freezing. Thereafter, the content was slightly reduced and maintained at a similar level in all freezing regimes until day 90 of storage. After freezing, the content of methanethiol decreased in all freezing regimes and a slight increase was observed after 90 days of storage, especially when freezing at -20°C. After 90 days of storage, the level of methanethiol was more than three times higher than on day 0, which could be explained by the decrease of other compounds in the overall aromatic composition.

Table 3. Volatile sulphur compounds in fresh and frozen truffles at appropriate temperatures.

No.	Volatile compounds	RI	Fresh truffle	Days	Relative share during storage (%)			
					-20°C	Liquid N_2 -20°C	-80°C	Liquid N_2 -80°C
1.	Methanethiol	666	0.4	0	0.2	0.2	0.2	0.2
				30	0.8	1.4	1.1	1.1
				60	0.5	0.8	1.0	0.9
				90	1.2	0.8	0.9	0.7
2.	Dimethyl sulphide	737	3.97	0	0.3	0.3	0.3	0.3
				30	0.2	0.2	0.1	0.3
				60	0.2	0.2	0.1	0.3
				90	0.2	0.1	0.1	0.3
3.	3-(Methylthio) propanal	1724	0.17	0	0.05	0.03	0.02	0.01
				30	/	/	/	/
				60	/	/	/	/
				90	/	/	/	/

Sulphur-containing compounds derive from the catabolism of L-methionine, their major precursor (Spinnler et al., 2001). Sulphur compounds constituted the largest group of volatile compounds (thiols, thioesters, sulphides, thioalcohols and thiophenones), but generally they have a very low olfactory detection limit,

although they represent major contributors to the final aroma of truffle fruiting bodies. Methanethiol, also known as methyl mercaptan, is a product of methionine degradation. It has an unpleasant odor and a low threshold value of approximately 1 ppb (Devos et al., 1990). Volatile compounds such as DMS were present in most truffle species and were probably formed as a result of the Ehrlich pathway. These sulphur compounds could be conditioned by microbial activity (Martin et al., 2010; Splivallo et al., 2011), whereas DMS could be formed as a result of yeast activity (Buzzini et al., 2005). Furthermore, sulphur compounds, 2-methylbutanal, 3-methylbutanal, 2- methylbutan-1-ol, 3-methylbutanol and oct-1-en-3-ol were detected in most truffle species (Vahdatzadeh et al., 2019).

Table 4. Volatile aldehydes in fresh and frozen truffles at appropriate temperatures.

No.	Volatile compounds	RI	Fresh truffle	Days	Relative share during storage (%)			
					-20°C	Liquid N_2 -20°C	-80°C	Liquid N_2 -80°C
1.	Acetaldehyde	686	6.32	0	3.0	3.0	3.0	3.0
				30	0.8	2.1	1.2	1.2
				60	1.7	1.8	2.8	2.7
				90	1.1	1.2	1.3	1.1
2.	2-methyl-1- propanal	816	5.26	0	11.1	11.1	11.1	11.1
				30	7.9	19.1	8.7	10.6
				60	7.6	7.2	13.4	12.8
				90	0.5	0.4	0.4	0.3
3.	2-methyl-butanal	889	9.55	0	10.6	10.6	10.6	10.6
				30	13.8	15.0	17.4	13.8
				60	14.7	13.2	15.8	14.7
				90	13.1	7.6	11.7	13.1
4.	3-methyl-butanal	911	7.43	0	7.6	7.6	7.6	7.6
				30	0.4	0.2	0.3	0.4
				60	0.4	0.8	0.3	0.4
				90	9.8	6.6	9.3	9.8

The main volatile aldehydes were detected in all truffle samples: acetaldehyde, 2-methyl-propanal, 2-methyl-butanal and 3-methyl-butanal. The concentration of acetaldehyde decreased two times immediately after freezing compared to fresh truffle, and it continued to decrease under all storage conditions. The content of 2-methyl-propanal doubled after freezing the truffle samples, and after 90 days of storage the relative concentration dropped approximately twenty times. Likewise, the concentration of 2-methyl-butanal in frozen truffles was higher on day 0 and increased slightly during storage, except for truffle samples treated with liquid nitrogen and stored at -20°C on day 90 of storage where concentration decreased. For the component 3-methyl-butanal, a slight increase was observed after 90 days of storage in all freezing regimes.

Aldehyde compounds such as 2-methylbutanal and 3-methylbutanal represent common constituents of all truffle aromas (Costa et al., 2015) and have been reported as predominant compounds in the volatile profile of truffles, with qualitative fluctuations depending upon variables such as truffle type and geographical origin (Fiecchi et al., 1967; Gioacchini et al., 2005).

Table 5. Volatile ketones in fresh and frozen truffles at appropriate temperatures.

No.	Volatile compounds	RI	Fresh truffle	Days	Relative share during storage (%)			
					-20°C	Liquid N_2 -20°C	-80°C	Liquid N_2 -80°C
1.	2-Butanone	880	1.67	0	48.7	48.7	48.7	48.7
				30	35.4	29.3	32.4	39.4
				60	39.8	43.3	24.0	39.8
				90	39.2	41.9	37.5	35.2
2.	2,3-Butanenedion	994	0.56	0	0.2	0.2	0.2	0.2
				30	0.1	0.2	0.1	0.1
				60	0.1	0.1	0.2	0.1
				90	0.1	0.1	0.1	0.1
3.	2,3-Pentanedion	1056	0.67	0	0.4	0.4	0.4	0.4
				30	0.1	0.2	0.2	0.1
				60	0.2	0.1	0.3	0.2
				90	0.2	0.1	0.1	0.2
4.	3-Octanon	1257	2.49	0	0.1	0.1	0.1	0.1
				30	0.2	0.2	0.3	0.3
				60	0.2	0.9	0.3	0.2
				90	0.6	0.8	0.7	0.7

Ketones are very important components of the aromatic profile of truffles, synthesized via the fatty acid *β*-oxidation pathway. The results show that the dominant ketones appeared to be: 2-butanone, as the predominant volatile ketone component, 2,3-butanedione, 2,3-pentanedione and 3-octanone. The concentration of 2-butanone increased multiple (24) times in all frozen truffle samples after freezing on day 0 and remained at the same level during 90 days, while the concentration of 2,3-butandione reduced twice on day 0 and was maintained during 90 days of storage. The compounds 2,3-pentanedione and 3-octanone slightly decreased after freezing and remained at the same level during the storage period. The component 2-butanone was detected in different *Tuber* species and was found in the fruiting bodies of *T. magnatum*. The results of the study by Strojnik et al. (2020) indicate that component 2-butanone is a quality marker, since samples with low amounts (<15%) had an unpleasant rotten odor, while samples with high amounts of 1-octen-3-ol (≤80%) had a pleasant odor. Likewise, similar ketone compounds were detected in the fresh truffle species *T. sinensi*, *T. sinoalbidum* and T. *sinoexcavatum* (Feng et al., 2019).

Table 6. Volatile alcohols in fresh and frozen truffles at appropriate temperatures.

No.	Volatile compounds	RI	Fresh truffle	Days	Relative share during storage (%)			
					-20°C	Liquid N_2 -20°C	-80°C	Liquid N_2 -80°C
1.	Ethanol	922	18.36	0	0.7	0.7	0.7	0.7
				30	1.2	1.3	1.5	1.2
				60	1.1	2.1	0.9	1.1
				90	0.8	2.6	1.4	0.8
2.	2-butanol	1023	17.90	0	14.7	14.7	14.7	14.7
				30	29.3	21.9	28.6	29.3
				60	29.2	24.1	34.0	29.2
				90	24.1	22.0	19.4	22.1
3.	1-propanol	1034	0.64	0	0.5	0.5	0.5	0.5
				30	1.1	1.6	0.7	1.1
				60	1.1	0.4	1.2	1.1
				90	0.4	0.5	0.4	0.4
4.	2-methyl-1-propanol	1089	1.02	0	0.5	0.5	0.5	0.5
				30	0.3	0.3	0.6	0.3
				60	0.4	0.6	0.5	0.4
				90	0.5	0.6	0.7	0.4
5.	2-methyl-1-butanol	1215	12.13	0	0.6	0.6	0.6	0.6
				30	1.1	0.2	2.2	0.3
				60	1.0	0.8	2.1	0.3
				90	1.3	0.8	2.0	0.8
6.	1-octen-3-ol	1467	2.49	0	2.1	2.1	2.1	2.1
				30	2.3	4.9	2.8	5.3
				60	2.4	2.4	2.7	4.6
				90	2.6	2.5	2.4	3.6

In terms of alcohol content, 6 volatile compounds were monitored during 90 days of storage: ethanol, 2-butanol, 1-propanol, 2-methyl-1-propanol, 2-methyl-1-butanol, 1-octen-3-ol. The obtained results showed that 2-butanol was the predominant volatile alcohol compound. The freezing regimes had no effect on the content of 2-butanol on day 0. The highest concentration of 2-butanol was obtained after 90 days of storage at -20°C, while the lowest concentration was noticed at -80°C. Nevertheless, the concentration of 2-butanol was higher by 50–70% in all freezing regimes, compared to fresh truffle. Similar results were gained for 1-octen-3-ol, which is believed to have a signalling effect on plants (Barbieri et al., 2005). The content of 1-octen-3-ol was reduced in all freezing regimes on day 0 comparing to fresh truffle (from 2.49% to 2.1%). After 30 days of storage, the

content of this volatile component had doubled under freezing conditions with liquid N_2 (-20°C and -80°C). The content of 1-octen-3-ol in frozen truffle with N_2 at -80°C was higher than in fresh truffle, while the content of this solution was similar to fresh truffle in all other regimes. Based on the obtained results, it is believed that freezing in liquid N_2 has a positive effect on 1-octen-3-ol, by increasing the content of this solution comparing to fresh truffle. This alcohol compound is generally the flavor of fresh mushrooms found in human breath and sweat (Rajarathnam and Shashirekha, 2003).

The concentration of 2-methyl-1-butanol decreased 20 times comparing to fresh truffle on day 0 and then it showed a growing trend for samples stored at -20°C and -80°C during 90 days of storage. The concentration of this component was higher in freezing regimes without liquid N_2 after 60 and 90 days of storage. The gained results indicate that freezing with liquid N_2 had a negative impact on the concentration of 2-methyl-1-butanol. This alcohol has been reported to be the major contributor to the final aroma of *T. melanosporum*. Furthermore, 2-methyl-1-butanol is derived from fatty acid catabolism and is well represented among truffle species. Moreover, alcohols can be oxidized to the corresponding component; 2-butanol could be transformed into 2-butanone and further transformation may lead to 2-methylpropanal, 3-methylpropanal or 2-butanal (March et al., 2006).

Table 7. Volatile furans in fresh and frozen truffles at appropriate temperatures.

No.	Volatile compounds	RI	Fresh truffle	Days	Relative share during storage (%)			
					-20°C	Liquid N_2 -20°C	-80°C	Liquid N_2 -80°C
1.	Furan	804	0.97	0	0.4	0.4	0.4	0.4
				30	0.4	0.8	0.5	0.5
				60	0.4	0.4	0.7	0.6
				90	0.1	0.1	0.1	0.1
2.	2-Furanmethanol	1673	0.2	0	0.2	0.2	0.2	0.2
				30	0.2	0.3	0.5	0.2
				60	0.2	0.6	0.2	0.1
				90	0.4	0.8	1.0	0.4

Studies have shown that eight carbon compounds contribute to the specific truffle aroma, since they impart a characteristic odor to the mushroom (Combet et al., 2006). Also, authors Splivallo et al. (2011) have detected alcohol compounds in several truffle species (*T. borchii* and *T. indicum*). Applied freezing regimes after 90 days contributed to the formation of truffle aroma, since the content of these alcohols increased during storage compared to the content of fresh truffles.

The obtained results show that the furan derivatives were furan and 2-furanmethanol. Of these two compounds, furan showed the greatest differences in concentration depending on the working conditions. After freezing on day 0, the concentration of furan decreased by 50% proportionately in all freezing regimes in regard to fresh truffle. The concentration of this compound after 90 days of storage was nine times lower than in fresh truffle.

