

Diversity of indigenous arbuscular mycorrhizal fungi in rhizosphere of upland rice (*Oryza sativa* L.) varieties in Southwest Nigeria

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Arbuscular mycorrhizal fungi (AMF) have the potential to increase crop productivity and play a key role in the functioning and sustainability of most agroecosystems. However, limited information is available on the diversity of AMF associated with upland rice varieties in Southwest Nigeria. Field survey was conducted to investigate colonization and diversity of AMF in 13 upland rice varieties commonly grown in Southwest Nigeria. Root and soil samples were collected from rice fields in 2012. The results showed natural root colonization of all the rice varieties by AMF with highest root colonization in ITA 157 and Ofada. The spore densities retrieved from the different rhizospheres were relatively high, varying from 13 spores in UORW 111 to 174 spores in Ofada with a mean of 67.6 spores per 20 g dry soil. *Glomus* was observed to be the most abundant AMF genus. *Funneliformis mosseae* was the most frequently occurring AMF species (96.2%) with relative density (RD) of 32.2%, followed by *Glomus intraradices*, *Claroideoglomus etunicatum*, and *Glomus clareium*. This study showed that AMF naturally colonized the roots of these rice varieties and diversity of different AMF genera in rice rhizosphere. This study will help draw attention to natural colonization of AMF in rice producing areas of Nigeria that can influence future possibility of using inocula of the dominant AMF species in upland rice cultivation.

Keywords: Arbuscular mycorrhizal fungi, community structure, diversity, upland rice, spore density

1 Introduction

Rice (*Oryza sativa* L.) is one of the world's most important cereal crops. In Nigeria, rice is a major staple food consumed in many households. It is the only crop that is grown nation-wide in all agro-ecological zones, from the savannah to the coastal mangrove swamps of Nigeria. One of the limiting factors in sustainable utilization of agricultural land for rice production in Nigeria is the declining soil fertility. Arbuscular mycorrhizal fungi (AMF) are important and widespread components of soil microbial communities and agricultural ecosystems forming symbiotic relationships with roots of over 80% terrestrial plants, including many agricultural crops such

as rice, soybean etc. (Brundrett and Tedersoo, 2018; de Andrade Júnior et al., 2018). They are generally essential for many important ecosystem functions and processes, including nutrient cycling, plant productivity and sustainability (Van Der Heijden et al., 2015; Souza et al., 2016).

In many agricultural plants, AMF have shown the potential to increase crop productivity, thereby playing a key role in the functioning and sustainability of agroecosystems (Brundrett and Tedersoo, 2018; Silva-Flores et al., 2019). The most reported function of these symbiotic associations involves the transfer of nutrients such as organic carbon (C), in the form of sugars and

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lipids (Jiang et al., 2017; Luginbuehl et al., 2017), to the fungi by the plants, and the transfer of phosphorus (P) and nitrogen (N) to the plants by the fungi (Smith and Read, 2008; Van Der Heijden et al., 2015). AMF-mediated improvement in nutrient uptake may lead to increased growth and development of plants, and may confer resistance to abiotic and biotic stress (Ventura et al., 2018; de Moura et al., 2018). They enhance host growth and survival by improving tolerance to drought, salinity effects and to some root pathogens and nematodes (Chen et al., 2018). AMF may improve soil structure, ameliorate drought and salinity stress, and affect the diversity of plant communities (de Moura et al., 2018). Thus, the widespread benefits of AMF may be critical to increasing agricultural yields and productivity in a low-input manner.

AMF share a long history of coevolution with plants in various ecosystems, resulting in their adaptation to specific areas (Oehl et al., 2017). A number of factors have been shown to act as environmental filters, structuring AMF communities, such as host plants, land use, fertilization and soil pH (Lin et al., 2012; Peyret-Guzzon et al., 2016; Oehl et al., 2017). During the last two decades, different aspects of the association of crop plants with AMF have been studied extensively in different geographical regions and under different agricultural conditions (Gianinazzi et al., 2010). Those studies have shown variable effects of AMF on crop plants, ranging from mutualistic to parasitic. The effects of AMF can depend on soil moisture, the inorganic nutrient available in the soil, pH, species of AMF and the host plant species. Along with these factors, a number of agricultural management practices affect the soil environment, and therefore, mycorrhizal abundance and activity. Host preferences have also been demonstrated to exist to a certain extent in AMF (Pivato et al., 2007), but strict host specificity seems to be rare. However, it is well established that individual AM fungal species can differ in their associations with different plants (Adeyemi et al., 2019). Most studies on AMF community structure have been conducted at a small scale, with only a few authors reporting AMF diversity at the regional scale or larger (Hazard et al., 2013). Some AMF taxa have been reported to be surprisingly widespread (Davison et al., 2015), however, many cannot yet be directly linked to a certain set of agricultural practices or environmental conditions.

Colonization by indigenous or native AMF species in cereal crops in general and rice in particular has been reported earlier (Campos-Soriano et al., 2010). Despite this, in Nigeria, little attention has been paid to AMF associations in rice fields. Thus, it will be necessary to evaluate the natural association of AMF with rice plants, particularly in cultivars that are commonly cultivated

by farmers, to facilitate agricultural exploitation of the symbiosis. Given the paucity of information on the natural association of AMF with rice in different production areas of the southwest Nigeria, the aim of this study was to survey fields of different rice cultivars grown across the southwest Nigeria to determine the extent of AMF colonization and community structure in the rhizosphere. The study tested the hypotheses that AMF establish natural association with rice roots, and that the AMF colonization would differ among rice cultivars. This study was carried out on most of the rice cultivars grown in southwest Nigeria and demonstrated natural colonization of AMF in rice fields, which may have practical implications for increasing rice production and sustainability.

2 Material and methods

Study sites

The study was conducted across different rice fields in Southwest Nigeria in 2013. The region has a tropical climate with two main seasons, namely rainy (March – November) and dry season (December – February).

Field sampling

Root and soil samples were collected from rhizosphere of thirteen upland rice varieties grown in different locations in Southwest Nigeria (Table 1). Approximately 2 kg of soil and roots were collected from a depth of 0–30 cm using soil auger and stored in the refrigerator (10 °C) until processing. There were four sampling points in each field. At each sampling point, four subsamples (250 g) were collected, mixed and pooled to produce composite soil

Table 1 Upland rice varieties and coordinates of samples collection in 2013

No	Varieties	Location	Soil origin
1	Igbomo	Ekiti	Lat. 7° 79' N Long. 5° 51' E
2	ITA 157	Osun	Lat. 7° 62' N Long. 4° 73' E
3	ITA 150	Osun	Lat. 7° 62' N Long. 4° 73' E
4	NERICA 1	Ogun	Lat. 6° 95' N Long. 3° 50' E
5	NERICA 2	Ekiti	Lat. 7° 79' N Long. 5° 51' E
6	NERICA 8	Ogun	Lat. 6° 95' N Long. 5° 51' E
7	Ofada	Ogun	Lat. 6° 95' N Long. 3° 50' E
8	UDM 4A11	Ogun	Lat. 6° 95' N Long. 5° 51' E
9	UDRW 111	Ogun	Lat. 6° 95' N Long. 3° 50' E
10	UN 111	Ogun	Lat. 6° 95' N Long. 3° 50' E
11	UORG 311	Ekiti	Lat. 7° 79' N Long. 5° 51' E
12	UORW 111	Ekiti	Lat. 7° 79' N Long. 5° 51' E
13	WITA 4	Osun	Lat. 7° 62' N Long. 4° 73' E

samples. A total of 52 soil samples were thus collected. From these samples, the roots of the respective crop species were carefully freed from adhering soil and immediately fixed in 50% ethanol.

Soil analysis

Soil chemical and physical parameters were determined and analyzed using the following procedures; the soil pH was determined by the glass electrode in a 1 : 1 soil: water and in 1 : 1 KCl suspensions, particle size by the hydrometer method (Bouyoucos, 1951), organic carbon by the chromic acid oxidation procedure (Walkey and Black, 1934). Exchangeable bases were determined using the neutral ammonium acetate saturation, Na and K in solution were measured by flame photometer while Ca and Mg by Atomic Absorption Spectrophotometer, Exchangeable acidity was determined using the 1 M KCl extraction followed by 0.01 M NaOH titration. Total nitrogen was determined using the regular macro-Kjeldahl method, and available P by Bray 1 extraction method.

Staining and estimation of AMF root colonization

Roots in ethanol were rinsed thoroughly in tap water, cut into approximately 1 cm segments and cleared in hot KOH solution (10% w/v, at 90 °C) for 1 hour. The bleached roots were rinsed to remove excess KOH and stained in acidic glycerol containing methyl blue lacto-glycerol (1 : 1 : 1 : 0.5 g) at 90 °C for 30 minutes (Phillips and Hayman, 1970). The stained root segments were mounted on microscopic slides and examined for AMF structures (hyphae, vesicles and arbuscules) under light microscope to determine percentage root colonization (Adeyemi et al., 2017, 2020):

$$\text{Percentage root colonisation} = \frac{\text{number of root infected}}{\text{total number of roots}} \times 100$$

AMF spore isolation and identification

AMF spore extraction was done in triplicates for individual soil sample. The spores were extracted by the modified wet sieving method (Błaszowski, 2012). A sample of 25 g of air-dried field soil was mixed with distilled water. The resulting mixture was passed through 250, 150 and 40 µm sieves. The fraction retained in the 500 µm sieve was checked for large spores, spore clusters, sporocarps and organic matter debris. Soil materials retained by the 150 and 40 µm sieves were washed into centrifuge tubes using a small stream of distilled water. Tubes were centrifuged at 4,000 rpm for 2 minutes. The supernatants were decanted and subjected to sucrose centrifugation (70% (w/v)) gradient and centrifuged at 4,000 rpm for 2 minutes. The supernatant was passed through the

40 µm sieve, washed with distilled water and transferred to new Petri dishes. Spores, spore clusters and sporocarps were recovered and counted at 40× magnification. For identification, spores were picked under the dissecting microscope with a glass micropipette and subsequently mounted on slides with polyvinyl-lactic acid-glycerol (PVLG) or polyvinyl-lactic acid-glycerol mixed with Melzer's reagent (1 : 1 (v/v); Brundrett 2002) to get permanent slides for spore observation and identification under a compound microscope at up to 400× magnification. The spores were identified at the genus level on the basis of size, spore-wall structure, Melzer's reaction, colour and presence or absence of subtending hyphae and compared with descriptions of fungal genera according to taxonomic criteria (Oehl et al., 2011) and information available on the international network for vesicular arbuscular mycorrhizal (INVAM, 2018). The relative abundance, which is the ratio of the number of spores from a particular genus with respect to the total number of spores recovered was calculated based on percentage.

Ecological AMF diversity indices

To determine differences in the structure of the AMF communities on different crops, the following parameters were calculated: The isolation frequency (*IF*) of occurrence was calculated as the percentage of samples in which a genus or species occurred among all samples, and it reflects the distribution status. Relative spore density (*RD*) was defined as the ratio between the spore densities of a particular genus or species to the total AMF spore densities and it shows the degree of sporulation ability of different AMF in a given soil. The importance value (*IV*) was used to evaluate the dominance of AMF species based on *IF* and *RD* and was calculated as $IV = (IF + RD)/2$. An $IV \geq 50\%$ indicates that a genus or species is dominant; $10\% < IV < 50\%$ applies to common genera or species; an $IV \leq 10\%$ indicates that a genus or species is rare (Chen et al., 2012). Species richness (*SR*) is the number of AM fungal species recovered from each site per sample collection. Simpson's Index of Diversity ($D = 1 - \sum(P)^2$ where $P = n/N$, n is the relative abundance of the species calculated as the proportion of individuals of a given species to the total number of individuals in a community N . Shannon diversity index (*H*) by Shannon and Wiener is commonly used to characterize species diversity in a community, accounting for both abundance and evenness of the species present:

$$H = -\sum(P \ln(P))^2$$

Statistical analysis

Spore density and root colonization (%) were subjected to log $e(x + 1)$ and square root transformation respectively

for normalization of the data. Analysis of Variance was conducted to determine significant differences among the means at 5% probability level. Significant means were separated using Least Significant Difference (LSD). Pearson correlation analysis was used to detect the relationship between spore densities, percent root colonization and AMF relative abundance using the statistical package Genstat 12th edition

3 Results and discussion

Chemical and physical soil characteristics of the surveyed fields

The physico-chemical soil analyses are summarized in Table 2. The pH ranged from 5.1 to 6.8, and cation exchange capacity (CEC) values varied from 0.16 to 10.71 cmol kg⁻¹, while total amount of nitrogen (N) and available P contents were low. Organic carbon present in the soil was adequate.