The concentrations of 2-furanmethanol were unchanged in all freezing regimes on day 0. However, after 60 and 90 days of storage, the concentration of this compound in the regime with liquid N_2 at -20°C was three times higher than in fresh truffle. Furthermore, after 90 days of storage at -80°C, the concentration of 2-furanmethanol was five times higher than that of fresh truffle, while the concentration was two times higher when stored with liquid N_2 at -80°C, indicating that the freezing regime without liquid N_2 had a better effect on the concentration of this compound. Considering furans and furanones, Díaz et al. (2003) detected 2-pentylfuran in *T. escavatum*, *T. aestivum* and *T. melanosporum* via SPME. These compounds have a characteristic odor of fruits, green and earthy plants with a nuance similar to vegetables. Effenberger et al. (2019) found that 2 (5H) furanone had typical odor of caramel or burned sugar at low concentrations. Furaneol, often described as an attribute of "caramel" and "sweet" scent (Culleré et al., 2010; Ong and Acree, 1998), is associated with sweetness, along with γ-decalactone, which other authors have described as having "fruity" and "sweet" notes (de Andrade et al., 2017).

In this study, among 19 selected significant volatile compounds, 2-butanone and 2-butanol were quantitatively dominant and accounted for more than 50% of the total aroma in all frozen truffle samples.

Conclusion

Most of the identified bacteria belonged to the genus *Bacillus* sp., 56.3% from the ascocarp and 6.2% from the gleba. Other isolates belonged to *Pseudomonas* sp., *Staphylococcus* sp., *Brevibacterium* sp., *Microbacterium* sp., *Arthrobacter* sp., and *Enterococcus* sp. Yeast isolates were identified as *Cryptococcus* sp., *Debaromyces hanseinii*, *Candida fermentati* and *Rhodotorula mucilaginosa*. Further analysis of different samples from other regions in Serbia is required to better understand the microbiota of *T. aestivum.*

The GC/MS analysis revealed differences in the volatile profile of fresh and frozen truffles, both immediately after freezing and during 90 days of storage. The frozen samples were richer in the compounds 2-butanone, 2-methyl-butanal, methanethiol and 2-butanol after freezing or during storage, of which 2-butanone and 2-butanol quantitatively dominated and accounted for more than 50% of the total aroma in all frozen truffle samples. The content of DMS, acetaldehyde, 3-

octanone, ethanol, and 2-methyl-1-propanol significantly decreased immediately after freezing. Freezing could be a good preservation method regardless of the temperature of the freezing regime. Likewise, liquid N_2 generally showed no significant effect on the volatile profile of truffles.

Further analysis could provide better insight into the microbiota of truffles from other regions of Serbia, as well as the influence of bacterial and yeast microbiota on the volatile profile of truffles. Furthermore, the application of other techniques for the determination of volatile compounds, their quantification, as well as sensory analysis of fresh and frozen truffle samples, would provide significantly better insight into the changes in the relevant volatile compounds and the volatile profile of truffles.

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SRPSKI CRNI TARTUF *TUBER AESTIVUM*: MIKROBIOTA I UTICAJ RAZLIČITIH REŽIMA SMRZAVANJA NA ISPARLJIIVA AROMATIČNA JEDINJENJA TOKOM SKLADIŠTENJA

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R e z i m e

Primena tartufa u hrani se uglavnom zasniva na dodavanju veštačkih aroma, sa ciljem postizanja intenzivne arome tartufa u proizvodima. S obzirom na to da su tartufi proizvodi sa nutritivnim i funkcionalnim karakteristikama, važno je pronaći optimalan način za skladištenje tartufa. Pošto mikrobiota doprinosi aromi tartufa, sastav bakterija i kvasaca u rizosferi i plodonosnom telu srpskog tartufa, kao i uticaj različitih metoda smrzavanja na isparljiva jedinjenja tartufa *Tuber aestivum* ispitivan je tokom 90 dana skladištenja. Bakterije i kvasci su izolovani iz svežeg tartufa i izolati su identifikovani korišćenjem 16s rRNK i 18s rRNK. Uticaj smrzavanja tartufa na -20°C i -80°C sa i bez prethodnog potapanja u tečni N_2 na sadržaj isparljivih jedinjenja ispitivan je korišćenjem GC/MS. Rezultati ispitivanja su pokazali da izolovane bakterije pripadaju carstvu *Proteobacteria*, *Firmicutes* i *Actinobacteria*, pri čemu identifikovane vrste uglavnom pripadaju *Firmicutes,* rod *Bacillus* sp. Izolovani kvasci su identifikovani kao *Cryptococcus* sp., *Debaromyces hanseinii*, *Candida fermentati* i *Rhodotorula mucilaginosa*. Analiza GC/MS je ukazala na razlike u profilu isparljivih jedinjenja svežeg i smrznutog tartufa. Smrznuti uzorci su više sadržali komponente kao što su 2-butanon, 2-metil-butanal, metanetiol i 2-butanol nakon smrzavanja ili tokom skladištenja. Sadržaj DMS, acetaldehida, 3-octanona, etanola, 2-metil-1-propanola značajno se smanjio odmah nakon smrzavanja. Dobijeni rezultati ukazuju da smrzavanje tartufa kao metoda konzervacije ima značajan uticaj na isparljiva jedinjenja, pri čemu potapanje u tečni N_2 nije pokazalo značajan uticaj na isparljiva jedinjenja tartufa *Tuber aestivum*.

Ključne reči: aromatična jedinjenja, bakterije, gljive, konzervacija, kvasci.

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COMPETITIVENESS OF THE AGRI-FOOD SECTOR OF THE REPUBLIC OF SERBIA

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Abstract: The competitiveness of the agri-food products is one of the key factors for promoting export and economic development, especially in developing countries. Competitiveness can be influenced by increasing productivity, applying modern knowledge, innovating processing capacities and producing a high-quality final product with high added value. The agriculture of Serbia is very important for economic growth. In order to improve the competitive advantage of the agri-food sector in foreign markets, it is important to increase its efficiency by introducing modern technologies and approaches. The subject of the paper is to examine the most important export agri-food products of Serbia, as well as the most important markets for their exports. Using the RCA and ARCA indices, the comparative advantages of these products in trade were examined, which is the goal of the research. Multiple regression was then also used to examine the impact on exports. Although significant agri-food products achieve comparative advantages, the decline in their competitiveness has a negative impact on exports. Low pricing policies, as well as inadequate product quality cannot maintain competitiveness in the long run. The structure of the export of agricultural products is also unsatisfactory, bearing in mind that they are products with a low level of processing, and low added value. Therefore, it is necessary to invest in modern equipment, develop innovative approaches, such as organic production and the introduction of innovations to improve quality standards, differentiate products and create a final product with high added value, as important prerequisites for improving competitiveness and promoting the export of the agri-food sector.

Key words: competitiveness, agri-food sector, RCA, ARCA.

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Introduction

Agriculture of the Republic of Serbia (RS) is an important sector of the economy, which is characterized by a number of specificities of production, resources, and tradition, and for this very reason has the potential to improve its competitive position. Many strategic documents recognize agriculture as one of the priorities for economic growth. Increasing productivity and exports, especially foreign trade cooperation with countries with which there is already intensive cooperation in the field of agriculture, can contribute to this. The modern approach to competitiveness is evaluated based on costs and market share of the business entity, but also based on modern technologies and innovations that contribute to market positioning.

The development of agriculture in the RS is already to some extent oriented to the modernization and changes in the production structure, greater market orientation and improvement of productivity in agriculture. Restructuring of production and technology, productivity growth in agriculture, and greater competitiveness in national and international markets are increasingly guided by environmental, energy, and economic criteria (Cvetković and Petrović-Ranđelović, 2017). Productivity and efficiency are often cited as important factors of competitiveness. Within a market economy, competition contributes to economic growth. In practice, it is described as market rivalry to achieve advantages based on economic activity. In the age of globalization, the importance of competition is increasing in all sectors of the economy, including agriculture.

The subject of the paper is to examine the most important export products of the agri-food sector, as well as the most important markets for their export. The goal of the paper is to point out the comparative advantages of the RS in trade of agri-food products and their impact on exports.

Based on the subject and goal of the research, a research hypothesis was established:

H_1: The most competitive agricultural products have a statistically significant impact on the export markets of the most important trading partners of RS.

The competitiveness of a country is considered as its ability to produce goods or services that meet the conditions of international competition, and at the same time that this positively affects the growth of living standards (Tyson, 1992). Porter's concept of competitive advantage (Porter, 1998) uses the "diamond" model, which includes components such as supply and demand, microeconomic and management factors. The global competitiveness of agri-food exports can be viewed according to the concept of revealed comparative advantage, developed by Balassa (1965). Due to the globalization and liberalization of trade in the global market of agri-food products, the long-term sustainability of the export competitiveness of these products is very important. Although developed countries

tend to be less focused on agricultural products, they are more competitive in the long-term export of such final products, where the added value is higher. Long-term competitiveness can be the result of favorable trade conditions, because more economically developed countries operate in a more favorable environment. Accordingly, long-term competitive advantage is positively correlated with the level of economic development of the country (Bojnec and Fertő, 2017).

The structure of agricultural exports in developing countries is a significant factor affecting the competitiveness of agricultural products. As some developing countries have increased their productivity by cooperating with some foreign companies, the export competitiveness of agriculture has become an important issue (Huo, 2014).

The growth of international trade has given rise to new global actors with multiple comparative advantages. The development of international trade has conditioned the difference between developed countries, which are characterized by advantages in industrialization, and developing countries, which have a comparative advantage in labor-intensive sectors. At the same time, the agricultural sector as a labor-intensive activity and the export of agricultural products gives many developing countries a certain advantage in global competition (Escaith, 2010). Globalization of the world economy has indeed increased the competitiveness of agriculture in certain developing countries (Esterhuizen and Rooyen, 2006). Developed countries also stand out in terms of the competitiveness of the agricultural sector. There is a connection between agricultural competitiveness and economic development. At the same time, the instruments that have a potential impact on increasing agricultural competitiveness, especially in countries that are at a low level of development, include opportunities to apply innovations, measures to improve the transfer of knowledge, advisory services, investments and cooperation (Nowak and Kaminska, 2016).

Horizontal integrations as a form of cooperation are one of the factors that increase the competitiveness of the agricultural sector (Pawlak et al., 2019). For example, cooperatives are often a successful business model for organizing and uniting farmers, and contribute to their competitiveness (Nedanov and Žutinić, 2015). Also, the formation of agro-food clusters can increase the competitiveness and sustainability of agribusiness (Dorzhieva and Dugina, 2015), so there is a need for support for agricultural entities, which should consist of a series of state subsidies, benefits, etc. (Zakharchenko et al., 2020).

Economic entities in agriculture achieve competitiveness through adequate cost management, product differentiation, etc. Among the sources of competitiveness, the following should be highlighted: production technology, input costs, economies of scale, product quality, product differentiation, advertising, etc. Competitiveness is associated with efficient use of resources and factors of production, as well as structural changes. Competitiveness is a feature of economic

growth. The factors affecting the competitiveness of agricultural entities are numerous and can be classified in different ways. Some of them are based on resources in agriculture, such as labor, capital, and land. There are also factors related to intangible resources (such as relational capital, technological resources, know-how, management skills, etc.) or external conditions, such as climate, legal-political issues, etc. (Matyja, 2016).

Productivity can be increased through the introduction of innovations and modern technologies, which also has a positive impact on competitiveness. In addition, competitiveness can be improved by differentiated supply of high-quality agricultural products as well as by their processing. Competitiveness factors are numerous, and the focus is often on the product quality, that is, food safety, environmental protection, quality standards, etc. With the application of new knowledge, investments and modern technology, it is possible to rationally use the available resources, with the aim of increasing productivity and efficiency in production, thus increasing the competitiveness of agricultural and food products on the world market (Milojević et al., 2011).

A certain technical change in the agri-food sector may have a long-term impact on efficiency and competitiveness (Bezat-Jarzębowska and Rembisz, 2013). Innovation and research and development (R&D – Research & Development) are considered key factors of competitiveness in many segments of agricultural development, as they are part of the agribusiness system (Buccirossi et al., 2002). Namely, many contemporary problems in agriculture can be overcome with effective R&D programs and innovative solutions (Maienfisch and Stevenson, 2015). At the same time, public allocations for R&D, i.e., for increasing agricultural productivity and production, are of great importance (Khanal et al., 2017; Fuglie, 2018), so that economic entities that properly manage R&D can better take advantage of numerous opportunities for structural changes, development (Fuglie, 2016), and increased competitiveness.

Modern information and communication technologies, entrepreneurial skills, and cooperation between companies in the vertical supply chain are becoming increasingly important factors in the agri-food sector (Lans et al., 2004), which faces global challenges that must be addressed. Agri-food enterprises need to use modern technologies in production to face a very dynamic and complex environment (Fisher et al., 2000), because the application of these technologies and innovations, as well as the connection and coordination between the different levels of the agri-food sector, plays an important role in increasing its competitiveness (Streeter et al., 1991; Boehlje et al., 2011).