AMF root colonization and spore density in the soil

The result showed that all the rice varieties root samples surveyed in this study were colonized by AMF. The mean percentage of AMF colonization was 50.1%, ranging from 33.6% to 76.2% (Figure 1). Percent AMF root colonization was highest in roots collected from ITA 150 (50%) and lowest in UORW 111 (33.6%). The spore densities (expressed as per 20 g dry soil) retrieved from different rhizosphere crops were relatively high, varying from 13 in UORW 111 to 174 in Ofada with a mean of 67.6 (Figure 2).

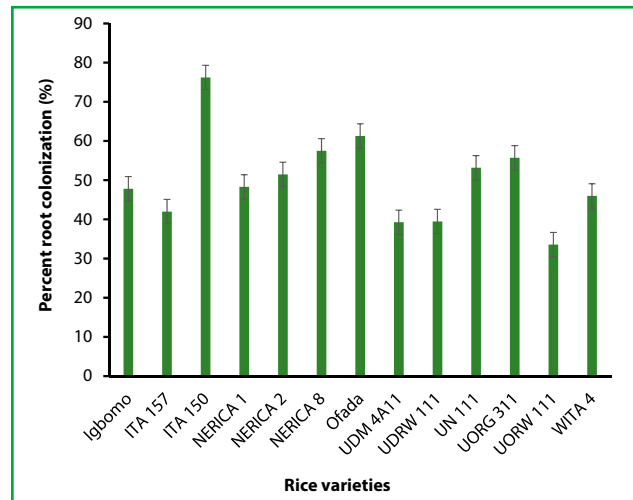


Figure 1 Percent AMF root colonization of 13 rice varieties

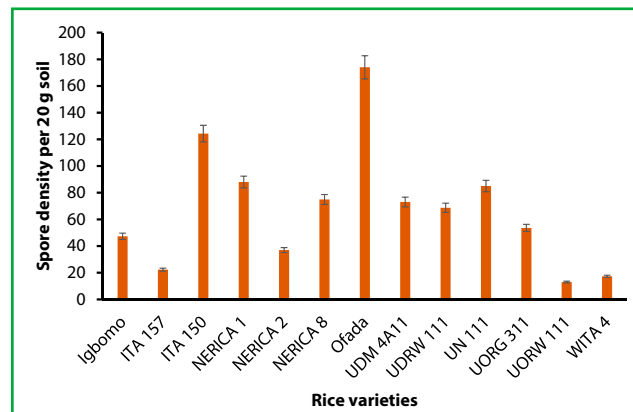


Figure 2 AMF spore density in rhizosphere of 13 rice varieties

Table 2 Chemical and physical soil characteristics of the surveyed fields

Varieties	pH	Sand (%)	Silt (%)	Clay (%)	OC (%)	N (%)	P (mg kg ⁻¹)	Ca (cmol kg ⁻¹)	Mg (cmol kg ⁻¹)	K (cmol kg ⁻¹)	Na (cmol kg ⁻¹)	CEC (cmol kg ⁻¹)	Exch. acidity (cmol kg ⁻¹)
Igbomo	6.16	76.4	12.8	10.8	1.05	0.11	1.02	2.20	1.22	0.30	0.35	4.17	0.10
ITA 157	6.48	86.4	8.80	4.80	1.73	0.17	8.88	4.01	1.83	0.40	0.37	6.70	0.09
ITA 150	6.37	85.2	8.80	6.0	1.95	0.20	1.34	2.61	0.81	0.32	0.29	4.12	0.09
NERICA 1	7.10	56.4	28.8	14.8	0.96	0.10	2.41	3.41	2.44	0.39	0.38	6.68	0.06
NERICA 2	6.17	76.4	17.6	6.0	1.15	0.12	2.22	1.20	0.41	0.82	0.43	2.96	0.10
NERICA 8	6.80	88.4	4.80	6.80	1.50	0.15	1.94	3.21	2.20	0.16	0.30	5.94	0.07
Ofada	5.96	86.4	8.80	4.80	1.62	0.16	1.16	2.61	0.81	0.23	0.32	4.08	0.11
UDM 4A11	7.13	86.8	6.80	6.40	2.19	0.22	2.45	4.01	1.62	0.19	0.29	0.16	0.05
UDRW 111	5.1	60.4	24.8	14.8	1.06	0.11	1.11	1.60	0.61	0.57	0.43	3.33	0.12
UN 111	6.16	76.4	12.8	10.8	1.05	0.11	1.02	2.2	1.22	0.30	0.35	4.17	0.1
UORG 311	7.73	86.0	7.20	6.80	2.99	0.30	5.56	8.22	1.62	0.53	0.32	10.71	0.02
UORW 111	6.66	86.4	6.80	6.80	1.06	0.11	1.19	1.80	0.61	0.19	0.29	2.97	0.08
WITA 4	6.14	86.8	8.80	4.40	0.78	0.08	0.51	2.0	0.41	0.41	0.39	3.33	0.10
Mean	6.46	79.88	12.12	8.00	1.47	0.15	2.37	3.01	1.22	0.37	0.35	4.56	0.08

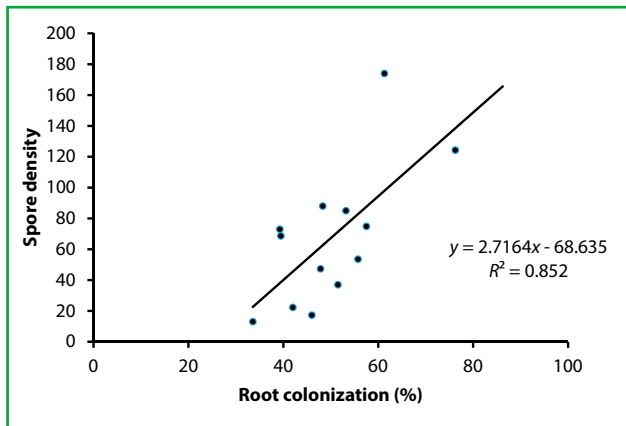


Figure 3 Relationship between AMF spore density and root colonization

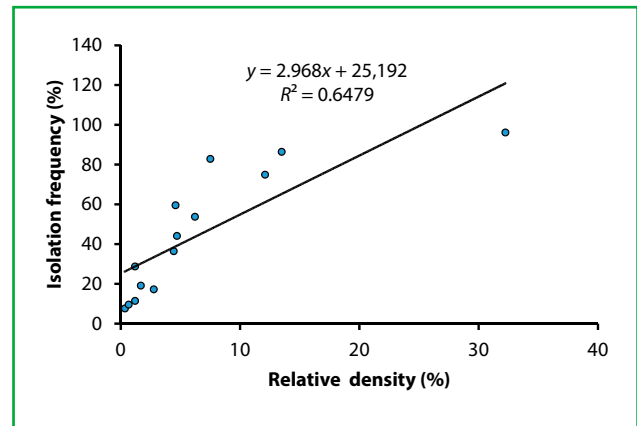


Figure 4 Relationship between AMF isolation frequency and relative abundance

There was a positive correlation between spore densities in the soil and the root colonization rates ($r^2 = 0.45$) (Figure 3).

AMF species richness and diversity

A total of 6810 AMF spores were identified and classified from the 52 rhizosphere soil samples. A total of 14 morphologically classifiable species of AMF were detected from this study. The most abundant genus was *Glomus* (Table 3). The relative density of AMF ranged from 0.34% to 32.2%, the isolation frequency from 7.9% to 96.2%, and the importance values ranged from 4.02 to 64.21 (Table 3). Relative density was positively correlated with the isolation frequency ($r^2 = 0.65$, $P < 0.05$) (Figure 4). In addition, *Funneliformis mosseae* was the most

frequently occurring AMF species (96.2%) with an RD of 32.2%, followed by *Glomus intraradices* (IF = 86.5%, RD = 13.5%), *Claroideoglomus etunicatum* (IF 82.9%, RD 7.5%), and *Glomus clareium* (IF 75%, RD 12.1) (Table 3). Maximum AMF diversity was recorded in Ofada and ITA 150 (Figure 5).

The widespread importance of AMF in rice production have received some attention in recent times (Vallino et al., 2014; Wang et al., 2015; Zhang et al., 2015), but no study has investigated how native AMF species naturally colonize rice and to what extent the natural colonization by AMF differs among commonly grown rice varieties in the southwest of Nigeria. In the present study, the results indicate natural AMF colonization in all the sampling

Table 3 Spore count per species, relative spore density, isolation frequency and importance value of arbuscular mycorrhizal fungi (AMF) identified from 52 soil samples from 13 upland rice varieties in South-west Nigeria.

AMF species	S	RD (%)	IF (%)	IV (%)
<i>Acaulospora scorobiculata</i>	81	1.19	28.8	14.9
<i>Acaulospora</i> sp	23	0.34	7.69	4.02
<i>Claroideoglomus claroideum</i>	312	4.58	59.6	32.1
<i>Claroideoglomus etunicatum</i>	511	7.50	82.9	45.2
<i>Funneliformis mosseae</i>	2,195	32.23	96.2	64.21
<i>Glomus intraradices</i>	918	13.48	86.5	49.9
<i>Glomus clareium</i>	823	12.09	75.0	43.5
<i>Glomus aggregatum</i>	423	6.21	53.8	30.0
<i>Glomus fasciculatum</i>	320	4.70	44.2	24.4
<i>Glomus</i> sp	302	4.43	36.5	20.5
<i>Glomus geosporus</i>	114	1.67	19.2	10.4
<i>Gigaspora gigantea</i>	188	2.76	17.3	10.0
<i>Scutellospora pellucida</i>	81	1.19	11.5	6.34
<i>Scutellospora</i> sp	45	0.66	9.61	5.14
Total AMF species richness: 14 species	6,810	100		

S = absolute number of spores identified per species; RD = relative spore density; F = isolation frequency; IV = importance value

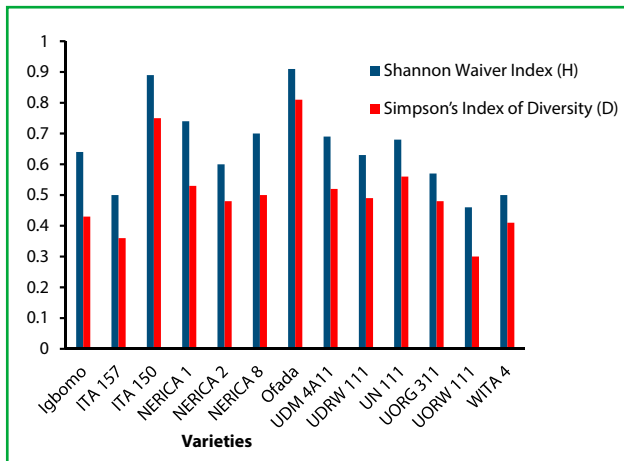


Figure 5 AMF diversity in rhizosphere of rice varieties

rice fields, confirming our hypothesis that natural AMF colonization is widespread in most soils.

In this study, variation in the spore density and root colonization of AM fungi naturally associated with the rice cultivars varieties was observed with ITA 157 and Ofada having highest root colonization and spore density respectively. Host preferences have been demonstrated to exist to a certain extent in AMF, but strict host specificity seems to be rare (Pivato et al., 2007). This could possibly be attributed to the differences in rooting habits and nutrients demands of the crops (Oehl et al., 2017) or amount of carbon transfer from the hosts to AMF. It has also been reported that AMF community composition depends on host plant species and, therefore, plant species may have varying degrees of selectivity on AMF species that range from selective specialists to non-selective generalists (Davison et al., 2015; Oehl et al., 2017).

Environmental factors and agricultural management practices in rice fields such as fertilizer input and water management have been reported to influence the root colonization and diversity of AMF communities (Lumini et al., 2011; Barber et al., 2013). Soil P has been known to have a negative impact on AMF symbiosis and at lower soil P concentrations; plants tend to allocate a higher fraction of available carbon to AMF, thus stimulating AMF colonization (Johnson, 2010). In general, AMF spore density was found to be positively correlated with root colonization in this study. This could be due to the fact that some AMF species rely more on extensive formation of hyphal networks in roots and survival through spore formation as primary infective propagules in soils.

There was a prominent distribution of AMF species among the rice varieties, with higher isolation frequency of *Funneliformis mosseae*, *Glomus intraradices* and *Claroideoglomus etunicatum*. High diversity may

be important for buffering an ecosystem against disturbances. Occurrence of maximum number of species results in higher index of diversity. Maximum diversity observed in the Ofada and ITA 150 indicated shared dominance of many AM fungal genera. The genus *Glomus* was the most dominant and widely distributed. It has been previously reported that *Glomus* species are the most abundant among the glomeromycotan genera in tropical areas (Snoeck et al., 2010; Oehl et al., 2017), regardless of the type of hosts and intensity of disturbance in the different ecosystems. Furthermore, *Glomus* species have the ability to produce a relatively high number of spores within a very short period of time (Oehl et al., 2017). The significant reduction in relative spore abundance of *Gigaspora* and *Scutellospora* may be due to soil disturbances due to agricultural practices such as tillage. Furthermore, Gigasporaceae have been reported to rely on spores as their primary infective propagules. Moreover, soil fertility and physical properties also play a key role for their occurrence in tropical soils (Lekberg et al., 2007).

4 Conclusions

All the rice varieties are managed with each variety having its own cultivation practice. The difference in cultural practices can cause changes in the suitability for growth of AM fungi. In the present study, dominance of *Glomus* genus was recorded in the rhizosphere of all the varieties. The information gathered from this study suggests the suitability of some of the AMF species to be used as inocula, and can be used to further investigate the impact of the symbiotic relationship between AMF and rice, which is becoming increasingly relevant for sustainable agriculture, where soil organisms may be useful for crop production.

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Genetic structure of breeds of goats bred in Slovakia

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The genetic structure of five goat breeds bred in Slovakia was characterized using a visible genetic profile and biochemical polymorphic systems. Tree dairy goat breeds – White Shorthaired, Brown Shorthaired and Alpine goats – and two wool goat breeds – Angora and Cashmere goats – were evaluated. Calculated were the heterozygosity and the effective number of alleles for each locus based on the allele frequencies of eight genes determining type traits and biochemical polymorphic systems, as well as their average values as indicators characterizing the genetic variability of each breed. The genetic differences between breeds were also determined.

Keywords: Goats, White Shorthaired, Brown Shorthaired, Alpine, Angora Cashmere, genetics, Slovakia

1 Introduction

Goat farming in Slovakia is a specific livestock sector where most goats are concentrated in small-scale breeding. At the end of 2018 of 36.9 thous. goats were bred in Slovakia. Goat-farming is of minor importance and has features of self-subsistence. An extensive production system with one kidding per year is applied according Oravcová (2013).

2 Material and methods

Using a visible genetic profile and biochemical polymorphic systems, we characterized the genetic structure of 5 goat breeds bred in Slovakia. We evaluated three dairy goat breeds (White shorthair goat – BKK, Brown shorthair goat – HKK, Alpine goat – AK), and two wool goat breeds (Angora goat – ANK, and Cashmere goat – KK). The goats of the BKK breed came from 3 herds ($n = 53–205$ pieces depending on the analyzed polymorphic system, HKK, AK, ANK and KK from one herd ($n = 7–54$).

In all individual breeds, we found the frequency of alleles for polledness (Ho), the occurrence of wattles of the goats (Wa), occurrence of the beard (Br) and length of ears (EL). In ANK and KK we have also determined the phenotype representation for the type (shape) of horns.

In the biochemical polymorphic of transferrin (Tf), X-protein (X), plasma arylesterase (Es) and haemoglobin (Hb) were calculated allele frequencies. Based on the allele frequencies of all eight genes determining type traits and biochemical polymorphic systems, were calculated the heterozygosity and the effective number of alleles for each locus as well as their average values. The genetic difference between individual breeds was also determined.

3 Results

We found that the frequency of the dominant Ho^p allele for polledness was 0.487 for BKK and 0.209 for HKK and AK; thus, the proportion of polled goats in BKK was higher

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than for HKK and AK. All evaluated KK and ANK were horned ($Ho^P = 0$). Three types of horns were observed in wool-type goats: markhor, ibex and intermediate type. The largest part of ANO and KK were goats with the type of markhor horns.

The occurrence of wattles on the neck of goats was observed in all breeds except for ANK ($Wa^+ = 1$). The frequency of the dominant Wa^w allele for the occurrence of wattles was low in other breeds (0.051–0.136), except for AK (0.567).

The recessive allele of the Br^B beard (0.633–0.870) prevailed in the female sections of the studied populations. Only in the HKK breed (0.378 and AK (0.250)) the frequency of the Br^B allele was lower than that of the dominant Br^+ allele for the lack of beard.

The occurrence of short-haired goats with reduced ear was observed in only one BK^K herd. The recessive EL^r allele frequency was 0.017 for the whole evaluated population.

In the analysis of biochemical polymorphic systems we found that all evaluated populations resp. the breeds were monomorphic in the haemoglobin system, with the fixation on Hb^A allele. All studied subjects also reacted negatively without dark colouring of plasma when testing arylesterase activity with the so-called tube test; i.e. all evaluated breeds were monomorphic in the plasma arylesterase system.

In the Tf system, the polymorphic breeds were BKK ($Tf^A = 0.898$; $Tf^B = 0.102$) and KK ($Tf^A = 0.914$; $Tf^B = 0.086$), but these populations can be considered panmictic in terms of the Tf system.

Other breeds were monomorphic in the Tf system ($Tf^A = 1$). In the X protein polymorphic system, the X^+ phenotype was higher in all populations.

The frequency of the dominant X^+ allele ranged from 0.342 to 0.863.

The highest genetic variability assessed based on the eight analyzed systems and expressed as mean heterozygosity (H) was found in BKK and AK ($H = 0.207$) with an average effective allele count only in AK ($Ne = 1.362$). The smallest genetic difference (D) was found between KK and ANK ($D = 0.0075$) and HKK and AK ($D = 0.0323$). The largest difference was found between AK and KK ($D = 0.1209$) and AK and ANK ($D = 0.0970$). The observed genetic differences primarily reflected the phylogenetic relatedness of the evaluated breeds and focus on their produce.

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Epiphyllous bryophytes in Arboretum Mlyňany (Slovakia)

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In this work we screened for the diversity of epiphyllous bryophytes covering leaves of evergreen plants growing in temperate region of Arboretum Mlyňany (Slovakia). We identified five taxa of not typically epiphyllous bryophytes, all belonging to mosses: *Brachythecium salebrosum* (Hoffm. ex F. Weber & D. Mohr) Schimp, *Hypnum cupressiforme* Hedw., *Hypnum cupressiforme* var. *filiforme* Brid., *Platygyrium repens* (Brid.) Schimp., *Pylaisia polyantha* (Hedw.) Schimp. All these taxa are considered as obligate members of bryoflora of Slovakia at low risk of extinction. The most abundant was the generalist *H. cupressiforme*, while the rarest was the typical epiphyte *P. polyantha*. All identified epiphylls occurred on nine species of evergreen angiosperm phorophytes: *Prunus laurocerasus* L., *Hedera helix* L., *Mahonia aquifolium* (Pursh) Nutt., *Ilex aquifolium* L., *Rubus caesius* L., *Viburnum × burkwoodii* auct., *Rhododendron catawbiense* Michx., *Viburnum rhytidophyllum* Hemsl., *Aucuba japonica* Thunb.; on one gymnosperm phorophyte *Cephalotaxus harringtonii* var. *drupacea* (Siebold. & Zucc.) Koidz; and on one fern *Asplenium scolopendrium* L. The most often species of phorophyte for epiphyllous bryophytes was *P. laurocerasus*, while the rarest taxa were *R. caesius*, *V. rhytidophyllum*, *R. catawbiense*, *A. japonica*, *V. × burkwoodii*.

Keywords: epiphyllous bryophytes, epiphylls, epiphytes, phorophytes

1 Introduction

Bryophytes are simple and small land plants. They have numerous important adaptations, including the alternation of gametophytic and sporophytic generations, specialization of gametangia, and the adaptation to desiccation. The bryophytes are considered to be the closest extant relatives of the plants that first colonized land. Due to their phylogenetic position, they are crucial for understanding the evolutionary transition from freshwater algae to land plants and from structurally relatively simple early land plants to more complex forms (Bennici, 2008; Ligrone et al., 2012; Bowman et al., 2017). Thus, they adapt to various environments ranging from harsh Antarctic conditions to extremely drought niches (Glime, 1982). Bryophytes play important roles in nutrient cycling and can act as bio-indicators of air and water pollution especially by heavy metals (Blagnyté and Paliulis, 2010).

Phyllosphere of vascular plants represents a complex micro-habitat inhabited by a diverse spectrum of epiphyllous organisms such as bacteria, fungi, algae, cyanobacteria, lichens and bryophytes (Pócs, 1996). Epiphyllous bryophytes are considered as plant semi-parasites because they lightly (in comparison with lichens) reduce photosynthesis, phosphorous content and hydration of host leaves (phorophytes), but can also be beneficial: deter herbivores, provide suitable micro-habitat for N-fixing cyanobacteria, provide some nutrients to plant, for example carbon (Berrie and Eze, 1975; Lepp, 2012; Zhou et al., 2014).

Many epiphyllous species are typically epiphylls, but some may also often be found on other plant parts (twigs, branches, trunks) or even on non-plant substrates such as soil or rocks. The typically epiphyllous species are only confined to the tropics. Majority of them are liverworts. But not typically epiphyllous species have been found also in sub-tropical to temperate regions in various

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parts of the world (Gradstein, 1997) however, their distribution and community structure remains largely unexplored. Epiphyllous bryophytes are widespread and often quite common in the tropical areas, but they occur in extratropical regions only amongst the most oceanic humid climatic conditions – e.g. Japan, China, Southern Appalachians (USA), Macaronesian Islands, Caucasus Mountains (Russia), British Columbia (Canada), Blue Mountains (Australia) and even Great Britain (Vitt et al., 1973; Smith, 1982; Pócs, 1989; Porley, 1996; Risk et al., 2011; Malombe et al., 2016). Leaf wetness having a large influence on phyllospheric organisms usually depends more on atmospheric than on soil humidity (Burkhardt and Hunsche, 2013). Thus foliicolous bryophytes need more humid microclimatic conditions than epigeic, epilithic or epiphytic ones. Therefore epiphylls are more vulnerable than other bryophytes. So, in the tropics with high humidity, but also high solar radiation, the invasion of exotics or the replacement of the original canopy by plantation trees usually means the total loss of the epiphyllous flora (Pócs, 1996). In temperate zones the diversity, roles and interactions of epiphyllous bryophytes are largely unknown. Thus, a question can be raised: does the partial replacement of the original canopy by more dense exotic trees and shrubs in temperate regions cause appearance de novo of epiphyllous bryophytes? For the answer on this question we have chosen Arboretum Mlyňany (Slovakia). So our goal was to estimate the diversity of bryoepiphylls on leaves of evergreen plants in the Arboretum, where is one of the largest collection of leafy evergreens in Eastern Europe (Hořka and Barta, 2012).

2 Material and methods

The study site, Arboretum Mlyňany, Detached Branch of the Institute of Forest Ecology, Slovak Academy of Sciences, is located in Vieska nad Žitavou near the town Nitra (Podunajská pahorkatina upland, foot of the Western Carpathians – Tribeč Mts. and Pohronský Inovec Mts., lat: 48.319656, long: 18.368701). It is situated in one of the warmest and driest areas of Slovakia with an average annual temperature of 9.8 °C and an average annual rainfall of 577.1 mm. The area is characterized by a prolonged dry periods during the height of summer with, sometimes, almost no rain at all (Hořka and Barta, 2012).

Arboretum Mlyňany is considered to be the first evergreen park in Central Europe established in 1892 (Hořka and Barta, 2012). Old Semper Vireo Park of 40 hectares as the main part of Arboretum Mlyňany was founded 127–105 years ago near to the native oak-hornbeam forest. The neighboring plot of Eastern Asian dendroflora of 14 hectares was established in 1965. The age of the

majority of adult trees growing there is determined by the time of the plots foundation.

The undergrowth in these areas consisted mainly of native species *Hedera helix* L., *Rubus caesius* L. and artificially planted *Prunus laurocerasus* L., *Mahonia aquifolium* (Pursh) Nutt., *Ilex aquifolium* L. (Hořka and Barta, 2012). Less frequently occurs *Viburnum × burkwoodii* auct., *Rhododendron catawbiense* Michx., *Viburnum rhytidophyllum* Hemsl., *Aucuba japonica* 'longifolia' Thunb., a conifer *Cephalotaxus harringtonia drupacea* (Siebold. & Zucc.) Koidz. and a fern *Asplenium scolopendrium* L. The undergrowth densely covered the ground (about 1 individual per a m²) around the trunks of mature trees.

In the territory of Arboretum Mlyňany bryophytes grew abundantly on rocks of several alpine gardens and trunks of old trees, especially on the oaks *Q. cerris* and *Q. robur* (Figure 1), but also on *Acer campestre* L., *Quercus × turneri* 'Pseudoturneri', *Ilex aquifolium* L., *Rhododendron catawbiense* Michx., *Malus* sp., *Taxodium distichum* (L.) Rich., *Carpinus betulus* L., *Cercidiphyllum japonicum* Siebold & Zucc., *Lonicera maackii* (Rupr.) Maxim., *Prunus serrulata* 'Amanogawa'.

The investigation was carried out in July 2019. The occurrence of epiphyllous bryophytes was surveyed on all leaves of undergrowth plants (0–2.5 m above the ground level) growing along all paths of old Semper Vireo Park and on the plot of Eastern Asian dendroflora. The leaves were considered as covered by a bryophyte only if the bryophyte was firmly attached to the leaf. All collected specimens were identified (Atherton et al., 2010; Danyluk et al., 2002; Frahm, 2009) after their macroscopic (under 5×, 10×, 16× magnification) and microscopic evaluation (under 200× magnification). Surface area (*S*) of bryophyte mats was measured.