The international competitiveness of agri-food products is extremely important for the development of the agricultural sector and, consequently, for the use of modern information and communication technologies to coordinate and modernize activities within this sector (Streeter et al., 1991). Since the export of

agri-food products is the basis of agricultural development, it is important to increase the technological level of production, productivity and efficiency in this sector (Vlahović et al., 2011). Apart from the level of agricultural households, competitiveness also plays a significant role at the level of companies in the agricultural sector (Sachitra, 2017), where competitiveness should be based on the successful use of adequate technological and personnel solutions to increase the productivity and performance of the company, as important sources of growth competitiveness (Lukić, 2017).

Material and Methods

In general, the components that significantly determine competitiveness are the quantity and quality of goods exports. In practice, the export competitiveness is often determined by considering comparative advantages, as well as other factors. The Balassa index is often used to analyze the comparative advantages. This index is also known as the revealed comparative advantage index or RCA index (Revealed Comparative Advantage index) (Cvetković and Petrović-Ranđelović, 2017). The index is constructed in the following form (Balassa, 1965; Fischer, 2010; Lee, 2011):

$$RCA = \left(\frac{Xij}{\sum iX} ij\right) / \left(\frac{Xi}{\sum iXi}\right) \quad \text{за i= 1, 2 ...., I; j=1, 2, ..., J} \qquad (1)$$

where X_{ij} is the export of product j from country i, and $/\sum_i X_{ij}$ is the total export of country i; X_i is the world export of that product, and $/\sum_i X_i$ is the total world export (Equation 1). This index compares the market share of the country in the export market of product j ($X_{ij}/\sum_i X_{ij}$) with its market share in total world exports ($X_i/\sum_i X_i$). The country has a revealed comparative advantage if RCA > 1, and a comparative disadvantage if RCA < 1.

In addition to the RCA index, there are a number of alternative measures of comparative advantage. The index of an additional comparative advantage (Additive Revealed Comparative Advantage index – ARCA) is one of them. This index was proposed by Hoen and Oosterhaven (2006) and indicates that this index has greater stability compared to the RCA index. The ARCA index is calculated according to the following formula:

$$\text{ARCA}JA = \left(\frac{XJA}{XA}\right) - \left(\frac{XJREF}{XREF}\right) \qquad (2)$$

where X_J^A is the sector's j export to country A; X^A is the country's total exports; X_J^{REF} is the sector's j export of reference countries; X^{REF} is the total export of the reference countries of the sector (Equation 2). The index values range from -1 to +1. A country has a revealed comparative advantage if ARCA>0, and a revealed comparative disadvantage if ARCA<0. In addition, ARCA=0 if the share of exports of sector j in country A is equal to the share of reference countries.

Table 1. The definition of variables relevant to the competitiveness of the most important agri-food products for export in the RS.

Label	Definition
Dependent variables	
Exp	Total export of all products
Exp_EU (28)	Export to the European Union
Exp_Ger	Export to Germany
Exp_Ita	Export to Italy
Exp_B&H	Export to Bosnia and Herzegovina
Agricultural independent variables	
RCA_cor	RCA – corn (excluding seeds for sowing) – (6 digits – 100590)
RCA_fro_fru	RCA – frozen raspberries, blackberries and other fruits – (6 digits – 081120)
RCA_app	RCA – apples, fresh – (6 digits – 080810)
ARCA_cor	ARCA – corn (excluding seeds for sowing) – (6 digits – 100590)
ARCA_fro_fru	ARCA – frozen raspberries, blackberries and other fruits – (6 digits – 081120)
ARCA_app	ARCA – apples, fresh – (6 digits – 080810)

Source: Authors' research, based on ITC – Trade Map, 2020.

This research was conducted using RCA and ARCA indices to examine the competitiveness of the most important agri-food products for export in the RS (Table 1). The examined competitiveness was used to examine its importance for the total export of the RS and for the most important export markets using multiple regression.

Results and Discussion

The RCA index was used as part of the regression equations to examine the impact of competitiveness on the exports from the RS:

$$Exp,t = \alpha + \beta 1 RCA_{cor} i,t + \beta 2 RCA_{fro_{fru}} i,t + \beta 3 RCA_{app} i,t + \varepsilon i,t \quad (3)$$

$$Exp_{EU},t = \alpha + \beta 1 RCA_{cor} i,t + \beta 2 RCA_{fro_{fru}} i,t + \beta 3 RCA_{app} i,t + \varepsilon i,t \quad (4)$$

$$Exp_{Ger},t = \alpha + \beta 1 RCA_{cor} i,t + \beta 2 RCA_{fro_{fru}} i,t + \beta 3 RCA_{app} i,t + \varepsilon i,t \quad (5)$$

$$Exp_{Ita},t = \alpha + \beta 1 RCA_{cor} i,t + \beta 2 RCA_{fro_{fru}} i,t + \beta 3 RCA_{app} i,t + \varepsilon i,t \quad (6)$$

$$Exp\_B\&H,t = \alpha + \beta 1 RCA_{cor} i,t + \beta 2 RCA\_fro_{fru} i,t + \beta 3 RCA_{app} i,t + \varepsilon i,t \quad (7)$$

The robustness check of the data, i.e., the examination of the impact of the competitiveness of agricultural products on the dependent variables was carried out using the ARCA index.

$$Exp,t = \alpha + \beta 1 ARCA_{cor} i,t + \beta 2 ARCA_{fro_{fru}} i,t + \beta 3 ARCA_{app} i,t + \varepsilon i,t \quad (8)$$

$$Exp_{EU},t = \alpha + \beta 1 ARCA_{cori},t + \beta 2 ARCA_{fro_{frui}},t + \beta 3 ARCA_{appi},t + \varepsilon i,t \quad (9)$$

$$Exp_{Ger},t = \alpha + \beta 1ARCA_{cori},t + \beta 2ARCA_{fro_{frui}},t + \beta 3ARCA_{appi},t + \varepsilon i,t \quad (10)$$

$$Exp_{Ita},t = \alpha + \beta 1ARCA_{cori},t + \beta 2ARCA_{fro_{frui}},t + \beta 3ARCA_{appi},t + \varepsilon i,t \quad (11)$$

$$Exp\_B\&H,t = \alpha + \beta 1ARCA_{cor}i,t + \beta 2ARCA\_fro_{fru}i,t + \beta 3ARCA_{app}i,t + \varepsilon i,t \quad (12)$$

The most important export agri-food products of the RS are grain corn, fresh apples, frozen fruits (raspberries, blackberries, currants, etc.), of which the most important export is frozen raspberries without sugar (SORS, 2020). Corn belongs to the cereal sector (10), and this sector was the most important agricultural export sector, followed by sector 08 (edible fruits and nuts), where frozen fruits dominated exports, followed by fresh apples (ITC – Trade Map, 2020). Table 2 shows the competitiveness of the most important agri-food export sectors.

Table 2. Determining the competitiveness of the most important agricultural export sectors of the RS (cereals and edible fruits).

Year	RCA -10- Cereals	ARCA -10- Cereals	RCA -08- Edible fruits	ARCA -08- Edible fruits
2006	7.12	0.03	6.94	0.03
2007	3.72	0.01	7.67	0.03
2008	2.10	0.01	6.87	0.03
2009	6.16	0.03	6.93	0.03
2010	7.81	0.04	7.24	0.03
2011	7.28	0.04	7.84	0.03
2012	9.08	0.05	6.67	0.03
2013	5.08	0.03	6.20	0.03
2014	6.39	0.03	6.66	0.03
2015	5.73	0.03	6.90	0.04
2016	5.72	0.03	5.95	0.03
2017	3.86	0.02	5.79	0.03
2018	4.16	0.02	4.73	0.02
2019	5.13	0.02	4.57	0.02

Source: Authors' research, based on ITC – Trade Map, 2020.

Table 2 shows that both sectors (cereals and edible fruits) were competitive, considering the RCA and ARCA indices. Next, the competitiveness of the most important export agri-food products within each sector was examined (Table 3).

Table 3. Determining the competitiveness of the most exported agricultural products of the RS.

Year	Sector 10		Sector 08				Rate of change, 2006=100					
	Corn		Frozen fruits		Apples		Corn		Frozen fruits		Apples	
	RCA	ARCA	RCA	ARCA	RCA	ARCA	RCA	ARCA	RCA	ARCA	RCA	ARCA
2006	26.67	0.026	500.12	0.019	4.82	0.001	0.00	0.00	0.00	0.00	0.00	0.00
2007	5.64	0.006	426.14	0.019	6.52	0.002	-78.84	-75.16	-14.79	0.14	35.21	58.45
2008	5.63	0.007	339.63	0.019	3.24	0.001	-78.89	-72.16	-32.09	-3.57	-32.77	-36.12
2009	21.66	0.030	404.45	0.025	3.82	0.001	-18.76	14.94	-19.13	28.46	-20.73	-8.15
2010	22.53	0.030	397.09	0.019	11.30	0.004	-15.52	17.19	-20.60	-0.61	134.15	199.61
2011	21.25	0.035	397.56	0.018	13.77	0.005	-20.33	34.57	-20.51	-7.60	185.45	259.29
2012	26.56	0.046	392.80	0.016	9.04	0.003	-0.42	75.83	-21.46	-16.83	87.30	127.85
2013	6.48	0.009	314.49	0.016	8.36	0.003	-75.71	-64.44	-37.12	-17.83	73.34	121.61
2014	19.44	0.029	343.04	0.019	13.47	0.005	-27.10	10.44	-31.41	-3.19	179.24	251.72
2015	15.66	0.023	358.31	0.023	18.08	0.007	-41.28	-10.02	-28.36	19.76	274.70	410.87
2016	13.49	0.021	305.59	0.019	18.94	0.008	-49.41	-19.10	-38.90	-2.77	292.53	465.30
2017	10.20	0.014	312.56	0.016	17.26	0.007	-61.76	-44.98	-37.50	-18.96	257.82	384.86
2018	6.99	0.010	302.66	0.014	13.32	0.005	-73.78	-63.05	-39.48	-29.64	176.10	236.58
2019	14.11	0.023	303.28	0.014	16.17	0.006	-47.08	-11.01	-39.36	-28.16	235.17	294.45
average rates of change, 2006=100							-45.30	-15.92	-29.28	-6.22	144.42	212.79

Source: Authors' research, based on ITC – Trade Map, 2020.

The observed products (Table 3) are competitive, given that their RCA index is greater than 1. Considering competitiveness and according to the ARCA index, all observed products are also competitive given that the ARCA index is greater than 0 for all products. However, a decrease in competitiveness was observed for corn and frozen fruits, while an increase was observed for apples, with the average rate of change in competitiveness according to both the RCA and ARCA indices.

Since there was an increase in the export of the most important agricultural export products of the RS in the most important export markets of these products, Table 4 examines the competitiveness of these products in these markets, i.e., the competitiveness of corn in the most important export markets, namely Romania and Italy, the competitiveness of frozen fruits exported mainly to Germany and France, and the competitiveness of apples exported mainly to Russia and the United Arab Emirates (UAE).

From Table 4, it can be concluded that corn was competitive in both the Romanian and Italian markets, viewed according to both indices, i.e., according to the RCA index, which was greater than 1 in all cases, and to the ARCA index, which was greater than 0 everywhere. The same was true for frozen fruits, competitive in the German and French markets, considering both the RCA and ARCA indices, and for apples competitive in the market of Russia and the United Arab Emirates, also considering both the RCA and ARCA indices.

Table 4. The competitiveness of the most important agricultural export products from the RS in the most important markets

Year	RCA of corn		RCA of frozen fruits		RCA of apples		ARCA of corn		ARCA of frozen fruits		ARCA of apples	
	Romania	Italy	Germany	France	Russia	UAE	Romania	Italy	Germany	France	Russia	UAE
2006	34.69	889.87	1452.48	1470.22	268.98	/	0.026	0.027	0.019	0.019	0.002	/
2007	4.22	70.46	1311.17	1530.66	357.18	13.11	0.006	0.008	0.019	0.019	0.003	0.002
2008	3.04	106.62	838.49	1511.73	260.21	8.79	0.006	0.009	0.019	0.019	0.001	0.001
2009	4.31	1227.86	1150.72	1918.76	320.22	/	0.024	0.031	0.025	0.025	0.002	/
2010	3.68	450.10	1092.43	1933.81	4657.96	/	0.023	0.032	0.019	0.019	0.005	/
2011	3.55	462.14	921.67	1941.94	4113.77	/	0.026	0.037	0.018	0.018	0.006	/
2012	4.57	1101.48	767.24	1592.01	2399.12	29.90	0.037	0.047	0.016	0.016	0.004	0.004
2013	0.97	471.47	657.09	1614.86	2316.63	27.80	0.000	0.011	0.016	0.016	0.004	0.003
2014	2.96	1147.12	754.76	1537.58	5244.17	72.40	0.020	0.030	0.019	0.019	0.005	0.005
2015	1.68	358.27	699.89	1466.58	1225.42	101.81	0.010	0.025	0.023	0.023	0.008	0.008
2016	2.41	634.82	620.67	1041.70	533.12	186.79	0.013	0.023	0.019	0.019	0.009	0.009
2017	1.70	387.20	551.25	757.94	391.57	177.52	0.007	0.016	0.016	0.016	0.007	0.007
2018	1.06	421.06	626.18	669.48	573.35	21.35	0.001	0.011	0.014	0.014	0.005	0.005
2019	1.61	1338.88	554.75	685.83	508.01	27.14	0.009	0.025	0.014	0.014	0.006	0.006

Source: Authors' research, based on ITC – Trade Map, 2020.