3 Results and discussion

There occurred only mosses on leaves growing on understory twigs approximated or touching rocks or trunks densely covered by mosses (Figure 1). Far from such rocks or tree trunks (more than 1.5 m) no epiphyllous bryophytes were found. All observed moss species were not typically epiphylls. No epiphyllous liverworts were found in the investigated area.

In Arboretum Mlyňany we determined *Brachythecium salebrosum* (Hoffm. ex F. Weber & D. Mohr) Schimp., *Hypnum cupressiforme* Hedw., *Hypnum cupressiforme* var. *filiforme* Brid., *Platygyrium repens* (Brid.) Schimp., *Pylaisia polyantha* (Hedw.) Schimp. growing on leaves (Figure 2). The same moss taxa were found on the adjacent rocks or trunks of the trees. All these taxa are considered as



Figure 1 The occurrence of moss carpets on neighboring rocks (left) and *Quercus cerris* trunk (right) with bushes of *Prunus laurocerasus* (both photos) and an individual of the fern *Asplenium scolopendrium* (left photo)

obligate members of bryoflora of Slovakia at low risk of extinction (Mišíková et al., 2020).

The values of the surface area (S) of bryophyte mats, the percentage of available for moss branchlets (1.5 m around the rocks or tree trunks covered by the mosses) phorophyte leaf area bearing the epiphylls (P) and the maximal surface area per a leaf (S_{max}) for are presented in Table 1 and 2.

According to Table 1 the most abundant epiphyllous moss in Arboretum Mlyňany was *H. cupressiforme*. The leafy surface area covered by its mats (S) was $51.8 \pm 2.5 \text{ cm}^2$ (it was in 5 times higher than the sum of the surface areas of the other epiphylls). The percentage of available phorophyte leaf area bearing *H. cupressiforme* (P) was $4.7 \pm 0.5\%$ (it was in 6 times higher than the sum of the quantities P for the other epiphylls) and the maximal surface area per a leaf (S_{max}) was $3.0 \pm 0.4 \text{ cm}^2$ (it was almost equal with the sum of S_{max} for the other epiphylls). *H. cupressiforme* is considered as generalist (Nowińska et al., 2009; Mišíková et al., 2015; Wierzgoń and Fojcik, 2014) and occurred in Arboretum Mlyňany on the bark of different tree species (for example *A. campestre*, *C. betulus*, *I. aquifolium*, *L. maackii*, *P. serrulata* 'Amanogawa', *Q. cerris*, *Q. robur*, *R. catawbiense*), on the ground, on stones

and also on the leaves of seven phorophyte species: *A. scolopendrium*, *H. helix*, *I. aquifolium*, *M. aquifolium*, *P. laurocerasus*, *R. catawbiense*, *R. caesius*.

The second place for the surface area was possessed by *B. salebrosum*. The value of S_{max} for this species was of the same order with that for *H. cupressiforme*, while the orders of the quantities S and P for *B. salebrosum* were lesser than these for *H. cupressiforme*. Thus we can assume that *B. salebrosum* has not so good ability to bind with a leaf surface as *H. cupressiforme*, but like the latter taxon can grow there. Mišíková et al. (2015) reported this species as epigeic for several Slovakian villages, while in Poland and in Ukraine *B. salebrosum* is considered as generalist (Danylkiv et al., 2002; Wierzgoń and Fojcik, 2014). In Arboretum Mlyňany *B. salebrosum* abundantly grew not only on ground, but also on stones, on the *C. japonicum* and *L. maackii* trunks, on adjacent *C. harringtonii* var. *drupacea* needles and on *I. aquifolium* leaves correspondently.

The third place for the moss mats surface area belongs to *H. cupressiforme* var. *filiforme*. The quantities S and P for this species were of the same order with these for *B. salebrosum*, while the order of the value of S_{max} for *H. cupressiforme* var. *filiforme* was lesser than that for



Figure 2 Occurrence of non-specialized epiphyllic moss *Hypnum cupressiforme* (A–C) firmly attached to the leaves of (A) *Prunus laurocerasus*, (B) the fern *Asplenium scolopendrium*; (C) *Mahonia aquifolium*; (D) the moss *Brachythecium salebrosum* on needles of *Cephalotaxus harringtonii* var. *drupacea*

B. salebrosum. It allows us to assume that *H. cupressiforme* var. *filiforme* can poorly grow on the leaf surface, but like *B. salebrosum* it can easily bind to such a surface. Wierzgoń and Fojcik (2014) considered this species as epiphyte, which can grow also on logs of fallen trees, while Frahm (2009) stated that *H. cupressiforme* var. *filiforme* can grow also on rocks. In Arboretum Mlyňany *H. cupressiforme* var. *filiforme* grew on the trunks of *Q. cerris*, *Q. × turneri* 'Pseudoturneri', *T. distichum*, and also on adjacent leaves of 3 phorophyte species: *H. helix*, *P. laurocerasus*, *A. japonica* correspondently.

The next species in the list of recorded epiphyllous taxa was *P. repens*. The quantities S and S_{max} for this species were of the same order with these for *H. cupressiforme* var. *filiforme*, while the order of the quantity P for *P. repens* was lesser than that for *H. cupressiforme* var. *filiforme*. So we can think that the abilities of *P. repens* to bind with the leaf surface and grow there are considerably limited. For several villages in Slovakia *P. repens* reported as epiphyte (Mišíková et al., 2015), while this species commonly is

considered as facultative epiphyte (Király and Ódor, 2010).

In Arboretum Mlyňany it was found on leaves of 2 phorophyte species: *P. laurocerasus*, *V. rhytidophyllum*, and on the adjacent trunks of *C. betulus* and *A. campestre* correspondently. The quantities S , P , S_{max} for *P. polyantha* were the smallest in the list. This species was reported only as epiphyte (Danylkiv et al., 2002; Wierzgoń and Fojcik, 2014; Mišíková et al., 2015). Probably therefore in Arboretum Mlyňany it occurred incidentally on the leaves of two phorophyte species: *I. aquifolium*, *V. × burkwoodii*, adjacent to trunks of *I. aquifolium* and *Malus* sp., which abundantly covered by this moss.

According to Table 2 all phorophyte species for epiphyllous bryophytes in Arboretum Mlyňany can be divided into three groups.

To the first group belongs only the most abundant *P. laurocerasus*. Its leaf surface area covered by mosses (S) was $40.0 \pm 1.8 \text{ cm}^2$ (it was almost 2 times higher than the

Table 1 The list of recorded epiphyllous and phorophyte taxa, followed by the values of the surface area (S) of bryophyte mats, the percentage of available phorophyte leaf area bearing the epiphylls (P) and the maximal surface area per a leaf (S_{max})

Recorded epiphyllous taxa	Recorded phorophyte taxa	Surface area of bryophyte mats (S) (cm ²)	The percentage of available phorophyte leaf area bearing the epiphylls (P) (%)	Maximal surface area per a leaf (S_{max}) (cm ²)
<i>Brachythecium salebrosum</i> (Hoffm. ex F. Weber & D. Mohr) Schimp.	<i>Ilex aquifolium</i> L. 2. <i>Cephalotaxus harringtonii</i> var. <i>drupacea</i> (Siebold. & Zucc.) Koidz.	6.8 ±0.7	0.60 ±0.15	1.5 ±0.3
<i>Hypnum cupressiforme</i> Hedw.	1. <i>Asplenium scolopendrium</i> L. 2. <i>Hedera helix</i> L. 3. <i>Ilex aquifolium</i> L. 4. <i>Mahonia aquifolium</i> (Pursh) Nutt. 5. <i>Prunus laurocerasus</i> L. 6. <i>Rhododendron catawbiense</i> Michx. 7. <i>Rubus caesius</i> L.	51.8 ±2.5	4.7 ±0.5	3.0 ±0.4
<i>Hypnum cupressiforme</i> var. <i>filiforme</i> Brid.	1. <i>Aucuba japonica</i> Thunb. 2. <i>Hedera helix</i> L. 3. <i>Prunus laurocerasus</i> L.	1.7 ±0.4	0.10 ±0.05	0.7 ±0.2
<i>Platygyrium repens</i> (Brid.) Schimp.	1. <i>Prunus laurocerasus</i> L. 2. <i>Viburnum rhytidophyllum</i> Hemsl.	1.3 ±0.3	0.020 ±0.007	0.4 ±0.2
<i>Pylaisia polyantha</i> (Hedw.) Schimp.	1. <i>Ilex aquifolium</i> L. 2. <i>Viburnum</i> × <i>burkwoodii</i> auct.	0.5 ±0.1	0.020 ±0.008	0.20 ±0.08

Table 2 The list of examined phorophytes, followed by the values of the surface area (S) of epiphyllous bryophyte mats, the percentage of available phorophyte leaf area bearing the epiphylls (P), and the maximal surface area per a leaf (S_{max})

Leafy phorophyte name	Number of moss species	Surface area of epiphyllous bryophyte mats (S) (cm ²)	The percentage of available phorophyte leaf area bearing the epiphylls (P) (%)	Maximal surface area per a leaf (S_{max}) (cm ²)
<i>Prunus laurocerasus</i> L.	3	40.0 ±1.8	5.9 ±0.78	3.0 ±0.4
<i>Cephalotaxus harringtonii</i> var. <i>drupacea</i> (Siebold. & Zucc.) Koidz	1	6.0 ±0.6	0.6 ±0.15	1.5 ±0.3
<i>Mahonia aquifolium</i> (Pursh) Nutt.	1	5.7 ±0.5	0.6 ±0.31	2.0 ±0.3
<i>Hedera helix</i> L.	2	3.6 ±0.7	0.27 ±0.15	0.6 ±0.2
<i>Asplenium scolopendrium</i> L.	1	3.0 ±0.4	0.8 ±0.32	1.4 ±0.3
<i>Ilex aquifolium</i> L.	3	1.1 ±0.4	0.3 ±0.2	0.5 ±0.2
<i>Rubus caesius</i> L.	1	0.8 ±0.3	0.04 ±0.02	0.5 ±0.2
<i>Viburnum rhytidophyllum</i> Hemsl.	1	0.8 ±0.2	0.010 ±0.005	0.4 ±0.1
<i>Rhododendron catawbiense</i> Michx.	1	0.4 ±0.1	0.05 ±0.05	0.4 ±0.1
<i>Aucuba japonica</i> Thunb.	1	0.4 ±0.1	0.010 ±0.005	0.4 ±0.1
<i>Viburnum</i> × <i>burkwoodii</i> auct.	1	0.3 ±0.1	0.03 ±0.02	0.2 ±0.08

sum of the surface areas of the other phorophytes). The percentage of available *P. laurocerasus* leaf area bearing the epiphylls (*P*) was $5.9 \pm 0.78\%$ and the maximal surface area per a *P. laurocerasus* leaf (S_{\max}) was $3.0 \pm 0.4 \text{ cm}^2$. Thus we can assume, that *P. laurocerasus* leaves have the ability to bear mosses (especially *H. cupressiforme*, for which $S = 38.8 \pm 2.1 \text{ cm}^2$, $P = 6.1 \pm 0.81\%$, $S_{\max} = 3.0 \pm 0.4 \text{ cm}^2$) and may allow them to grow there.

The next five taxa can be corresponded to the second phorophytes group: *C. harringtonii* var. *drupacea*, *M. aquifolium*, *H. helix*, *A. scolopendrium*, *I. aquifolium*. The order of quantity *S* for them was 1 cm^2 , $P \sim 0.1\%$, $S_{\max} \sim 1 \text{ cm}^2$ (excepting *H. helix* and *I. aquifolium*, for which $S_{\max} \sim 0.1 \text{ cm}^2$). All these taxa had smaller, but not incidental ability to bear mosses and (excepting *H. helix* and *I. aquifolium*) may allow them to grow there. Further five taxa belong to the third group: *R. caesius*, *V. rhytidophyllum*, *R. catawbiense*, *A. japonica*, *V. × burkwoodii*. The quantities S_{\max} for the species of this group were of the same order with these for the taxa of the second group, while the orders of the quantities *S* and *P* for the members of the third group were lesser than these for the members of the second. Thus we can think that their leaves have only incidental ability to bear mosses and don't allow them to grow there.

We found no epiphyllous species growing far from the sites abundantly covered by mosses which indicate that in Arboretum Mlyňany epiphyllous mosses probably do not develop from the spores, they reproduce vegetatively. All found moss species are not parasites in their common status (Wierzgoń and Fojcik, 2014), the leafy area shading by them is not bigger than 3% of the whole leafy area per an individual (Table 1, 2). We can assume that in the Arboretum Mlyňany there are suitable microclimatic conditions for the growth of epiphyllous mosses:

1. subtropical evergreen understory plants densely planted near old trees;
2. high density of old trees and understory shrubs providing the damping of wind and the high humidity level near the ground in relatively arid region of Slovakia.