All the examined models (Table 5) were statistically significant. It can be concluded that the already established decline in the competitiveness of corn and frozen fruits had a negative impact on both total exports and exports to the most important export markets, and this impact was statistically significant in the case of frozen fruits. The competitiveness of apples had a statistically significant and positive influence on the export of the RS (statistical significance was not recorded only in the fifth model). It can be concluded that the increase in the competitiveness of apples (Table 4) had a positive impact on both the total export of the RS and the export to the most important export markets, in contrast to corn and frozen fruits, because the decrease in their competitiveness (Table 4) had a negative effect on the export of the RS (Table 5).

All models presented in Table 6 were statistically significant. The robustness of the data was proven, since the same impact of competitiveness on the exports of the RS was obtained using the ARCA index, i.e., the decline in the competitiveness of corn and frozen fruits had a negative impact on exports, while the increase in the competitiveness of apples had a positive impact. Statistical significance for corn was not confirmed in the first, third, and fifth models, and for frozen fruits in the fourth model.

Table 5. The impact of the competitiveness of the most important agricultural export products on the export of the RS using the RCA index.

Label	Model 1	Model 2	Model 3	Model 4	Model 5
Dependent variables	Exp	Exp_EU (28)	Exp_Ger	Exp_Ita	Exp_B&H
Intercept	***28516742.03 (5.25)	***18001646.28 (4.36)	***3438538.17 (4.78)	**3104162.95 (2.63)	***2290361.97 (7.55)
RCA_cor	-3399.37 (-0.04)	-8156.34 (-0.12)	-158.85 (-0.01)	-8925.15 (-0.46)	-1683.44 (-0.34)
RCA_fro_fru	***-49360.25 (-3.45)	***-32544.59 (-2.99)	***-6495.86 (-3.43)	*-5122.29 (-1.65)	***-2956.42 (-3.70)
RCA_app	*213796.95 (1.73)	*192701.73 (2.05)	**39602.12 (2.42)	*50839.93 (1.89)	945.34 (0.14)
Adjusted R^2	0.81	0.79	0.83	0.64	0.76
F-statistic	***18.94	***17.22	***22.61	***8.61	***15.06

Note: beta coefficients in parentheses, t-values in parentheses; *, **, *** indicate statistical significance at the 10%, 5%, and 1% levels, respectively.
Source: Authors' research, based on ITC – Trade Map, 2020.

Table 6. The impact of the competitiveness of the most important agricultural export products on the export of the RS using the ARCA index.

Label	Model 1	Model 2	Model 3	Model 4	Model 5
Dependent variables	Exp	Exp_EU (28)	Exp_Ger	Exp_Ita	Exp_B&H
Intercept	***21999547.56 (5.51)	***14396593.98 (5.16)	***2569680.98 (4.75)	**2078223.80 (2.68)	***1675035.01 (5.91)
ARCA_cor	-71683627.02 (-1.35)	*-51306281.46 (-1.38)	-9393906.24 (-1.31)	*-16554845.19 (-1.61)	-5159089.94 (-1.37)
ARCA_fro_fru	***-646709379.38 (-3.22)	***-458173610.94 (-3.27)	***-83719686.19 (-3.08)	-46068658.81 (-1.18)	*-28412520.49 (-1.99)
ARCA_app	***983792643.86 (3.68)	***75745932.77 (4.06)	***15722576.06 (4.35)	***18622619.836 (3.60)	*35649433.35 (1.88)
Adjusted R^2	0.71	0.73	0.74	0.56	0.42
F-statistic	***11.41	***12.78	***13.06	***6.61	**4.20

Note: beta coefficients in parentheses, t-values in parentheses; *, **, *** indicate statistical significance at the 10%, 5%, and 1% levels, respectively.
Source: Authors' research, based on ITC – Trade Map, 2020.

Investments in modern equipment and accompanying production processes are essential for improving competitiveness and promoting exports. The space for increasing competitiveness lies in the significant natural resources of the RS, in the sphere of improving quality standards and product range, faster penetration of innovations, etc. (Cvetković and Petrović-Ranđelović, 2017). Developing

innovative approaches, such as organic production, in modern conditions, is of particular importance, both from the aspect of quality and in terms of increasing the export competitiveness of the agricultural sector.

Although there are natural resources for the development of organic agriculture, the export of organic products from the RS was at a low level (Table 7). Therefore, we should work to introduce innovative production methods of this type so that the competitiveness of products that are of great importance for the export and economic development of the RS does not decrease, because such a development approach is sustainable. In the RS, organic production is still insufficiently developed. Also, buying organic food in Serbia is still at a very low level (Mitić and Čolović, 2022). On the other hand, organic agriculture is constantly growing in the world (Savić, 2022). It is encouraging that the area under organic production in the RS is growing year by year, but the number of producers decreased in 2019 compared to 2018 (SORS, 2020).

Table 7. The export of organic products of the RS.

Year	Export of organic products, in million euro	% of the total merchandise exports of the RS
2012	4	0.05
2013	10	0.09
2014	10	0.09
2015	19	0.16
2016	18	0.13
2017	18	0.12
2018	18	0.11
2019	18	0.10

Source: FIBL statistics, 2020.

Development policies aimed at increasing the competitiveness of agrarians, through innovations, simultaneously raise the issue of improving the quality of agricultural and food products (Curzi et al., 2014). Consequently, the role of organic agriculture is determined by whether it can be more competitive than conventional agriculture, which depends on the productivity of organic agriculture and the demand for organic products (De Ponti et al., 2012).

Despite the pronounced comparative advantages of the RS, the export structure of the agricultural and food sector is unsatisfactory (raw materials, products with a lower degree of processing, a small share of livestock products, etc.). The example of the RS shows that expressed comparative advantages are not at the same time a reflection of a strong competitive position, but only a reflection of a certain "vitality" of the agricultural sector (Đukić et al., 2018). The structure of RS exports includes labor-intensive products such as raw materials and products with a low level of processing. Exports of technologically intensive products are in the minority. In the

international market, few products are competitive in terms of high quality. Prices for certain products have also decreased, making it difficult for agricultural products from the RS to compete with bidders from other countries. Accordingly, it is necessary to work integrally and continuously to increase productivity, quality and quantity of production, to improve the structure of production and export, in accordance with demand and other relevant factors (Milojević et al., 2011).

The investment in modern technologies is an important prerequisite for the competitiveness of farmers and agri-food export. The increase in the value of exports of agri-food products encourages primary agricultural production and processing of primary products, the import of modern equipment and cleaner technology for the needs of agri-food production, processing or marketing of these products. The RS, from the aspect of agriculture, must promote the maintenance and increase competitiveness in the EU markets, with which it achieves the biggest part of foreign trade, but also in the markets of other countries. The space for increasing competitiveness lies in the significant natural resources that are still underutilized in agriculture, in the sphere of improving product quality standards, improving the product assortment, introducing new types of production and faster penetration of innovations in the agri-food sector (Cvetković and Petrović-Ranđelović, 2017).

Conclusion

The product quality of the RS, on the basis of which frozen fruits and apples are competitive at the world level, and the low price, on the basis of which corn becomes competitive, are not sufficient to achieve this competitiveness in the long term. It is also necessary to renew processing capacities, as well as to create a final high-quality product with high added value. In addition, it is important to organize production and join clusters in order to achieve greater bargaining power as well as chances for financing. It is precisely the lack of the mentioned factors, as observed in this research, that led to a decrease in the competitiveness of the observed products, i.e., corn and frozen fruits, which had a negative impact on the exports from the RS. At the same time, apples, for example, experienced an increase in competitiveness, which had a positive impact on exports. Accordingly, the hypothesis that the most competitive agricultural products have a statistically significant impact on the export markets of the most important trading partners of the RS was confirmed. It was also found that the improvement of product quality had a positive impact on the export and competitiveness of the agri-food sector.

Despite the pronounced comparative advantages of the RS, the export structure of the agri-food sector was unsatisfactory. In addition to improving the quality of agricultural products, it is important to innovate the production process itself and process primary agricultural products into products of higher processing levels, which can influence the increase of the competitiveness and exports of the agri-food sector.

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KONKURENTNOST POLJOPRIVREDNO-PREHRAMBENOG SEKTORA REPUBLIKE SRBIJE

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R e z i m e

Konkurentnost poljoprivredno-prehrambenih proizvoda je među ključnim faktorima u podsticanju izvoza i privrednog razvoja, posebno za zemlje u razvoju. Povećanjem produktivnosti, primenom savremenih znanja, inoviranjem prerađivačkih kapaciteta i proizvodnjom kvalitetnog finalnog proizvoda sa većom dodatom vrednošću može se uticati na konkurentnost. Poljoprivreda Srbije je veoma značajna za ekonomski rast. U cilju poboljšanja konkurentske prednosti poljoprivredno-prehrambenog sektora na inostranim tržištima bitno je povećati njegovu efikasnost uvođenjem savremenih tehnologija i pristupa. Predmet rada je da istraži najznačajnije izvozne poljoprivredno-prehrambene proizvode Srbije, kao i najznačajnija tržišta njihovog izvoza. Pomoću indeksa RCA i ARCA ispitane su komparativne prednosti ovih proizvoda u trgovini, što je cilj istraživanja, a zatim višetrukom regresijom i uticaj na izvoz. Iako značajni poljoprivredno-prehrambeni proizvodi ostvaruju komparativne prednosti, opadanje njihove konkurentnosti se negativno odražava na izvoz. Politika niskih cena, kao i neodgovarajući kvalitet proizvoda ne mogu da očuvaju dugoročnu konkurentnost. Takođe, struktura izvoza poljoprivrednih proizvoda je nezadovoljavajuća, imajući u vidu da su to proizvodi niže faze prerade, sa niskom dodatom vrednošću. Stoga je neophodno ulaganje u modernu opremu, razvijanje inovativnih pristupa poput organske proizvodnje i brži prodor inovacija u cilju poboljšanja standarda kvaliteta, diferencijacije asortimana proizvoda i stvaranja finalnog proizvoda sa visokom dodatom vrednošću, kao bitne preduslove za poboljšanje konkurentnosti i podsticanje izvoza poljoprivredno-prehrambenog sektora.

Ključne reči: konkurentost, poljoprivredno-prehrambeni sektor, RCA, ARCA.

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COOPERATION BETWEEN AGRICULTURAL EXTENSION SERVICES AND COOPERATIVES – THE STATE AND POSSIBILITIES

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Abstract: One of the key links in the system of knowledge and information transfer in Serbian agriculture is the extension service, which acts as a mediator between scientific institutions and different user groups. Extension officers prefer to cooperate with already formed user groups, such as agricultural cooperatives, because they unite farmers with similar production and are convenient for group methods of extension work. This paper aims to examine the quality of cooperation between agricultural extension services and agricultural cooperatives in Serbia and to evaluate the views of farmers (cooperative members) on the success of this cooperation. For this research, a survey was conducted with 220 respondents, members of agricultural cooperatives. The analysis showed that over 80% of cooperative members cooperated with extension service, and that they were more satisfied with the quantity of extension services than with the quality. More than half of the respondents believe that extension services need to be adapted to the needs of cooperatives, and that the cooperatives should be given more attention in the media appearances of extension officers. This research provides an important insight into the form and specifics of cooperation between agricultural extension services and agricultural cooperatives, on the basis of which measures for their continuous improvement can be proposed.

Key words: agricultural extension service, agricultural cooperatives, attitudes of farmers, cooperation.

Introduction

Modern society is characterized by continuous development and innovation in all spheres of production and everyday life, including agriculture. The modern business is conditioned by the application of scientific and technological innovations, information and communication technologies, but also by the application of innovative business solutions, especially when connecting with external partners in the production chain (Milojević et al., 2015). Agricultural

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production implies a practice based on the use of technology and the implementation and adaptation of new knowledge.