Usually liverworts prevail as epiphyllous bryophytes in tropical rain forests (Gradstein, 1997; Pócs, 1989; Pócs, 1996). But in temperate regions it is not always so. Thus in Canada Vitt et al. (1973) reported four species of mosses, all in the genus *Orthotrichum* growing on *Thuja plicata* L. leaves. In Arboretum Mlyňany it was find similar situation: all epiphyllous taxa belonged to mosses and among phorophytes there was one conifer species.

4 Conclusions

1. The partial replacement of the original canopy by more dense exotic trees and shrubs in Arboretum Mlyňany (Slovakia) probably created microclimatic conditions for the appearance of epiphyllous bryophlora.
2. In the Arboretum Mlyňany (Slovakia) five taxa of epiphyllous bryophytes were found. All of them were facultative epiphyllous mosses: *Brachythecium salebrosum* (Hoffm. ex F. Weber & D. Mohr) Schimp., *Hypnum cupressiforme* Hedw., *Hypnum cupressiforme* var. *filiforme* Brid., *Platygyrium repens* (Brid.) Schimp., *Pylaisia polyantha* (Hedw.) Schimp.
3. All these taxa are considered as obligate members of bryoflora of Slovakia at low risk of extinction.
4. The most abundant epiphyllous moss was the generalist *H. cupressiforme*.
5. The rarest epiphyllous moss was the typical epiphyte *P. polyantha*.
6. The most often species of phorophyte for epiphyllous bryophytes was *P. laurocerasus*.
7. The rarest taxa of phorophytes for epiphyllous bryophytes were *R. caesius*, *V. rhytidophyllum*, *R. catawbiense*, *A. japonica*, *V. × burkwoodii*.

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Growth of beef cattle as prediction for meat production: A review

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Increased interest in the breeding of beef cows results from the trends of society, especially in the consumption of quality raw materials of animal origin. Breeding of beef cattle is often encountered as part of a modern rural lifestyle. The good growth ability of calves is a decisive factor in the profitability of breeding of suckling cows and decides on the breeder's satisfaction in setting purchase prices. This quantity is expressed mainly by the average daily gains and the live weight of calves under one year of age. In addition to the achieved weight of beef, is very important shaping of individual body parts representing the most valuable meat parts of animal, to which the body measurements of sires must correspond. Weight gains point to the degree of adaptation of a specific breed to the farming conditions. Equally, the genetic basis of an individual influences the achieved weight of animal. Genetic improvement of meat performance depends on breeding programs that exploit genetic variability between breeds and within the breed. Moreover, the breeding conditions and animal handling could influence the increasing of live weight. Breeding efficiency will always be a summary of factors that determine the own cost and the purchase price of weaned calves. In view of the above, this review is focuses on the main intrinsic and extrinsic factors influencing the growth characteristics of different cattle breeds as well as its relationship with slaughter characteristics.

Keywords: body measurements, body weight, breed, factor, growth characteristics

1 Introduction

Natural way of cattle breeding on pastures is acquiring increased public interest derived from current social trends in consuming safe food of high quality. Beef cattle are most commonly bred this way, which is also linked with a modern rural lifestyle, agro tourism and culture. Mentioned reasons linked to require meat of high nutritional quality with favourable price. Bovine meat satisfies these demanding consumers' requirements, especially for its nutritional composition, juiciness and tenderness. Meat producers effort to quantify variability of parameters conveying the quality of the slaughter product allows breeders usage of feeding systems, which lead to the potential of producing meat of required quality whilst satisfying animal welfare. Beef producers may adjust production systems to better monitoring of meat quality, while breeders can preferably use variability among animals, by choosing animals with higher genetic

potential for producing meat of greater quality. It is desirable for the final product to show signs of certain quality during the life of the animal, which means using easily measurable biological components that relate to sensory attributes of quality. Many different biological mechanisms take part in expressing meat quality. These mechanisms show joint effects of different production factors (gender, age, breed, feeding, etc.) on sensory attributes (texture, colour, flavour), as well as biological characteristics of muscles (fibres, collagen, enzymes, lipids, etc.; Renand et al., 2001). Manipulation with beef quality for economic advantages requires understanding how factors as muscle type, sex and breed influence the muscle characteristics of a growing animal (Schreurs et al., 2008).

One of the most important parameters of beef production is the growth ability of animals (Toušová et al., 2014). Growth is fundamental and deciding factor

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in meat utility, especially in categories intended for fattening, growth intensity is of deciding importance. Muscle and fat production of slaughter animals is chiefly the result of growth function, thus the average development of an individual consists of a proportion of functional changes in growth, relative bone, muscle and fat ratio (Owens et al., 1995). The growth scale of animal is given by its genetically defined mature weight. Many characteristics of carcasses develop depending on achieved degree of maturity; the breed effect is often the result of differences at maturity. In the final stage of fattening, growth rate becomes an important economical aspect, since it determines feeding amount and length of fattening (Schreurs et al., 2008). Measuring body weight and composition allows for more accurate estimation of nutrients retention, while measuring weight and quality of carcasses allows for better determining economy of production (Owens et al., 1995). To improve not only the production of beef, but carcasses as well, genetic sources are important included differences among breeds. No single one breed excels in every attribute important for meat production (Bartoň et al., 2006). Increase in adult weight and size of cattle is nowadays aim of modern selection programmes. Furthermore, regional preferences of consumers are considered, especially in terms of the eventual product quality. Therefore, the body size, carcass composition, as well as the indicators of beef quality should be considered for regarding the biological relation between these parameters (Albertí et al., 2008).

In relation to the importance of bovine growth for meat production, the aim of this paper was to bring findings about growth intensity of some substantial beef breeds and factors influenced the growth. Concurrently, the review provides findings about slaughter and meat quality indicators, without which the characteristics of bovine meat production would not be complete.

2 Intrinsic factors influenced the growth of cattle

Growth intensity has decisive meaning, with growth function producing muscle and fat. The average development of an individual is dependent on functional changes in growth and ratio of bones, muscles and fat. Quality carcass is characterized by a large amount of muscle mass, minimal amount of bones and a relative amount of fat (Dikeman and Devine, 2004). Economically and production favourable is growth intensity and carcass composition showing in young animals, although the growth and development of young cattle is unequal. Growth intensity is also influenced by age of animal. With increasing age, the growth intensity is decreasing; the consumption of nutrients for weight gain is increasing

as well as individual tissues in the body are changing (Bobček, 2002).

The total muscle weight and mass increases until the end of growth in the process of proteosynthesis, as describe Dikeman and Devine (2004). Proteosynthesis is influenced by three factors: changes in muscle fiber (the ratio of individual muscle components changes), hormone-level control mechanisms (somatomedins, thyroid hormones, sex hormones, insulin) and environmental factors (nutrition, age, gender).

Application of the multiphase function allows detailed explanation of the pattern of live weight growth, body size of groups or individuals (Koops et al., 1989). Growth intensity during the ontogenesis is different, while growth allometry indicates the result. Growth allometry expresses the growth rate of tissue or organ in relation to entire growth. According to Flak and Antal (1980), the allometric function expressing the relation of the specific growth rate of a part of the organism to the growth of the entire organism has a special position in the evaluation of animal growth. Partial growth rate of organs and tissues vary from birth to adult; this change in growth is marked as differential growth. Since the growth of individual organs and tissues occurs at different times, these differences will be manifested by a change in body shapes and proportions of animals. The point at which the weight changes in the animals' fat content can be defined as the mature size (Dikeman and Devine, 2004). As describes Owens et al. (1995), the average fat content of animals' body at this stage is approximately 25%. Proportional growth was found to be measured as the weight of tissue, organ or part (individual muscles) relative to the entire or the tissue. Huxley (1993) was able to develop a mathematical method for detection of changes in growth of various tissues relative to an entire animal or tissue, which can compare relative growth of animal or tissue on a log scale using eqn:

$$y = bx^k$$

where:

- y – tissue weight
- b – represents the y intercept
- k – the slope or growth impetus

According to the above equation, the allometric growth of body measurements was also calculated by Pontecorvo (1939). Authors found a linear allometric relationship of withers height to the body weight of 6 cattle breeds at the level $a = 0.675-0.785$. Other authors (Kidwell et al., 1952) reported higher level of heritability of the constants "a" and "b" of this function at body

measurements characterizing skeletal development in comparison with measurements related to fleshiness.

Dikeman and Devine (2004) noted that live weight is the most important and most commonly measured parameter, probably because of the importance of weight for marketing. Weighting is important for breeding documentation, growth control and is closely related to utility and cattle size. There are differences in the live weight of cattle in terms of sexual dimorphism (bulls about 100–300 kg heavier), within the day (according to the filling of the digestive and urinary system up to 10%), during transport (weight loss 10–15% over 300 km). Measurements that are determined by the withers height and the body length of the animal may be expressed by an index. A Dimension – weight index is changing during growth of animal with highest value at birth. In terms of sex of animals, males exhibited a higher dimension – weight index than females (Yapp, 1924). Body measurements are important characteristics in dairy cattle as well as in beef cattle (Bene et al., 2007). They are often used to estimate animals' maturity and other characteristics, i. e. live weight. Moreover, body dimensions can also serve as important selective factors with a relatively high level of heritability.

Growth is influenced by the neuro-humoral system and the production of hormones with different effects on tissue growth. Hypophysis through somatotropic *growth hormone* affects growth and production of proteins, thyroid gland through thyroxine stimulates bones growth and metabolic activity, insulin affects growth and production of proteins and fat, transport of glucose, acetate and amino acids into cells (Dikeman and Devine, 2004). The adrenal medulla hormones, epinephrine and norepinephrine help to mobilize glycogen to provide muscle energy. In addition, their effects also affect muscle protein metabolism and lipid metabolism. Adrenalin can activate certain tissue receptors known as β -receptors, which shift available nutrients away from fat deposition and towards to muscle accumulation (Irshad et al., 2013).

Sex hormones such as androgens and estrogens can affect growth, especially growth and production of proteins and fats; glucocorticoids (cortisol and corticosterone) affect metabolism of carbohydrates, fats and proteins (Dikeman and Devine, 2004). Different growth rate as well as tissue composition are influenced by the sex of the animals, while males grow faster with later maturation. Compare to females, carcasses of males have higher muscularity and less fat content (Irshad et al., 2013). Muscle growth is stimulated by androgens as increase of protein synthesis, thereby reducing fat deposition. Higher development of the musculature, especially the forequarter, neck and back can be observed in bulls. In addition, androgen and

oestrogen stimulate bone salt deposition, which causes increased growth of bones in males compared to females or castrates. In general, oestrogens have minimal effect on skeletal muscle protein synthesis, but are effective in promoting body fat deposition, with their specific effects dependent on puberty and oestrogen concentration (Irshad et al., 2013). The hormonal state of cattle is directly related to the distribution of fats and proteins in muscles (Blanco et al., 2008).

Growth and reproduction as important economic features should be included in each breeding program. Before implemented a selection program, it is necessary to know not only the genetic parameters but also the relationship between them (Chin-Colli et al., 2016). One of the most important tasks of higher productivity in beef production is genetic improvement of growth. Heritability as a proportion of genetic variability to phenotypic variability is still a topical subject of genetics. Heritability, i.e. the heredity of bovine growth attributes, has been estimated in various breeds and populations, traditionally based on pedigree information. This traditional estimate is called "classical heritability" (Ryu and Lee, 2014). Nowadays, powerful genotyping is currently used, and the use of a commercial DNA microarray chip is already routine in identifying genomic association signals for complex phenotypes (Lu et al., 2013). Identification of molecular markers associated with gene expression and growth properties are under great attention (Dikeman and Devine, 2004). Genetic markers are of great importance for indicators of growth and meat utility. As part of the research on genetic markers, efforts have been made to develop genetic maps that allow the use of chromosomal regions and genes for selection. A number of papers deal with the identification of unknown quantitative traits locus (QTL) affecting economically significant properties (Louda et al., 2009). Indicators of meat utility are controlled by an unknown number of genes. Some of these individual genes have a greater effect on the traits than others. The number of QTLs detected for meat quality was found in pigs 4018, cattle 512 and for meat production in pigs 573 and cattle 973. A number of markers that are associated in relation to meat utility are used in commercial tests targeting for certain traits (Knoll, 2010). Table 1 represents selected markers of nucleotides for quantitative traits of cattle associated with meat quality, carcass composition and growth.

The *LIM-homeobox gene 3 (LHX3)* plays an essential role in the development of hypophysis and nervous system. The *LHX3* transcription and growth abilities in cattle are influenced by sequence variants in coding and non – coding regions of *LHX3* (Huang et al., 2015). The authors found the association of the SNPs 1-6 genotype

Table 1 Selected markers of QTN (nucleotides for qualitative traits) related to meat quality, growth and carcass composition of cattle

Trait category	Gene name	Gene symbol
Meat quality	fatty acid-binding protein 4	FABP4
	fatty acid synthase	FASN
	pituitary-specific transcription factor	<i>Pit-1</i> (POU1F1)
	stearoyl-CoA desaturase	SCD
	thyroglobulin	TG
	calpastatin	CAST
	micromolar calcium activated neutral protease (calpain 1)	CAPN1
Growth, feed intake	fatty acid-binding protein 4	FABP4
	G-protein-coupled receptor 137	GPCR137
	growth hormone	<i>GH</i>
	growth hormone receptor	GHR
	growth hormone-releasing hormone	GHRH
	insulin-like growth factor-1	IGF-1
	neuro peptide Y	NPY
	pituitary-specific transcription factor	<i>Pit-1</i> (POU1F1)
Carcass composition	<i>leptin</i>	LEP
	myostatin (growth differentiation factor 8)	MSTN (GDF8)

Source: Ibeagha-Awema et al. (2008); modified

with body weight at 6, 12 and 18 months of age in the Nanyang breed ($P < 0.01$; $P < 0.05$). Analysis by Sun et al. (2015) exhibited a strong linkage of SNP T1694A and C2213 G ($r^2 > 0.33$) in the study of the association of *cofilin2* (*CFL2*) polymorphism with growth abilities in Chinese Quinchuan cattle. For adipogenesis and regulation of the expression of fatty acid biosynthesis genes is responsible the *SREBP1c* gene. The level of its expression increases in parallel with the increase in fattening. Growth attributes analysis of Nanyang breed showed significant effect of SNPs in the *SREBP1c* gene on body weight and average daily gain at birth, 6 and 12 months of age (Huang et al., 2010). In the European Piemontese breed, the C313Y mutation in the *myostatin* gene responsible for muscularity and tenderness has been demonstrated. The developed double muscling evolves at the age of 3 months and thus does not affect the calving difficulty (Káčer, 2016). Also, *fibroblast growth factor 21* (*FGF21*) can be used as a gene for marker-assisted selection (MAS). This hepatic hormone regulates peripheral glucose tolerance, energy balance and lipid metabolism (Sun et al., 2013). Transcription of this gene in bovine liver is stimulated by *growth hormone* (*GH*), which is thought to be the major regulator of animal growth, development and metabolism (Yokoo et al., 2010). Sun et al. (2013) found the association of SNPs of bovine *fibroblast growth factor 21* with higher body weight after 18 months of

age in Nanyang cattle. In adult cattle, weight change is largely due to a change in fat deposition into tissues. *FGF21* is considered as a mediator of fat storage in cattle due to its function in regulating energy homeostasis and glucose metabolism, explaining its association with body weight control. *Leptin* is considered to be a candidate gene in the management of bovine performance, carcass quality and meat quality. The concentration of *leptin* is associated with adiposity and feed intake, therefore the changes in nutrition caused by a change in weaning period can affect both *IGF-1* and *leptin* concentrations and thus change growth and development (Blanco et al., 2009). Liu et al. (2010) reported *melanocortin-4 receptor* (*MC4R*) as the candidate gene for final body weight and carcass weight.

The heritability of most carcass traits is generally moderately high, while technological measures are more heritable than sensory characteristics (Irshad et al., 2013). Growth inheritance level reaches low values ($h^2 = 0.12-0.27$). As describe in Table 2, heritability of the live weight ranges between 0.10 in age of 6 months and 0.69 in 21 months of age. According to Szabó et al. (2007), the heritability of average daily gains of calves before weaning is $h^2 = 0.27$ and $h^2 = 0.30$ at weaning. While evaluating the direct and maternal effects, Duangjinda et al. (2001) found in the Charolais breed at weaning the value

$h^2 = 0.33$ for direct heritability, and $h^2 = 0.15$ for maternal heritability. There is a different correlation between direct and maternal genetic effects (Szabó et al., 2007). Knowing the dependence and correlation coefficient values makes it possible to determine the characteristics that support a particular production (Szabó et al., 2006). Authors Stålhammar and Philipsson (2008) evaluated components of variance according to gender for gains in weaning time and after weaning time in Swedish beef cattle. They found that genetic relationship between the direct effects observed in bulls and heifers was in most cases moderately to highly positive, on average $r = 0.7$. In terms of maternal effects, genetic and environmental, the correlations between genders was highly positive ($r = 0.7$ to 1.0). Bene et al. (2007) reported correlations between live weight and wither height $r = 0.84$ and between live weight and hip height $r = 0.70$.

The slaughter parameters are characterized by medium to high heritability, so they can be significantly influenced by the breed of animal (Irshad et al., 2013). Crossbreeding appears to be as a convenient alternative to change these characteristics because of wide variability between bovine breeds (Albertí et al., 2008). In contrast, dressing percentage, as a proportion of carcass weight from live weight of animal, achieves low to moderate heritability (Irshad et al., 2013; Coleman, 2016). There are differences in dressing percentage values between animals due to changes in digestive fill and weight, fat content in the carcass and other factors which influence the live weight (Coleman, 2016). In comparison with continental European breeds, traditional British beef breeds (Angus, Hereford, Shorthorn) tend to have lower dressing percentage values, because they are early maturing and they carcasses have higher proportion of fat in non-carcass depots (Irshad et al., 2013). Coleman (2016) mentions that the crossbreeding of British or Dairy breeds with European beef breeds, which mature later, potentially increases growth rate of calves as well

as dressing percentage and lean meat yield. Albertí et al. (2008) reported significant genetic variation of fat distribution in different parts of the cattle body. According to Casasús et al. (2000) in cattle it will occur also under similar nutritional conditions. When compare beef breeds which transform the nutrient mainly into proteins, dairy breeds with different hormonal and metabolic profile, deposit more intra-abdominal fat (Albertí et al., 2008). Relative to the deposition of subcutaneous fat, large late-maturing breeds (British beef breeds) have more intermuscular fat compare to continental European – small early maturing breeds, which have higher levels of intramuscular fat (Irshad et al., 2013). On the other hand, the differences in the physiological age of breeds at the same chronological age as well as differences in the growth potential between breeds could affect the variability of fat tissue in the body. The development of fat tissue is influenced by multiple factors; a relationship between fatness and breed type is still not clear (Albertí et al., 2008).

Genetics also plays a key role in determining average daily gains (ADGs). In general, continental cattle breeds achieve the required weight faster than other breeds. The expected differences in progeny within the breeds are based on individual genetics and thus overcome the differences in growth rate. Average daily gains can be enhanced by heterosis, with better offspring manifestations compared to parents (Drovers, 2013). Economic efficiency of beef cattle production systems is associated with the body weight and weight gain of cattle (Caetano et al., 2013). Genetic progress in field of body weight and weight gain can be reached by changes in these attributes explained by the additive action of genes and positive genetic correlation between them (Martínez-González et al., 2010). There also exist publications on a positive genetic correlation between sexual maturity and body weight of animals in both – young and adult ages. Due to the favourable genetic

Table 2 Coefficients of heritability of selected bovine growth indicators

Cattle		Coefficient of determination h^2	Range
ADG (prenatal)		0.38	–
ADG from birth to 7 months of pregnancy		0.29	0.27–0.30
Gains in the field test (85.–365. days of life)			
The live weight	6 months	0.31	0.10–0.53
	12 months	0.37	0.30–0.49
	21 months	0.44	0.22–0.69
Withers height	6 months	0.38	0.22–0.56
	12 months	0.51	0.44–0.64

ADG – average daily gain; Source: Krausslich, 1994

association within different age groups of animals, it is possible to improve body weight and weight gain at sexual maturity and reproductive performance of cows through selection for high body weight in young ages of animals (Caetano et al., 2013).

2.1 Maturation of cattle

By the term of physiological age, we mean the stage of development of the animal. Physiological age can be described by identifiable stages of the body development or body function, such as body height and weight, carcass composition or puberty onset. During growth, the composition and shape (form) of the body change dramatically and continually. Moreover, in genotype-dependent variation in carcass composition is stage of maturity an important factor (Irshad et al., 2013). Since different breeds vary in maturity extent and average weight, standardization of body composition measurement (muscle, fat and bone ratio) to the same level of mature body weight (actual weight to expected maturity) leads to much less variation in carcass composition than standardization to the same age or weight (Irshad et al., 2013). The higher weight of weaned calves coming from the crossing of dairy cows with beef sires is likely to reflect higher milk intake due to the impact of the dairy breed. Coleman (2016) found the faster growth rates after weaning of Hereford-sired cross straight-bred Angus steers, which indicate a tendency of crossbreds to grow to higher final weights and possibility of compensating the growth.

The ability of cattle to grow is correlated with the development of the body and the achievement of physical maturity, the calving ease as well as the maternal characteristics of the cow (Szabó et al., 2007). Several authors evaluated the relationship between calving ease and calf weight; they found that live birth weight as well as sex of the calf significantly influences calving difficulty, mainly in primiparous cows (Krupa et al., 2005; Strapák et al., 2000; Hradecká et al., 2000). Whereas the birth weight of calves influences calving ease in primiparous more than 71% and 61% in second calving, it represents one of the main selection criteria (Toušová et al., 2014). The highly significant influence of the year of birth, herd and sex of calves on the live weight at birth and weaning weight, as well as daily gain up to the weaning in the evaluation of Czech Fleckvieh and Beef Simmental crosses were determined by Vostrý et al. (2008). Calving ease statistically significantly influenced the live weight at the level of significance $P < 0.001$ and daily gains at the level $P < 0.05$. The genotype had a significant effect on birth weight. Papatungan and Makarechian (2000) based on their studies conclude that, calves from heavier cows in general, were heavier at birth and had a higher growth

rate before weaning. Calves from dams with average body condition score had faster growth than those born to cows with high scores of body condition.

The extent of growth of the animal is determined by its genetically defined mature weight. Mature weight is consistent with lifetime production. For most animals, a sigmoidal growth model is typical for reaching the maturity. However, animals exclusively used for meat production are often slaughtered before reaching the maturity. The slope of the growth curve indicates the growth rate and is usually expressed as the average daily gain (ADG, kg day^{-1}). The cattle with high weight gains produce more muscle fibres with greater glycolytic activity, which contributes to the meat aging processes and hence the tenderness that is considered as an important factor in consumer preferences (Albertí et al., 2008). Growth rate is an important economic aspect in the final stage of fattening, as it determines fattening time and feed quantity. Cattle with smaller body frame usually mature earlier and show slower growth rate at lower mature weight than later maturing cattle (Menchaca et al., 1996, Schreurs et al., 2008). Breeds with higher body weight need more time to reach puberty, so they can reach higher weight before reaching mature size (Papaleo et al., 2015).

2.2 Carcass characteristics associated with growth of cattle

Many carcass characteristics develop depending on the level of maturity reached; the result of differences in maturity is often the effect of the breed (Schreurs et al., 2008). Compared to later maturing breeds in the same age, earlier maturing breeds with smaller body frame are associated with lower muscularity and higher fat content (Schreurs et al., 2008, Scollan et al., 2006). Typical cattle breeds classified as early maturing are Aberdeen Angus, Hereford or Jersey (Table 3). Late maturing breeds are e.g. Limousin, Holstein, Charolais or Beef Simmental. Cattle of a smaller body frame are typically characterized by early adolescence at lighter mature weight, as described in Table 3 (Freer et al., 2007; Schreurs et al., 2008). According to Papaleo Mazzucco et al. (2016) when we compare breeds with different performance (British breeds, Continental breeds and cross-breeds), animals with a smaller mature size (British breeds) are characterized by lighter carcasses with greater fat thickness, smaller ultrasound rib-eye area and percentage of lean muscle at the same live weight.

According to paper of McMurry (2009) the weight of weaned calves is strongly affected by the influence of European breeds but also by using crossbreeding, complementarity and heterosis (McMurry, 2009). Producing calves for fattening and finishing from

Table 3 Standard values for carcass weight (kg) in different cattle breeds

Cattle breed	Cows	Steers	Bulls	Level of maturity
Jersey	400	480	560	early maturing
Aberdeen Angus, Hereford	500	600	700	early maturing
Limousine, Holstein	550	660	770	late maturing
Charolais, Beef Simmental	650	780	910	late maturing

Source: Freer et al., 2007

crossbreeding is nowadays a common practice due to the benefits of obtaining hybrid vigor (Papaleo Mazzucco et al., 2016). The productivity of beef cattle could be increased by utilising a cattle breed which is suited to the environment, mainly in terms of growth rate and carcass production (Albertí et al., 2008; Keane and Moloney, 2009). By means of carcass and meat quality parameters over different production systems, it is possible to estimate the potential value of a biotype (crossbreeds or pure-bred animals) for profitable beef production. Considering the purchase prices of products, the final weight of cattle is important characteristics for beef producers. There is a strong relationship between mature weight of cows and higher growth potential of calves that could achieve valuable carcass and purchase weight at an earlier age. The time required for preparing animal for sale or slaughter is an important economic attribute, hence the overall growth is the best available measure (Papaleo Mazzucco et al., 2016). On the other hand, the most reliable characteristic of animal growth potential is the live weight measured at the target age (i.e. one year old). Also carcass composition depends on the range of target weight and different growth curves of breeds (Albertí et al., 2008).