The adoption of new technologies and innovations is a prerequisite for the successful production and survival of farmers in the market (Oreszczyn et al., 2010). Innovation can be defined as the implementation of a new or significantly improved product or process, a new market method, or a new organizational method in business practice and production organization. The macroeconomic view sees innovation as a linear process from basic research to its commercial application (EU SCAR, 2015). One of the key challenges facing modern agriculture is the timely application of existing innovations in the production process, which is one of the tasks of AKIS (Agricultural Knowledge and Information System).

AKIS can be defined as a set of people, networks, and organizations, as well as connections and interactions between them, aimed at creating, transforming, transferring, storing, and applying knowledge and information, with the purpose to support decision making, problem solving, and innovation in agriculture (Röling and Engel, 1991). In the last decade, AKIS has also included support for the application of innovations that contribute to the improvement of agricultural production (EU SCAR, 2015).

One of the key elements of AKIS in most economies, including Serbia, is agricultural extension services (AESs), which have the task of educating farmers, providing them with new information and knowledge, and helping them develop new skills. AESs transfer knowledge, information, and technology from research institutions and organizations to agricultural producers. In performing these activities, AESs implement several methods, which can be divided into individual, group, and mass media methods of extension.

The group approach in extension involves working with groups of farmers. This method is desirable not only because of the greater coverage of the target group, but also because it allows an exchange of information between extension officers and farmers, as well as among the farmers themselves. The group approach makes an important contribution to changing farmers' behavior and influences the strengthening of farmers' awareness of the importance of AES existence and activities (Čikić et al., 2009). The group approach in extension work can be carried out with existing, already organized groups of agricultural producers, such as cooperatives.

Cooperatives are a specific form of members' organization found in almost all countries of the world in all sectors of the economy. They have a long tradition that began in the second half of the XIX century, when cooperatives emerged as a response of a socially neglected part of the population to difficult living conditions. Rooted in Western Europe, cooperatives quickly spread to other parts of the continent and the world. Today, there are more than 2.5 million cooperatives

globally with over one billion members and clients. Almost half of them (48.7%) are agricultural cooperatives, making them the most important ones (UN, 2014).

Cooperatives in Serbia have had a long and turbulent history, but at each phase of their development they played a more or less important role in agriculture and rural areas. In the light of this paper, cooperatives are important in agriculture not only because they empower farmers when entering the market, but also because cooperatives are by definition an organization of farmers with similar productions and similar problems. This implies that it is more effective for extension officers to approach a cooperative than a number of individual farmers.

The aim of this paper is to examine models of cooperation between Serbian AES and agricultural cooperatives, the quality of this cooperation, and the way cooperative members perceive the contribution of extension services to the improvement of production on their farms. The main hypothesis of this paper is that AES and agricultural cooperatives collaborate, and that cooperative members perceive this as useful for the improvement of agricultural production. Based on the conducted research, the segments where this cooperation was successful, the shortcomings and the proposed ways of improving the quality of AES work with agricultural cooperatives (ACs) in Serbia were studied.

The cooperation of AES and agricultural cooperatives through the prism of legal regulations

The importance of performing extension work is recognized in relevant strategic and legal documents. The Strategy of Agriculture and Rural Development of the Republic of Serbia for the period 2014–2024 (Official Gazette of the Republic of Serbia, No. 85/14) classifies the improvement of the system of knowledge transfer and the development of human resources in agriculture as one of the 14 priority areas of agricultural policy. The transfer of knowledge in the field of agriculture includes the system of formal education at all levels and various types of training organized by educational and research institutions, AES of Serbia, private companies, media, and other organizations. In evaluating the contribution of AES to this process, it is stated that *"the organized transfer of knowledge through AES reaches a relatively small number of users",* and that *"the existing structure and system of knowledge transfer are not efficient enough"*. It is necessary to improve the entire system of knowledge and information transfer, especially the work of AES, and to achieve greater coverage of users with its services. This can be accomplished by applying the group approaches in extension work, particularly with existing organizations of farmers, such as agricultural cooperatives.

The basic legal document that regulates the work of agricultural extension services in the Republic of Serbia is the Law on Providing Extension Service in

Agriculture (Official Gazette of RS, No. 30 of May 7, 2010), which provides general guidelines and sets rules for the work of these services. The more detailed regulation of AES work is given in secondary legal documents, primarily regulations on the medium-term development program, which are adopted for a period of five years, and in the annual regulation on establishing the development program of extension work in agriculture.

Table 1. The presence of cooperatives and associations in the work of AES.

		2017	2018	2019
The share of extension officers' working time devoted to the performance of the following advisory activities (%)				
Group methods		24.43	22.72	21.61
Work with cooperatives, groups, and associations		1.20	1.13	1.16
Number of users				
Work with cooperatives and associations	Number	2,712	2,733	2,766
Participation in the number of users covered by group methods	%	6.45	6.97	7.79
Share in the total number of users	%	3.27	3.10	3.44

Source: Authors' calculation based on data from the Regulation on the medium-term program for the development of extension services in agriculture for 2021 to 2025

The regulation on the medium-term program for the development of agricultural extension services for 2021 to 2025 states that the priority of agricultural extension work is to assist and help users, including cooperatives, associations and producer organizations. Cooperation with these types of users is achieved through group extension methods. In 2017–2019, extension officers spent an average of 1.16% of their working time providing services to this category of users. At the same time, the share of working time spent on group extension methods decreased from 24.43% to 20.63%. The number of cooperatives and farmers' associations covered by extension services in the same period increased to a maximum of 2,766 in 2019.

A more detailed overview of extension work with cooperatives is provided in the Regulation on Annual Program for the Development of Extension Services in Agriculture for 2022 (Official Gazette of the RS, No. 18 of February 11, 2022). Each extension service monitors at least one cooperative that operates in the area where the service provides extension work. The number of cooperatives with which an individual extension officer works can range from one to 12, and he or she is required to visit each of the selected agricultural cooperatives four times per year. In addition, extension officers are obliged to give at least one lecture, attend lectures of one winter school, and make one television appearance annually in the area of supporting the creation, development and revitalization of agricultural

cooperatives. The selection of cooperatives to be included in the extension work is made on the basis of a written proposal from the Cooperative Union of Serbia, which indicates that a certain form of cooperation already exists between the institutions in the agricultural sector.

Material and Methods

The results of the research conducted in December 2020 by the Cooperative Union of Serbia on a sample of 220 agricultural cooperatives from all over the country are presented in this paper. The first criteria were that the cooperative was involved in agriculture, that it was situated in rural areas, that it was a member of the Cooperative Union of Serbia and that it had an e-mail address in the database. A questionnaire was sent by e-mail to all cooperatives that met these criteria. After a week, the cooperatives that had not responded were contacted by phone, and kindly reminded to participate in the study. There were 220 responses, and all were valid, so they were included in the sample.

The opinions of farmers and members of agricultural cooperatives on the use of agricultural extension services were collected using a questionnaire distributed in direct contact with representatives of cooperatives and their members.

The collected data were analyzed in Excel using descriptive statistics, and the Pearson's linear correlation coefficient to determine the relationship between two observed variables. The comparative method was also used to compare the obtained results with other similar studies. In addition to the primary data collected in the described research, legal documents that regulate the work of agricultural extension services in the Republic of Serbia and available relevant literature were also used.

Results and Discussion

The survey included 220 respondents, of whom the majority (140 or 63.6%) were men. The average age of the respondents was 46 years, and the two extreme interval groups (younger than 30 and older than 60) were represented by only about 10% (Table 2).

Most of the respondents (almost half) had a secondary school degree, while 37.3% had a college education, and 14.1% had the highest educational qualification. This educational structure differs significantly compared to the total population in rural areas (the so-called other settlements), where, according to the results of The Census of Population, Households and Dwellings in the Republic of Serbia (2011), 42.4% of the population had secondary education, and only 6.1% higher education. The majority of respondents (78.2%) indicated a village as their place of residence, while about one-fifth of them lived in urban and suburban settlements (Table 2).

Table 2. The socio-demographic characteristics of the respondents.

Variable	Frequency	%
Gender		
Male	140	63.6
Female	80	36.4
Age		
Up to 30	20	9.1
31 to 40	48	21.8
41 to 50	88	40.0
51 to 60	40	18.2
Over 60	24	10.9
Degree of education		
Primary	7	3.2
Secondary	100	45.5
Tertiary	82	37.3
Master's degree and Ph.D degree	31	14.1
Place of residence		
Rural area	172	78.2
Urban area	48	21.8
Total respondents	220	100.0

Source: Authors' calculation.

Of the total number of respondents, 202 or 91.8% stated that they were employed, of whom 50.5% worked in a cooperative, about one-third (31.3%) were employed on their own farms, and 37 (18.3%) were employed in another company. Respondents who stated that they were not employed were mostly retired elderly people.

Of 220 respondents, 26 of them (11.8%) did not use agricultural extension services. Among them, there were slightly more women (42.3%, compared to 36.4% in the entire sample), and most of them had a higher level of education, i.e., there were no persons with only primary education in this group. This can be explained by the fact that these respondents have a certain level of knowledge or skills and abilities to collect the information they need, and therefore consider that they do not need AES. They are more likely to face the challenges on their own, without the support of AES. As most extension services face an insufficient number of extension officers and the increasing need to provide services to marginalized social groups in the rural population, one should not insist on the full coverage of farmers by extension services.

Contrary to this group of respondents, 194 (88.2%) stated that they cooperated with AES. This is significantly higher than in other similar studies, which have found that only 30% of farmers have contact with extension services, and that only when extension officers come to their farm (Dimitrijević and Stojić, 2019).

Although this could be interpreted as a weak effect of extension work, at the same time it indicates the rigidity of farmers and their unwillingness to proactively participate in increasing their knowledge.

It is interesting to note that 41.8% of the respondents stated that they had contact with AES for more than two years, while 36.6% of them cooperated with them for less than one year. According to the legal framework, an extension officer is required to visit the cooperative with which he or she cooperates four times per year. However, 40.7% of the cooperative representatives stated that their officer visited them less than required, while about one-fifth of them (21.6%) indicated that the frequency of visits was higher than prescribed (Table 3). In some cases, these two groups included cooperatives that belonged to the same extension service: for example, three cooperatives from Kruševac stated that they were visited by extension officers less than four times per year, while one cooperative from that territory had more than four visits. This implies that better coordination at the service level is needed in order to fulfill the stipulated criteria and provide an adequate level of service to all cooperatives.

Table 3. The frequency and quality of cooperation between AC and AES.

Variable	Frequency	%
The existence of cooperation with AES		
Cooperate	194	88.2
Do not cooperate	26	11.8
Total respondents	220	100.0
Length of cooperation*		
Less than 6 months	32	16.5
6 months to one year	39	20.1
One to two years	42	21.6
More than two years	81	41.8
Frequency of visits from extension officiers*		
Not applicable because I have been working with AES for less than 6 months	29	14.9
Less than four times per year	79	40.7
Four times per year	44	22.7
More than four times per year	42	21.6
Frequency of using AES services*		
Not applicable because I have been working with AES for less than 6 months	13	6.7
Less than once a month	26	13.4
One to three times per month	35	18.0
Whenever I need advice	92	47.4
Not applicable	28	14.4
Total respondents	194	100.0

*Only respondents who stated that they cooperated with AES were included. The total number of these respodents was 194.
Source: Authors' calculation.

The arrival of extension officers to the cooperative office is not the only form of cooperation between these organizations. Contacts can also be made on AES premises, at group meetings, or through the mass media. It is extremely significant that almost half of the cooperatives that had contact with AES considered that they received advice every time they needed a certain type of help. As many as 18.0% of the respondents stated that they had contact with extension officers several times a month, while a significant part of these contacts occurred through group extension methods.

In addition to providing extension services to direct users, extension officers prepare various materials that are available to all interested persons, whether or not they have direct contact with the service. These activities are included in the provision of extension services using mass media communication methods and are one of the basic techniques for increasing the information level of the entire population on selected topics and can also be an important initiator of the extension process.

The largest number of cooperative members reported using applications that provided them with market information, such as STIPS and Agroponuda (Table 4). The focus of agricultural cooperatives is on primary agricultural production, the lack of engagement in food processing and loose ties with the processing industry result in an increased need for information on the possibilities of marketing agricultural products. This situation provides a more vivid insight into the possibilities and limitations of the business of cooperatives than the quality of agricultural extension services.

The AES portal is used as a source of information by 20.0% of respondents, which is more compared to similar surveys conducted in Serbia. Dimitrijević and Stojić (2019) have found that about half of the respondents use different sources of information, such as radio and television, the local agricultural pharmacy, contact with other producers, and that only 15% read specialized magazines or consult an extension officer. At the same time, cooperatives should be a significant source of data. The task of agricultural cooperatives is not only to connect agricultural producers, but also to pool knowledge and information. At the end of the 20th century, it was recognized that cooperatives had valuable knowledge on the supply of inputs and the demand in the market for agricultural products, especially if they were specialized in one line of production (Van Den Ban, 1993). Today, the European Union insists that cooperatives should be organized regionally and used as a tool for marketing agricultural products, especially through direct contacts between producers and consumers (EU SCAR, 2015).