A quality product with the desired properties can only be produced in herds with high productivity (Bureš and Bartoň, 2012). Higher musculature is typical for European cattle with larger body frame, British breeds are characterized with marbling in meat; and carcasses of Zebu cattle have higher content of connective tissue (Blanco et al., 2008). Traditionally a selection of beef cattle breed or crossing has been on the basis of performance parameters, carcass market value, adaptability to the climate, availability of feedstuffs and personal preferences. However, the nutritional quality has recently received an increased attention, mainly in terms of health safety (Bartoň et al., 2008). For the consumer are important the quality attributes such as meat colour or fat content, which can be result of the combination of breeds or by the selection of an appropriate production system (Albertí et al., 2008). The beef quality and sensory properties are affected by several factors, such as nutrition, slaughter age and weight, slaughter or sex

category and pre-slaughter handling (Nogalski et al., 2017).

The genetic background belongs to the most important factors affecting meat quality (Prado et al., 2009). Meat from fairy breeds has been generally considered of inferior eating quality compared to British and European beef breeds (Muir et al., 2000). Nowadays, when dairy cattle is predominant in the cattle population, it is possible to effectively increase the quantity and quality of beef through commercial crossing of dairy cows with beef bulls in order to create herds for the beef production. Commercial crossing results in offspring which have higher fattening performance and higher slaughter quality (Nogalski et al., 2017). Coleman (2016) noted improved growth rate and meat yield of calves from crossbreeding European breeds over Angus or Hereford when compare to pure-bred Angus or Hereford cattle. For the beef calf production on the base of dairy cows, cattle breeds with a high growth potential such as Charolais are used (Bureš and Bartoň, 2012). Charolais cattle as a late-maturing breed could be fattened intensively to heavy body weights (Bartoň et al., 2008). Castration and beef production from steers are a common practice in beef production leader countries; such meat is in high demand (Vieira et al., 2007). Castration also improves the quality of beef by increasing the intramuscular fat content, which is a key determinant of the sensory properties of beef (Hocquette et al., 2010). In the same line, Nogalski et al. (2017) reported higher fat content in carcasses of steers slaughtered at higher body weights. Intensive fattening of steers until 18 months of age resulted in highest weight of most valuable meat cuts. Moreover, castration of the crossbred offspring is associated with lower slaughter weight and a shorter fattening period (Nogalski et al., 2017). Table 4 describe average values of slaughter age, slaughter weight and daily gain of different cattle breeds by several authors. Average daily gains of different breed ranged between 1.03 kg in Limousin cattle (Chambaz et al., 2003) and 1.97 kg in Angus cattle (Albertí et al., 2008).

The optimal slaughter ages and weights differ widely between breed types of cattle, what is particularly characterized by different fat deposition during the

Table 4 Basic slaughter characteristics of different cattle breeds

Breed	Slaughter age (days)	Slaughter weight (kg)	ADG (kg)	Source
Aberdeen angus	597.7	428.6	1.97	Albertí et al. (2008)
	433.7	562.3	1.17	Bartoň et al. (2006)
	381	–	1.30	Chambaz et al. (2003)
	510	662.5	1.23	Bureš and Bartoň (2018)
Charolais	634	460.6	1.53	Albertí et al. (2008)
	526.3	620.7	1.43	Bartoň et al. (2006)
	513	–	1.22	Chambaz et al. (2003)
	630	682.22	0.620	Vavrišínová et al. (2017)
Simental	621.8	455.9	1.49	Albertí et al. (2008)
	515.5	632.4	1.42	Bartoň et al. (2006)
	499	–	1.18	Chambaz et al. (2003)
Holstein	596.3	458	1.18	Albertí et al. (2008)
	515	655.6	1.32	Bureš and Bartoň (2018)
Holstein calves	–	155.0	0.825	Vavrišínová et al. (2010)
	151.80	150.30	0.740	Vavrišínová et al. (2019)
	198.20	179.00	0.710	Vavrišínová et al. (2019)
	203.40	210.00	0.840	Vavrišínová et al. (2019)
Limousin	594	–	1.03	Chambaz et al. (2003)
Limousin crossbreed	541.5	528.12	0.520	Vavrišínová et al. (2017)
Jersey	378.4	414.7	1.08	Albertí et al. (2008)
Hereford	482.5	540.1	1.32	Bartoň et al. (2006)
Fleckvieh	518.7	629.0	1.34	Bureš and Bartoň (2018)
Parda de montaña	309	447	1.65	Balnco et al. (2009)
Pirenaica	322	451	1.66	Blanco et al. (2009)
Gascon	539.7	659.2	1.29	Bureš and Bartoň (2018)
Charolais × simental	408.8	554.3	1.35	Bureš and Bartoň (2012)
	526	698	1.31	Bureš and Bartoň (2012)
Slovak pinzgau	600	471.00	–	Vavrišínová et al. (2009)

finishing period of fattening (Albertí et al., 2008). The slaughter age influences the beef tenderness in a greater extent than the rate of growth; hence later maturing cattle should be fattened to higher weights (Nogalski et al., 2017). When compare different cattle breeds, Holstein cattle tend to have more tough meat than British or European beef cattle, however, differences can be reduced by comparing animals at the same level of maturity or ageing the meat (Muir et al., 2000; Purchas and Zou, 2008). Increased likelihood of intramuscular fat from Jersey cattle results in tendency of Jersey cattle to have tender beef, often more tender than i.e. Angus or Holstein (Coleman, 2016).

Albertí et al. (2008) evaluated 436 young bulls from fifteen Western European breeds (beef, dairy and local types) to assess variability in live weight, total live weight gains, body measurements and carcass characteristics at different ages. They divided cattle into three groups in relation to their carcass characteristics. High meat producing breeds (Piemontese, Asturiana de los Valles, Pirenaica, Limousin, South Devon, Charolais, Aberdeen Angus) characterized by late maturing, short carcasses and high blockiness; animals with average meat production and intermediate characteristics (local and dairy breeds) Whereas breeds Jersey, Casina and Highland – breeds with low meat production corresponded to early maturing with long carcasses and low blockiness. Carcass

blockiness index expresses the relationship between length of carcass and hot carcass weight, while high index values indicate the high development of muscularity. Blockiness is reflected in the carcass conformation, which is an important criterion for carcass classification on the European market. Therefore, the carcass blockiness index can be a complementary tool for the classification of carcasses, especially in classifying of different breeds on the same market (Albertí et al., 2008). As noted in Albertí et al. (2005) the increase of blockiness from low to high meat producers in young bulls is rather constant; for the veal-type can be assessed a clear leap from medium to high meat producers. When comparing cattle of different breeds of similar age and with similar management, there are changes in carcass weight, while carcass composition depends on the range of total weights and differences in growth curves of each breed. According to Blanco et al. (2008), carcasses of animals with compensatory growth contain more fat depending on the length of fattening. Hence, compensatory of the growth could influence the tissue composition, while its variability may be originated in the length and quality of the restriction of the feed but also in the age during at restriction, the severity and the duration of restriction. In the weaning period, as describe Blanco et al. (2009) when is a predominance of muscle and bone tissues development, a nutritional restriction could compromise subsequent growth.

Growth parameters are also associated with the final weight and hip height at the end of fattening in feedlot tests (Morsy et al., 1998). Veal carcass quality and models of growth are influenced by different management and feeding conditions, as describes Domaradzki et al. (2017). To evaluate the growth rate and weight of livestock as well as feed utilization and carcass quality, the linear body measurement are used. These linear measurements better reflect animals body proportions than conventional methods of weighing (Essien and Adesope, 2003). Informations about animal weight and changes in weight are a key management tool; thus they are important for determine responses to genetic selection (Lukuyu et al., 2016). Using body linear measurements could be more reliable and offers advantages over subjective methods – weighing or visual assessment and scoring. These methods can be influenced by short-term effects such as urination, defecation or gut fill (Lukuyu et al., 2016). Lukuyu et al. (2016) reported a strong correlation between live weight and heart girth ($r = 0.84$) as well as body condition score ($r = 0.70$). Moderate correlation was found between hear girth and body length ($r = 0.66$). Body weight of livestock as a good indicator of animal condition is an important factor in selection for slaughter, breeding or determining feeding levels (Ozkaya and Bozkurt, 2009).

Many authors describe body measurements of cattle as important selection criteria for growth (Van Marle-Köster et al., 2000). There is a relationship between body measurements and body weight, which is influenced by breed of animal, age, utility type, body frame, body condition score or level of fattening. Body measurements are in close correlation with body weight of animal, while heart girth is often describes as best prediction parameter (Ozkaya and Bozkurt, 2009). Authors reported the highest correlation between body weight and heart girth in Brown Swiss cattle ($r = 0.95$) and body length ($r = 0.89$). In the same line correlation coefficients in Holstein cattle were 0.78 and 0.69. Evaluation of relationships between growth characteristics in beef cows were reported in Bene et al. (2007). Correlations between live weight and body measurements were moderate to strong positive ($r = 0.40$ – 0.83), between age and body measurements moderate positive ($r = 0.01$ – 0.46), between body measurements moderate to high positive ($r = 0.22$ – 0.81). According to Maiwashe et al. (2002) strong genetic correlation (0.76 ± 0.06) between shoulder width and body length indicates, that selection for measurements in shoulder area could lead to rapid progress in body length.

4 Conclusions

Meat performance is influenced by several factors, it is a function of fertility, is carried out in growth and development processes and is characterized by indicators of fattening, carcass value and meat quality. Among the most important factors influencing the growth of cattle above cited authors include internal properties (neurohumoral system, breed, utility type, sex, body frame) as well as external properties, especially length of fattening, nutrition and management. The most significant correlations of growth, carcass and meat quality characteristics were determined between live weight of cattle and heart girth, body length as well as between body weight and age of animal. Nowadays, the analysis of genes associated with gene expression and growth characteristics are of enormous importance in evaluation of meat performance. In beef production system, genetic improvement of cattle growth is currently one of the most important tasks. In addition, attention of geneticists is directed to identifying unknown QTLs associated with meat quality. In determining this area, number of QTLs for meat quality was detected – 4,018 for pork, 512 for beef, 573 for pork meat production and 973 for beef production.

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Performance evaluation of induced mutant lines of black gram (*Vigna mungo* (L.) Hepper)

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Present investigation was carried out to explore the possibility of inducing genetic variability for yield and yield contributing traits in well-adapted variety PU-19 of black gram (*Vigna mungo* (L.) Hepper) following mutagenesis with methyl methane sulfonate (MMS), sodium azide (SA) and hydrazine hydrate (HZ). A considerable increase in mean values for fertile branches per plant, pods per plant and total plant yield was noticed among the mutant lines in M₄ and M₅ generations. Estimates of genotypic coefficient of variation, heritability and genetic advance for yield and yield components were also recorded to be higher compared to control. MMS followed by SA and HZ showed highest mutagenic potential for improving total plant yield of black gram var. PU-19. Treatment concentration 0.3% was found to be most effective in generating significant increase in total plant yield of black gram var. PU-19. The increased genetic variability for yield and yield components indicates the ample scope of selection for superior mutants in subsequent generations due to preponderance of additive gene action.

Keywords: black gram, mutagenesis, chemical mutagens, genetic variability, yield components

1 Introduction

Grain legumes, commonly known as pulses, occupy a pivotal position in meeting the protein needs of masses in developing countries like India. These have proved to be nutrient dense food stuffs especially the source of vegetable proteins. Besides nutritional values, pulse crops are endowed with unique property of maintaining and restoring soil fertility through biological nitrogen fixation (Dewanjee and Sarkar, 2017). The cultivation of pulse crops is preferred in rainfed areas of the country having poor management conditions prevailing with high biotic and abiotic stresses. In spite of constraints like unfavourable environment, non-availability of quality seeds, poor post-harvest management and inadequate market, the country has raised the annual pulse production from 8.41 to 16.35 million tonnes attributable

to area expansion from 19.09 million ha in 1951 to 24.91 million ha during 2015–2016 and filled a yield gap from 441 to 656 kg ha⁻¹ (Annual Report, 2016–2017). With population growth, the demand for food and feed is consistently growing, while natural resources are limited. Erratic rainfalls, sudden and severe drought conditions even deteriorate the crop production conditions (Auti, 2012).