A significant part of cooperative members (41.2%) had no objections to the work of the extension service. Slightly less than one-fifth of the surveyed farmers pointed out that the topics analyzed by AES were poorly focused on the cooperative sector, and the same number believed that communication between

these organizations was insufficient. This is consistent with previous findings that the frequency of visits by extension officers to cooperatives is insufficient.

Table 4. Selected indicators of the quality of extension services for agricultural cooperatives and possibilities for their improvement.

Variable	Frequency	%
Used materials/applications		
STIPS	66	30.0
Agroponuda	51	23.2
Bulletin of AES	44	20.0
PIS Vojvodina (Forecasting and reporting service)	12	5.5
None of the above	47	21.4
Total respondents	220	100.0
What aspects of agricultural extension work do you criticize?		
I have no objections	80	41.2
They are not able to meet the needs of cooperative members	38	19.6
Low level of communication between AES and cooperative members	34	17.5
Lack of topics interesting to cooperative members	31	16.0
Other	11	5.7
What would you improve in the work of AES?		
Higher level of communication (more frequent visits to the cooperative, greater interest, etc.)	67	34.5
Selection of topics according to the needs of cooperative members	61	31.4
More presence of cooperatives in media used by extenstion officiers	42	21.6
Other	24	12.4
Total respondents	194	100.0

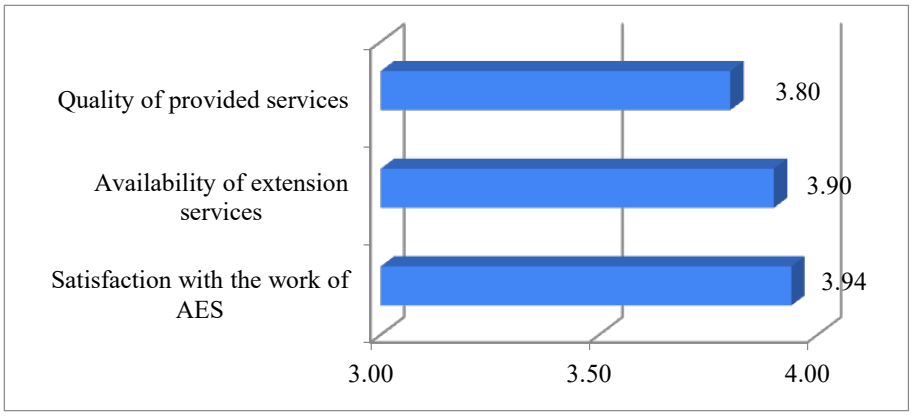
Source: Authors' calculation.

Recommendations that respondents gave to improve AES were to solve the aforementioned problems: more contacts between AES and AC, more attention to topics of interest to cooperatives, and more space in mass media focused on the work of cooperatives. This can be significant not only to the work of AES, but also to the overall promotion of association of farmers. Raising awareness of the general and rural population about the work of cooperatives is proclaimed according to the fifth basic cooperative principle, which combines education, training, and information and is carried out to create a conducive environment for the work of cooperatives and promote their achievements and results (Nikolić et al., 2021).

One of the models for providing extension services is organizing extension networks by farmers' associations or cooperatives. In such a way, an alternative extension service is created, which is primarily aimed at different forms of farmers' organizations. Such organizations have an important role and can be major drivers

of food system change (Dunning et al., 2012). However, for cooperatives to be able to support such an initiative, they need to reach a certain size, both in terms of number of members and available financial resources. From this point of view, the situation in Serbia is extremely unfavorable – cooperatives are predominantly micro or small enterprises, with a modest number of members. Over 50% of potential members do not see any significant advantages in joining a cooperative and therefore do not join these organizations (Simonovic et al., 2019). Currently, there is neither an initiative nor an opportunity to implement this form of extension services in Serbia since farmers' organizations are relatively weak, their representativeness is low, and they do not have sufficient resources to finance an independent extension service (Dimitrijević and Stojić, 2019).

Despite the mentioned shortcomings, the surveyed cooperative members showed a high degree of satisfaction with the work of AES, which they rated with an average score of 3.94. Other scores for the quality of given advice (3.80) and the availability of AES (3.90) are in line with previous findings. The lower scores for the quality of extension work can be linked to the shortcomings pointed out by the respondents, which are related to the low representation of topics of interest to the cooperatives. The availability of extension services would be rated higher if the frequency of their visits to agricultural cooperatives increased. Namely, more frequent contacts between extension officers and farmers are important to improve the efficiency of extension services, leading to higher user satisfaction (Elias et al., 2016; Sarnaik et al., 2020).



Graph 1. Scores of the cooperative members for the work of AES.

Similar findings were reached in other research. The availability of extension services is generally rated as satisfactory, but the variety of services provided is not adapted to the needs of farmers (Debnath et al., 2016; Kassem et al., 2021). It is evident that the quantitative elements of the cooperation between AES and AC,

reflected in the number of visits and contacts, can be further improved, but it would be preferable to focus on diversifying the advice provided, or in other words, on a qualitative approach. Petrović and Janković (2002) point out that changes in the program and organization of the work of extension stations are key factors for their better and more efficient work.

Using the Pearson's correlation coefficient, it was found that there was a weak positive relationship between the level of respondents' education and the rating of extension services availability (r=0.2474), the quality of the advice given (r=0.2454) and the degree of satisfaction with AES work (r=0.2554). The respondents with a higher level of education rated the mentioned indicators with a higher score. At the same time, there is no correlation between the scores given by the respondents and their characteristics (gender, age, place of residence, length of cooperation with AES, frequency of visits by extension officers).

This is contrary to other studies that found that the socio-demographic characteristics of respondents (gender, age, level of education, farm size, and number of cultivated plots) significantly affected their level of satisfaction with extension services (Agholor et al., 2013; Sarnaik et al., 2020). Younger farmers were also found to be more satisfied with the services they received than older farmers (Wayne et al., 2014). As a possible explanation, the authors state that younger producers do not insist on individual visits by extension officers, unlike older ones who consider this method the only reliable one. However, our study focuses on group methods (implemented with agricultural cooperatives), and the obtained results can be explained by the fact that cooperative members with a higher level of education are more willing and able to obtain the necessary information on their own, so they do not need help from AES.

Conclusion

The paper examines a special segment of agricultural extension work that focuses on cooperation with agricultural cooperatives. The research is based on the responses of the cooperative members about the existence, quality, shortcomings and opportunities for improving the cooperation between agricultural cooperatives and AES. The basic assumption of the paper is that some level of cooperation already exists between AES and agricultural cooperatives and that cooperative members perceive it as useful for improving their agricultural production.

The obtained results show that a significant part of cooperative members (88.2%) cooperate with AES, which is more compared to the total population of farmers. This further indicates that there are certain connections between these organizations, partly due to the legal regulation that obliges the extension service to provide services to cooperatives and is a good basis for the implementation of group extension methods, which are increasingly insisted upon. Although they

rated the availability and quality of extension services relatively well, the criticisms of cooperative members of the work of AES were directed primarily at the unadjusted structure of the extension services and their poor compatibility with the needs of cooperatives.

Based on the conducted research, it can be concluded that there is a long-term and relatively stable cooperation between AES and AC, but the quantity and quality can be improved. Further research should be focused on models to improve existing cooperation, but also on developing methods and studies to measure the impact that the extension services provided have on increasing agricultural production.

One of the ways to solve the observed problems is to involve agricultural cooperatives more in the creation of extension work, so that the needs of the cooperatives can be taken more into account. This is in line with European experience but requires strong and representative cooperatives that are respected not only by AES, but also by other competent institutions.

The second model also relies on empowering agricultural cooperatives, which could be implemented in two phases. In the first phase, which is already underway in Serbia, close links are established between AES and cooperatives in order to increase the membership and strengthen the market position of these organizations. Empowered agricultural cooperatives would then have better insight into the needs of their members and would be able to provide them with appropriate extension services, either by hiring external extension officers or by developing their own extension service. In this way, a higher quality of the extension services would be provided to cooperatives, and the existing AES could redirect its services to other potential users.

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SARADNJA POLJOPRIVREDNE SAVETODAVNE SLUŽBE I ZADRUGA – STANJE I MOGUĆNOSTI

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R e z i m e

Jedna od ključnih karika u sistemu transfera znanja i informacija u poljoprivredi Srbije su savetodavne službe, koje učestvuju kao posrednik između naučnih institucija i različitih grupa korisnika. Cilj ovog rada je da se ispita kvalitet saradnje između poljoprivredne savetodavne službe i zemljoradničkih zadruga u Srbiji, kao i da se sagledaju stavovi poljoprivrednika, članova zadruga, prema uspehu ove saradnje. Za potrebe istraživanja sprovedeno je anketiranje 220 ispitanika, članova zemljoradničkih zadruga. Utvrđeno je da preko 80% zadrugara ostvaruje saradnju sa savetodavcima, pri čemu su u većoj meri zadovoljni kvantitetom savetodavnih usluga, u odnosu na kvalitet. Preko polovine ispitanika smatra da je savete potrebno prilagoditi potrebama zadruga, odnosno posvetiti više pažnje zadrugama u medijskim nastupima savetodavaca. Ovo istraživanje pruža važan uvid u formu saradnje i specifičnosti rada poljoprivredne savetodavne službe sa zemljoradničkim zadrugama, na osnovu čega se mogu predložiti mere za njihovo kontinuirano unapređenje.

Ključne reči: poljoprivredna savetodavna služba, zemljoradničke zadruge, stavovi poljoprivrednika, saradnja.

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AN EMPIRICAL STUDY ON ASSESSMENT OF TREND ANALYSIS: FOOD GRAIN PRODUCTION IN INDIA

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Abstract: Considering the importance of the food grains to be specified—rice, wheat, coarse cereals, and pulses-is vital in developing the Indian economy. This paper carries out change point detection and trend analysis for the annual historical series of food grains in India. The change points were identified by the methods of the Pettitt's test (PT), the Buishand's range test (BRT), and the standard normal homogeneity test (SNHT). On the other hand, the Mann-Kendall (MK) test was used to determine the presence and absence of trends in food grain production, and their slopes were assessed by the Sen's slope estimator. The change point analysis result shows significant change points for food grains at a 95% level. The discrete change points were observed from 1982 to 2009, but the ideal change point, i.e., 1987, was captured in all food grains. In India's history, agricultural production in the year 1987 was vulnerable due to drought, and thus it is strong evidence of the need to consider methods based on proven facts. The MK test results reveal that the trend in food grain production was statistically significant and had an upward direction in all three segmentation periods after 1987.

Key words: food grains, change point, trend analysis, statistical analysis, statistical significance.

Introduction

Food is the most important item in terms of basic human needs. Since its independence, India has taken progressive steps towards food security. There is an inclination toward multigrain cereals in India, which has seen a significant ascent. These multigrain cereals are rich in a wellspring of proteins, minerals, iron, etc., with high levels of nourishing substances.

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Eating such varieties of food grains with a high source of proteins and minerals keeps one 'full and fulfilled' and supports the immune system, movement of functions, etc., in the human body. The assortment of whole grains such as whole wheat, rice, pulses, and coarse cereals offers a complete package of health benefits by providing nutrients, fiber, protein, vitamins, minerals, and other solid plant compounds that support a sound digestive system and lower the risk of heart disease. For instance, wheat provides energy, helps to control weight, and fights against some diseases such as cancer and cardiovascular disease, among others. Let us present the benefits of rice, coarse cereals, and pulses in turn. Rice provides energy that restores glycogen levels and is easy to digest. Coarse cereals aid in the prevention of cancer, type II diabetes, and other diseases. Pulses aid in the maintenance of a healthy heart as well as the reduction of body weight. A growing number of customers are placing an emphasis on healthy living, changing their metabolism in order to prevent chronic diseases, and using dietary supplements made by nature.

India is a global agricultural powerhouse, and agriculture forms the foundation and prime sector of the Indian economy. With the growth of the agricultural sector, the industrial sector also developed its economy by procuring raw materials from agriculture. However, in farming, the targets require quick expansion in food grain creation, as well as significantly faster development through broadening.

There are various time series models such as linear (ARIMA) and non-linear (ANN) methods used by some renowned professionals to build the mathematical models for food grain production and productivity (Mishra et al., 2015; Vijay and Mishra, 2018; Puneet, 2019). The trend pattern of food grains in India is analyzed by examining the growth rate and instability of food grain areas, production, and yield (Sanjay and Deepak, 2012; Sharma, 2013; Mukesh and Shallu, 2014; Ruche, 2017). Parul (2016) analyzed the trend in the compound growth rate for food grains in the pre-economic reform period. Kalpana and Kiran (2019) applied non-parametric techniques to assess the change point and trend of wheat in India.

However, from the above literature, we have observed that there is not much research done on food grains. A few other researchers considered that only parametric methods could build the best forecast models to predict future production and assess trends in food grains.

Most researchers have successfully used non-parametric methods to detect trends and find abrupt changes in longer-term data sets (Reshu et al., 2014; Tabari et al., 2014; Ijaz et al., 2015; Jamaludin and Zulkifli, 2018; Kalpana and Kiran, 2019; Kalpana et al., 2020; Zinabu and Michael, 2020; Annie and Madan, 2021; and many others). Therefore, in this paper, we considered non-parametric methods with the objective of studying the performance of food grains in India. Here, the main focus was on trend analysis by considering abrupt changes in the most important food grains in India.

Material and Methods

Considering the importance of food grains, the annual production of rice, wheat, coarse cereals, and pulses from 1950 to 2019 was used in this study. These food grains have the only major production share compared to other grains in India.

Tests to capture the change point

Within trend analysis, the prior task is verifying the nature of the time series data. This means whether the data are homogeneous or not. In this context, the three main homogeneity tests, such as the PT, BRT, and SNH tests, were conducted on the annual production of various food grains. The decision to consider the significant breakpoints followed the rules: 1. Assuming that the data represent homogeneity, it indicates no abrupt change has been recorded, and 2. In the case of inhomogeneity, it is possible to consider the breakpoint in time series data (Reiter et al., 2012; Ijaz et al., 2015; Arikan and Kahya, 2019). The non-parametric homogeneity methods are briefly discussed below.

Pettitt's test (PT)

The PT, established in the 1980s, introduced non-parametric methodologies to estimate the breakpoint in time series data. Later, most researchers adapted this method for trend analysis to compute shifts in various fields. The PT statistic is then defined by

$$K = \max_{1 \le k \le n} \left| U_k \right|, \text{ where } \mathrm{U}_k = 2\sum_{\mathrm{i}=0}^{n} R_i - k\left(n+1\right). \tag{1}$$

Here, R_i represents the rank of the i^{th} observation when the data is arranged in ascending order, and n is the number of observations in the data set, i.e., x_i (i=1, 2, …, n).

Buishand's range test (BRT)

The BRT is one of the most widely used homogeneity tests in its construction. The adjusted partial sum is computed as

$$S_k = \sum_{m=1}^{k} \left(x_m - \overline{x} \right) \tag{2}$$

where $x_m \, (m = 1, 2, ..., n)$ and $\overline{x}$ = mean of n observations.

The significant breakpoint can be computed by the method of rescaled adjusted range (*R*) if the $S_k = 0$, in this case, R is defined by

$$R = \frac{Max_{0 \le k \le n}\left(S_k\right) - Min_{0 \le k \le n}\left(S_k\right)}{\bar{x}} \tag{3}$$

Standard normal homogeneity test (SNHT)

Alexandersson (1986) developed the SNHT to study the abrupt change in the rainfall data. The SNHT is a likelihood ratio test that was first performed to study the homogeneity of rainfall. The test statistic is indicated as

$$Z_t = t\bar{z}_1 + (n\text{-}t)\bar{z}_2, \quad (1<t<n),$$
$$\text{where, } \bar{z}_1 = \frac{1}{t}\sum_{i=1}^{k}\left(\frac{y_i - \bar{y}}{\sigma}\right) \text{ and } \bar{z}_2 = \frac{1}{n\text{-}t}\sum_{i=t+1}^{n}\left(\frac{y_i - \bar{y}}{\sigma}\right) \tag{4}$$

Trend analysis

The main task in trend analysis is to assess the monotonic fluctuations in long-period data sets, which can be well performed through a non-parametric linear trend test, i.e., the Mann-Kendall test (Tabari et al., 2014; Jaiswal et al., 2015; Jamaludin and Zulkifli, 2018; Kalpana and Kiran, 2019; Kalpana et al., 2020; Zinabu and Michael, 2020; Annie and Madan, 2021). The test statistic of the Mann-Kendall test (S) is expressed as

$$S = \sum_{j=1}^{n}\sum_{k=1}^{j-1}\text{sign}\left(x_j - x_k\right)$$
$$\text{where, } \text{sign}\left(x_j - x_k\right) = \begin{cases} 1, & \text{if } \left(x_j - x_k\right) > 0 \\ 0, & \text{if } \left(x_j - x_k\right) = 0 \\ -1, & \text{if } \left(x_j - x_k\right) < 0 \end{cases} \tag{5}$$

Suppose the sample size is n>10 and S follows a normal distribution in a tied group with a mean E(S) and a variance V(S), then the MK test statistic is as follows:

$$E(S) = 0,$$

$$V(S) = \left\{ n(n-1)(2n+5) - \sum_{j=1}^{p} t_j (t_j - 1)(2t_j + 5) \right\} / 18 \quad (6) \text{ and}$$

$$Z = \begin{cases} \dfrac{S-1}{\sqrt{Var(S)}}, & \text{if } S > 0 \\ 0, & \text{if } S = 0 \\ \dfrac{S+1}{\sqrt{Var(S)}}, & \text{if } S < 0 \end{cases}.$$

Here, the detection of a trend depends on the value of Z. For example, if the Z value is zero, it means that the data does not follow any trend; if the value of Z is positive, it means that the trend is in an upward direction; and if the value of Z is negative, it means that the trend is in a downward direction.

Sen's slope estimator

Assuming that the time series data exhibits a significant trend, i.e., either a positive or a negative trend, then the Sen's slope estimator can be estimated. It means the slope of the trend line or the average rate of change of the trend (Hosseinzadeh, 2014; Guo and Xia, 2014; Gavrilov et al., 2016; Thenmozhi and Kottiswaran, 2016; Zinabu and Michael, 2020; Annie and Madan, 2021). The slope of the trend is estimated using the following test statistic:

$$T_i = \frac{y_j - y_k}{j - k} \quad \text{for } i=1, 2, \ldots, N \quad (7)$$

Here, y_j and y_k are the data values at time j and k (j > k), respectively. The median of these N values of T_i is the Sen's slope estimator, which is defined as follows:

$$\beta = \begin{cases} T_{\frac{N+1}{2}} & N \text{ is odd}, \\ \dfrac{1}{2}\left(T_{\frac{N}{2}} + T_{\frac{N+2}{2}} \right) & N \text{ is even} \end{cases} \quad (8)$$

Here, if the value of β is positive, it means that the trend is in a rising pattern, and if the value of β is negative, it means that the trend is in a declining pattern.

Results and Discussion

In order to explain the characteristics of the data, the researchers analyzed various food grains: rice, wheat, coarse cereals, and pulses, using descriptive statistics. The calculations are presented in Table 1.

Table 1. Statistical information on the production of various food grains (in million tons) from 1950 to 2019.

Food grain	Count	Minimum	Maximum	Mean	S.D.
Rice	70	20.580	118.400	63.432	28.645
Wheat	70	6.180	107.590	46.782	30.808
Coarse cereals	70	15.380	47.500	30.229	7.370
Pulses	70	8.350	25.230	13.109	3.451

The highest production, i.e., 118.4 million tons, was counted for rice in 2019, and the lowest production of rice was recorded in 1950 with a production of 20.580 million tons. The next highest share of food grain production was wheat. The maximum production of wheat was 107.59 million tons in 2019 and the minimum production was 6.180 million tons in 1951; coarse cereals had the highest production (i.e., 47.5 million tons) in 2019 and the lowest production (i.e., 15.38 million tons) in 1950; likewise, pulses had the lowest and highest productions of 8.35 million tons in 1966 and 25.23 million tons in 2017. On average, the production of various food grains such as rice, wheat, coarse cereals, and pulses was 63.432, 46.782, 30.229, and 13.109 million tons, respectively. When comparing all types of food grain production, rice took the top spot, followed by wheat, coarse cereals, and pulses.

FAO acknowledged this as true. It is noteworthy that among all the observed food grains, rice was one of the leading producers from 1950 to 2019.

Table 2 and Figure 1 show there was a shift in rice and wheat production in 1987 by the Buishand's test and the SNH test.

Table 2. Homogeneity test analysis on various food grains in India.

Food grains	PT		BRT		SNH Test	
	Sig.	Shift	Sig.	Shift	Sig.	Shift
Rice	< 0.001	1982*	<0.001	1987*	<0.001	1987*
Wheat	< 0.001	1984*	< 0.001	1987*	< 0.001	1987*
Coarse cereals	< 0.001	1987*	< 0.001	1987*	< 0.001	2002*
Pulses	< 0.001	1987*	< 0.001	1987*	< 0.001	2009*

*Significant at the 95% level.

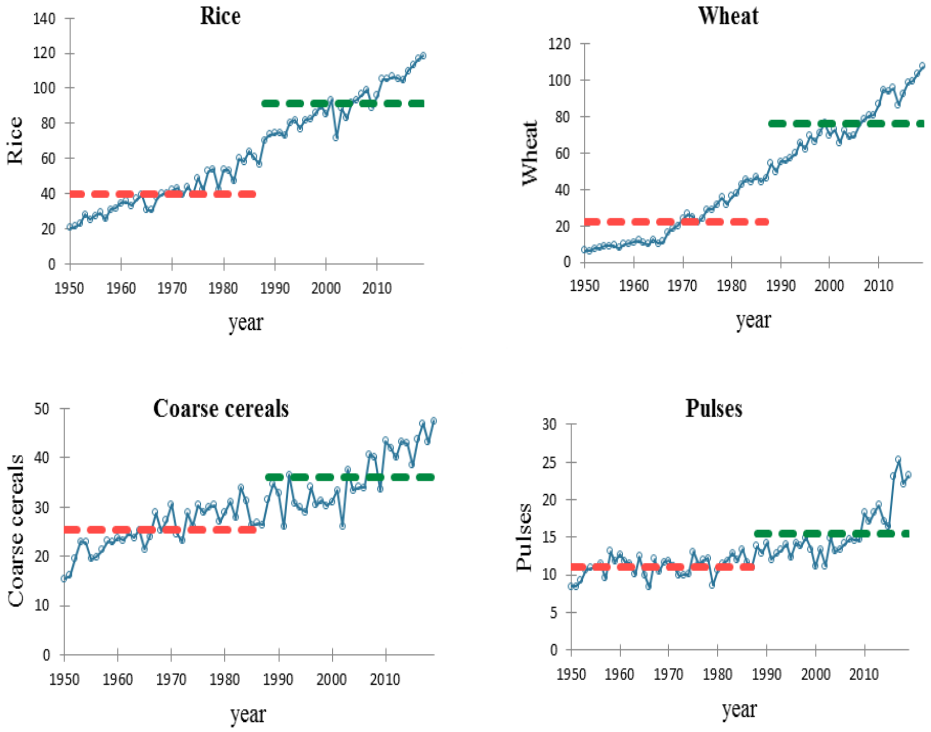

Figure 1. Identified shifts in food grain production in India.

The shifts were also detected for coarse cereals and pulses in the same year (1987) by the Pettit's test and the Buishand's test. Actually, the discrete change points were observed from 1982 to 2009, but the ideal change point, i.e., 1987, was captured in all food grains. In India's history, agricultural production was vulnerable in 1987 due to drought (Radhakrishna, 2002; Wilson, 2005); thus, there is strong evidence for considering methods that are well proven facts.

After detecting the significant change point, the annual data from 1950 to 2019 was divided into two-time intervals according to their shifts. The two periods were time period I (1950 to 1987), and time period II (from 1988 to 2019). The study was continued to analyze the performance of food grain production with different time periods such as period I, period II and overall data by applying trend analysis. The significance of monotonic trends and their magnitude were analyzed by the methods of the MK test and the Sen's slope estimator for three periods.

The data in Table 3 show that a trend analysis of the major types of food grain production and their segmentation year by year was performed. The trend patterns of various food grains such as rice, wheat, coarse cereals, and pulses were detected by

the MK test. The results reveal that there was a statistically significant upward trend in the annual production of all food grains. This indicates that the production of food grains was increasing over time. The study was extended to estimate the average rate of change or slope of the trend by using the Sen's slope estimator method.

Table 3. Trend analysis results of various food grain productions.

Food grains	Period	MK test statistic (S)	Sig.	Sen's slope
Rice	1950–1987	0.841	< 0.0001*	1.046
	1988–2019	0.827	< 0.0001*	1.427
	1950–2019	0.918	< 0.0001*	1.387
Wheat	1950–1987	0.915	< 0.0001*	1.169
	1988–2019	0.858	< 0.0001*	1.635
	1950–2019	0.946	< 0.0001*	1.508
Coarse cereals	1950–1987	0.656	< 0.0001*	0.317
	1988–2019	0.587	< 0.0001*	0.512
	1950–2019	0.754	< 0.0001*	0.33
Pulses	1950–1987	0.246	0.031*	0.045
	1988–2019	0.591	< 0.0001*	0.255
	1950–2019	0.642	< 0.0001*	0.107

*Significant at the 95% level.

From the above trend analysis, it can be seen that time period I represents the highest average growth rate of 1.169/year and the lowest average growth rate of 0.045/year for the food grains of wheat and pulses. Within time period II, the highest trend (1.635/year) was observed in wheat and the lowest trend (0.255/year) in pulses. Similarly, when considering the overall time period, it is clear that the maximum change rate was 1.508/year in wheat and the minimum change rate was 0.107/year in pulses. Moreover, in all three observed periods, the rate of change was increasing for all food grains.

In the view of overall trend analysis, the highest magnitude of trend (i.e., 1.635 million tons/year) was observed in wheat, and the lowest magnitude of trend (i.e., 0.045 million tons/year) was identified in coarse cereals. Here, it is noticeable that the trends were greater in time period II than in time period I and for the whole time period. In fact, the utility of food grains was booming based on their health benefits, medical purposes, and ability to sustain human life. For these reasons, the production of food grains failed to meet the needs for food grain utilization in India.

Finally, the surge in food grain production began after 1987, and food grain production has improved in the current scenario when compared to previous years, according to Indian agricultural history and trend analysis.

Conclusion

The present study examined trends and patterns in different major food grains in India. One important point that was noticed from the results is that food grain production, which includes rice, wheat, coarse cereals, and pulses, has increased since 1987, but it is not sufficient to meet human hunger. The gap between the availability of food grains and the requirements of a rising population, as well as the growth rate of food grains, was measured by the Sen's slope estimator. These results reveal that wheat and rice had the highest growth rate among coarse cereals and pulses. This suggests that rice and wheat comprise the predominant share of food grains produced in India.

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EMPIRIJSKA STUDIJA O OCENI TRENDA: PROIZVODNJA HLEBNIH ŽITA U INDIJI

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R e z i m e

Razmatranje važnosti određenih hlebnih žita-pirinča, pšenice, ostalih žitarica i leguminoza-od ključnog je značaja za razvoj indijske privrede. Ovaj rad otkriva trenutak promene i analizu trendova za godišnje istorijske serije hlebnih žita u Indiji. Trenuci promena su identifikovani metodama Petitovog testa (PT), Buišanovog testa opsega (BRT) i standardnog normalnog testa homogenosti (SNHT). S druge strane, Men-Kendalov (MK) test je korišćen za utvrđivanje prisustva i odsustva trendova u proizvodnji hlebnih žita, a njihovi nagibi su ocenjeni Senovom ocenom nagiba. Rezultat analize pokazuje značajne tačke promene za hlebna žita na nivou od 95%. Od 1982. do 2009. godine zabeleženi su pojedinačni trenuci promene, ali jedinstven trenutak promene zabeležen kod svih hlebnih žita je 1987. godina. U istoriji Indije, poljoprivredna proizvodnja u 1987. godini bila je ugrožena zbog suše, što je snažan dokaz potrebe da se razmotre metode zasnovane na dokazanim činjenicama. Rezultati Men-Kendalovog testa otkrivaju da je trend proizvodnje hlebnih žita bio statistički značajan i da je imao uzlazni smer u sva tri perioda segmentacije nakon 1987. godine.

Ključne reči: hlebna žita, trenutak promene, trend analiza, statistička analiza, statistička značajnost.

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U poglavlju „Rezultati i diskusija“ interpretiraju se podaci dobijeni na osnovu zapažanja i izvršenih eksperimenata. U komentaru rezultata treba se pozivati na literaturu koja se navodi na kraju rada, čime se obezbeđuje poređenje dobijenih rezultata sa dosadašnjim saznanjima u toj oblasti.

Zaključak

U zaključku treba ukratko navesti najznačajnije rezultate dobijene u radu. Izbegavati nabrajanje svih rezultata istraživanja sa ponavljanjem brojčanih vrednosti koje su prethodno već navedene u poglavlju „Rezultati i diskusija“. Zaključak ne sme da sadrži reference.

Zahvalnica

Zahvalnica treba da sadrži naziv i broj projekta, odnosno naziv programa u okviru koga je rad nastao, kao i naziv institucije koja je finansirala projekat ili program.

Literatura

Poglavlje „Literatura“ treba da sadrži samo radove citirane u glavnom tekstu. Rad citiran u tekstu treba da sadrži prezime autora i godinu. Ako citat obuhvata jednog autora on se navodi kao Jalikop (2010) ili (Jalikop, 2010). Kada citat obuhvata dva autora on se navodi kao Sadras i Soar (2009) ili (Sadras i Soar, 2009). Ako se u tekstu citiraju više od dva autora posle prezimena prvog autora navodi se skraćenica „et al.“, a zatim godina. Ovakav citat navodi se kao Lehrer et al. (2008) ili (Lehrer et al., 2008). Ako se za određeni problem istovremeno citira više radova onda se oni hronološki nabrajaju. Odvajanje većeg broja citiranih radova van

zagrade vrši se zarezom (,) a u zagradi tačkom i zarezom (;). Ako se citiraju dva ili više rada istog autora oni moraju biti poređani prema hronološkom redu (1997, 2002, 2006, itd.). Ukoliko se određeni autor pojavljuje nekoliko puta u istoj godini, dodaju se slova (2005a, b, c, itd.). Citate ličnih komunikacija i neobjavljenih podataka treba izbegavati, osim ako je to apsolutno neophodno. Takvi citati bi trebali da se pojave samo u tekstu (npr. Brown, lična komunikacija), ali ne i u spisku referenci.
Literatura koja je citirana u tekstu navodi se u spisku referenci u originalnom obliku, po abecednom redu, bez numeracije. Ako se citira veći broj radova istog autora najpre se navode radovi kada je autor sam, a zatim kada su prisutna dva i više autora. Ako se u nekoj od ovih kategorija javlja veći broj radova, treba ih hronološki srediti po godinama (1997, 2002, 2006, itd.), a ako se u istoj godini javlja veći broj radova dodaju se slova (2005a, 2005b, 2005c, itd.). Literaturni podatak treba da sadrži: prezime autora, početno slovo imena, godinu izdanja u zagradi, naslov rada, naziv časopisa, volumen i broj stranica (prva-poslednja). Prilikom citiranja knjiga navodi se izdavač i mesto izdavanja. Redovi svake reference posle prvog reda moraju biti uvučeni. U časopisu se koristi APA - Publication Manual of the American Psychological Association citatni stil.

Primeri navođenja referenci su sledeći:

Periodičan časopis
Gvozdenović, S., Saftić Panković, D., Jocić, S., & Radić, V. (2009). Correlation between heterosis and genetic distance based on SSR markers in sunflower (Helianthus annuus L.). *Journal of Agricultural Sciences*, *54*, 1-10.

Knjiga
Steel, R.G.D., & Torrie, J.H. (1980). *Principles and procedures of statistics*. New York: McGraw-Hill Book Company.

Poglavlje u knjizi
Bell, R.L., Quamme, H.A., Layne, R.E.C., & Skirvin, R.M. (1996). Pears. In J. Janick & J.N. Moore (Eds.), *Fruit breeding, Volume I: Tree and tropical fruits.* (pp. 441-514). New York: John Wiley and Sons, Inc.

Zbornik
Behera, T.K., Staub, J.E., Behera, S., Rao, A.R., & Mason, S. (2008). One cycle of phenotypic selection combined with marker assisted selection for improving yield and quality in cucumber. In M. Pitrat (Ed.), *Proceedings of the IXth EUCARPIA meeting on genetics and breeding of Cucurbitaceae* (pp. 115-121). Avignon.

Teza
Singh, N.K. (1985). *The structure and genetic control of endosperm proteins in wheat and rye*. University of Adelaide.

Izveštaj
Ballard, J. (1998). *Some significant apple breeding stations around the world*. Selah, Washington.

Veb sajt
Platnick, N.I. (2010). The world spider catalog, version 10.5. *American Museum of Natural History*. Retrieved February 12, 2016, from http://research.amnh.org/entomology/spiders/catalog/index.html

Rezime
Rezime na srpskom jeziku (za radove napisane na engleskom jeziku) ili na engleskom jeziku (za radove napisane na srpskom jeziku) navodi se na kraju rada i treba da ima od 200 do 250 reči. Ispred osnovnog teksta rezimea, navodi se naslov rada, puno ime, srednje slovo i prezime svih autora i naziv i adresa ustanove autora. Naslov „Rezime“ piše se razmaknuto i centrirano. Nakon naslova sledi jedan razmak, a zatim tekst rezimea, uvučen jednim tabulatorom. Neposredno nakon teksta rezimea, navode se ključne reči, sa tačkom na kraju. E-mail adresa autora za kontakt navodi se ispod crte, pri dnu stranice.

Tabele
Tabele obeležene arapskim brojevima (1, 2, itd.) praćene naslovom treba da se nalaze na odgovarajućem mestu u tekstu, u fontu 9. Maksimalna širina tabela treba da bude 13 cm. One treba da budu jasne, što jednostavnije i pregledne. Treba izbegavati vertikalne crte, a broj kolona ograničiti tako da tabela ne bi bila preširoka. Takođe, treba izbegavati nepotrebnu upotrebu horizontalnih crta. Naslov tabele, poravnat po levoj i desnoj margini, sa tačkom na kraju, navodi se sa jednim razmakom iznad tabele. Ispod tabele treba dati detaljno objašnjenje skraćenica, simbola i znakova korišćenih u samoj tabeli. Svaka tabela mora biti pomenuta u tekstu.

Ilustracije
Svi grafikoni, dijagrami i fotografije treba da se nazovu „Slika“ (1, 2, itd.). Prilažu se na odgovarajućem mestu u tekstu. Grafikone i dijagrame treba uraditi fontom 9, u crno-beloj tehnici i sa maksimalnom širinom od 13 cm. Voditi računa da oni budu čitki i jasni i nakon redukcije veličine. Za svaki grafikon i dijagram treba obezbediti detaljnu legendu bez skraćenica. Fotografije moraju biti visokog kvaliteta da bi se tehnički mogle dobro reprodukovati. Prilažu se u „TIF“ ili „JPG“ formatu, u crno-beloj tehnici. Naslov ilustracije, poravnat po levoj i desnoj margini, sa tačkom na kraju, navodi se sa jednim razmakom ispod ilustracije. Svaka ilustracija mora biti pomenuta u tekstu.

Skraćenice i jedinice
U radu treba koristiti samo standardne skraćenice. Merne jedinice treba izražavati u internacionalnom sistemu jedinica (SI). Kod navođenja jedinica posle broja treba da stoji razmak (osim za % i °C). Skraćenice se mogu koristiti i za druge izraze pod

uslovom da se ti izrazi navedu u punom obliku prilikom prvog pominjanja, sa skraćenim oblikom u zagradi. Vrednosti od 1 do 9 mogu se izražavati slovima, a ostali brojevi isključivo numerički.

Nomenklatura

Celokupna nomenklatura (hemijska i biohemijska, taksonomska, genetička itd.) mora biti usklađena sa međunarodnim kodeksima i komisijama, kao što su *International Union of Pure and Applied Chemistry, IUPAC-IUB Combined Commission on Biochemical Nomenclature, Enzyme Nomenclature, International Code of Botanical Nomenclature, International Code of Nomenclature of Bacteria* itd.

Formule

Sve formule i jednačine u radu moraju biti urađene pomoću programa „Word Equation". Pri pisanju formula, radi preglednosti, ostaviti dovoljno praznog prostora oko same formule. Subskripti i superskripti treba da budu jasni. Prilikom pisanja jednačina treba dati smisao svih simbola odmah posle jednačine u kojoj se simbol prvi put koristi. Jednačine treba da budu numerisane arapskim brojevima, serijski u zagradama, na desnoj strani linije. Svaka jednačina mora biti pomenuta u tekstu kao Eq. (1), Eq. (2), itd.

Nakon objavljivanja rada, autoru za kontakt će biti poslat jedan primerak časopisa.
Mole se svi budući saradnici da rad pripreme prema datom uputstvu, kako bi olakšali rad redakcije časopisa. Ukoliko se rad ne pripremi po navedenom uputstvu neće biti prihvaćen za objavljivanje.

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