Low yield arising from susceptibility of crops to diseases and pests and the absence of an effective mechanism to ensure remunerative returns have further forced the farmers to grow pulses on marginal lands. Development of high yielding and disease resistant varieties is the basic need of the time. Use of induced mutagenesis in breeding programmes for developing superior varieties has been used extensively for developing new crop

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cultivars and for changing the plant traits. During the past five decades, more than 3200 varieties have been released worldwide either as direct mutants or from their progenies (FAO/IAEA). Improvement of best available varieties by inducing mutations in one or two major traits without altering the basic genotypic and phenotypic design of the crop has been the background objective of mutation breeding (Ahloowalia et al., 2004; Shu et al., 2012; Mba, 2013; Tomlekova et al., 2014; Laskar et al., 2015; Raina et al., 2019). Genetic variability is one of the pre-requisites for crop improvement. The genetic variability in most of the pulse crops including black gram has been greatly reduced over the years due to natural selection under low level of management. Considering the several reported works in various crops (Giri et al., 2010; Dewanjee and Sarkar, 2017; Laskar and Khan, 2017; Wani, 2018; Laskar et al., 2018; Goyal et al., 2019; Raina et al., 2020), it is now quite clear that induced micro-mutations bring about significant genetic variability in the mutagen treated population(s). Micro-mutations basically create variability in quantitatively inherited traits in plants, which necessitates plant breeder's keen attention towards their utilization for improving complex trait(s) including yield.

The modes of action of different mutation causing agents are different (Laskar et al., 2019); hence wide variations can be observed in the resultant mutations induced in the crop population(s) exposed to different mutagens and their concentrations/doses. In general, chemical mutagens cause single base-pair (bp) changes or single-nucleotide polymorphisms (SNPs) while physical mutagens cause deletions or translocations in the exposed genome(s) (Sikora et al., 2011). In practice, multiple mutagens are used for inducing mutation(s) in target crop genotype(s) in a single experiment to amplify the scope of inducing wide range of desirable mutation(s). As reviewed by Laskar et al. (2019), among the various chemomutagens, alkylating agents like methyl methane sulphonate (MMS) are the most potent and commonly used category of chemical mutagens, which mediate through alkylation of DNA at various sites that result into micro-mutation(s) in the exposed genotype(s), useful for improvement of economically important quantitative trait(s). Sodium azide (SA) creates point mutation(s) in the targeted genome through the interaction between organic metabolite of azide and DNA, which is highly dependent on acidic pH. Hydrazine hydrates directly reacts to cause loss of the pyrimidines from DNA, G : C–A : T transitions and intermediate radical reactions, which results into wide variations in the subjected genotype(s). Mutagenicity of these highly potent chemomutagens was utilized in the present investigation to broaden the genetic

variations induced in the desirable quantitative traits of black gram var. PU-19.

Black gram [*Vigna mungo* (L.) Hepper], commonly called as urdbean, is an important legume crop of India. It belongs to family Leguminosae and sub-family Papilionaceae with Indian subcontinent as the centre of its origin. It is considered to be domesticated from its wild progenitor *Vigna mungo* var. *silvestris* (Gill et al., 2017). Urdbean possesses deep penetrating root system which enables it to utilize the limited available moisture content more efficiently than many other competitive crops and contribute substantially to the loosening of the soil. Due to this reason, farmers choose to grow urdbean under highly diversified conditions. Moreover, urdbean is an excellent source of high-quality dietary protein with good digestibility and contributes a major portion of lysine in vegetarian diets of most of the Asian population (Gill et al., 2017). As per the MoA&FW (2020), around 80 recommended black gram varieties are under cultivation at present in India. Black gram var. PU-19, used in this experiment, was developed by pedigree method of selection from the cross between UPU-1 X UPU-2 (MoA&FW, 2020). Among the several breeding strategies, mutation breeding has been successfully implemented in creating 8 mutant varieties in India till now (MVD, 2020). Keeping in view the economic and nutritional importance of black gram, the present investigation was carried out to estimate the improvement of yield and yield contributing traits in M_4 and M_5 generations of black gram var. PU-19 following mutagenesis with MMS, SA and HZ.

2 Materials and methods

Uniform and healthy seeds of black gram (*Vigna mungo* (L.) Hepper) var. PU-19 were pre-soaked in distilled water for 9 hours, prior to treatment with chemical mutagens viz., 0.1–0.4% of methyl methane sulphonate (MMS) and 0.01–0.04% of sodium azide (SA) and hydrazine hydrate (HZ) for 6 hours. The untreated seeds soaked in distilled water for 15 hours were sown as control. Initially, the concentrations of chemical treatments were determined based on preliminary germination and survival test, where inhibition above 50% was considered lethal. The solutions of MMS and HZ were prepared in phosphate buffer of pH 7, whereas SA solution was prepared in phosphate buffer adjusted to pH 3. All the chemically treated seeds were thoroughly washed in running tap water to get rid of the remaining mutagens from seed surface. The experiment was carried out at the Agriculture Farm, Aligarh Muslim University, Aligarh, India. The site of present study viz., Aligarh, is located in the north-western part of Uttar Pradesh, India and extends from 27° 29' to 28° 11' North latitudes and 77° 29' to 78° 38' East longitudes. Aligarh

has the characteristic semi-arid and subtropical climate with hot dry summers (~35 °C) and cold winters (~15 °C) with average rainfall of about 847.30 mm. One hundred seeds for every treatment and control were sown in the field in a randomized complete block design (RCBD) to raise M_1 generation. The distance between the seeds in a row and between the rows was kept at 30 cm and 60 cm respectively. Seeds harvested from individual M_1 plants were sown as M_2 families in three replicates in the field. For raising M_3 generation, such 10 M_2 progenies were selected which showed significant deviation in mean values in the positive direction from the mean values of control, particularly for the yield and its associated components. Seeds from each selected M_2 progeny were bulked by taking an equal amount of seeds from each M_2 progeny and thoroughly mixed. A random sample of this bulk was sown to obtain M_3 progeny. Progenies of each M_3 selection for seed yield trait were grown again as families in M_4 generation. Similar procedure was adopted to raise M_5 generation. The mutagenised populations were allowed to advance in subsequent generations based on yield statistics from M_2 onwards to narrow down the selection and attain yield stability. Finally, quantitative evaluations of yield traits were done at M_4 and M_5 generations to ascertain the yield performance of significantly stable mutant lines. Data collected for fertile branches per plant (counted at maturity as the number of branches which bore more than one pod), pods per plant (number of pods borne on a whole plant) and total plant yield (weight in grams of total number of seeds harvested per plant) of the mutant lines isolated in M_4 and M_5 generations were subjected to statistical analysis in order to assess the extent of induced variation. Analysis of variance was done according to Singh and Chaudhary (1985) to find out the variance between the families and within the families. The components of variance considered were:

1. within-family variation in the control and in the treated material which was an estimate of environmental variation;
2. between-families variation which was an estimate of between families genetic variation.

Genotypic Variance (σ^2g) – the genotypic variance (σ^2g) was estimated by the following formula:

$$\sigma^2g = \frac{(MS_{Bf} - MS_e)}{N}$$

where:

MS_{Bf} and MS_e – mean sum of squares for between families and within families or error, respectively

N – number of replications

Genotypic Coefficient of Variation (GCV):

$$GCV(\%) = \frac{\sqrt{\sigma^2g}}{\bar{X}} \times 100$$

Phenotypic Variance (σ^2p) – was estimated by summing the estimated genotypic variance (σ^2g) and the environmental variance (MS_e or σ^2e):

$$\sigma^2p = \sigma^2g + \sigma^2e$$

Phenotypic Coefficient of Variation (PCV):

$$PCV(\%) = \frac{\sqrt{\sigma^2p}}{\bar{X}} \times 100$$

Heritability (h^2) – It is the ratio of genotypic variance to the total phenotypic variance. The broad-sense heritability (h^2) was estimated by the formula suggested by Johnson et al. (1955).

$$h^2(\%) = \frac{\sigma^2g}{\sigma^2t} \times 100$$

where:

σ^2g – induced genotypic variance

σ^2t – total phenotypic variance ($\sigma^2t = \sigma^2g + \sigma^2e$) calculated from the treated population

Genetic Advance (GA) – the estimates of genetic advance (GA) with 1% selection intensity were based on the formula given below:

$$GA = k \times \sigma p \times h^2$$

where:

h^2 – broad sense heritability

σp – phenotypic standard deviation of the mean performance of treated population

K – 2.64, constant for 1% selection intensity

$$GA (\% \text{ of } \bar{X}) = \frac{GA}{\bar{X}} \times 100$$

Critical Difference (CD) – between the means of treated and control population was estimated from the error mean square and tabulated 't' value at 5% level of significance:

$$CD \text{ at } 5\% (p = 0.05) \text{ level} = \sqrt{\frac{2 MS_e}{r}} (t - \text{value at } 5\% \text{ level})$$

3 Results and discussion

In present investigation, a wide range of mutant phenotypes with altered characteristic affecting different plant parts were induced in the mutagenized populations of the black gram var. PU-19. The induced mutations for detectable phenotypes in yield attributing traits like branches and pods at different growth phases of plants were categorically inspected throughout the M_2 and M_3

season. Quantitative analysis of this induced phenotypic diversity facilitated the selection of desirable mutant progenies for advancement into subsequent generations from M_2 onwards and allowed up to M_5 generation for attainment of trait stability. The data recorded on fertile branches per plant, pods per plant and total plant yield is presented in Tables 1–3. It is clear from the data that mean values increased significantly for yield and yield

Table 1 Estimates of mean values (\bar{X}), shift in \bar{X} and genetic parameters for fertile branches per plant in M_4 and M_5 generations of urdbean var. PU-19

Treatment	Mean \pm S.E.	Shift in \bar{X}	PCV (%)	GCV (%)	h^2 (%)	GA (% of)
M_4 generation						
Control	6.26 \pm 0.12	–	12.12	7.14	18.24	6.10
0.1% MMS	6.62 \pm 0.22	+0.36	26.34	18.15	36.76	20.50
0.2% MMS	7.39 \pm 0.14	+1.13	28.20	20.19	40.92	24.20
0.3% MMS	7.62 \pm 0.17	+1.36	30.15	21.12	41.86	21.90
0.4% MMS	6.34 \pm 0.20	+0.08	21.10	16.86	38.72	16.22
CD ($p = 0.05$)	–	0.63	–	–	–	–
0.01% SA	6.36 \pm 0.16	+0.10	16.80	11.20	22.20	14.20
0.02% SA	7.10 \pm 0.21	+0.84	22.20	16.71	34.74	18.82
0.03% SA	7.27 \pm 0.18	+1.01	24.62	17.62	38.90	20.62
0.04% SA	6.42 \pm 0.19	+0.16	19.50	13.50	32.10	16.86
CD ($p = 0.05$)	–	0.73	–	–	–	–
0.01% HZ	6.35 \pm 0.13	+0.09	18.76	11.07	19.76	10.25
0.02% HZ	6.96 \pm 0.20	+0.70	20.92	16.95	30.26	16.77
0.03% HZ	7.15 \pm 0.16	+0.89	21.72	16.20	32.92	17.20
0.04% HZ	6.38 \pm 0.14	+0.12	18.82	11.60	21.76	14.90
CD ($p = 0.05$)	–	0.59	–	–	–	–
M_5 generation						
Control	6.30 \pm 0.18	–	11.50	6.86	22.11	8.11
0.1% MMS	7.10 \pm 0.28	+0.80	27.32	19.23	64.20	28.50
0.2% MMS	7.90 \pm 0.22	+1.60	29.27	21.09	72.66	30.17
0.3% MMS	8.10 \pm 0.26	+1.80	32.21	22.23	78.15	32.25
0.4% MMS	6.90 \pm 0.19	+0.60	22.76	17.76	62.11	27.12
CD ($p = 0.05$)	–	0.51	–	–	–	–
0.01% SA	6.95 \pm 0.25	+0.65	17.53	13.15	60.50	27.23
0.02% SA	7.35 \pm 0.18	+1.05	24.12	18.11	67.76	28.76
0.03% SA	7.70 \pm 0.16	+1.40	27.29	18.62	69.24	30.02
0.04% SA	6.82 \pm 0.21	+0.52	22.15	14.26	59.67	24.62
CD ($p = 0.05$)	–	0.44	–	–	–	–
0.01% HZ	6.85 \pm 0.23	+0.55	20.12	12.25	58.21	25.95
0.02% HZ	7.25 \pm 0.26	+0.95	21.92	17.34	63.25	26.32
0.03% HZ	7.48 \pm 0.16	+1.18	22.13	17.96	66.21	28.71
0.04% HZ	6.78 \pm 0.24	+0.48	19.76	13.07	57.15	23.92
CD ($p = 0.05$)	–	0.39	–	–	–	–

*CD ($p = 0.05$) mean Critical Difference at 5% level of significance

Table 2 Estimates of mean values (\bar{X}), shift in \bar{X} and genetic parameters for pods per plant in M_4 and M_5 generations of urdbean var. PU-19

Treatment	Mean \pm S.E.	Shift in \bar{X}	PCV (%)	GCV (%)	h^2 (%)	GA (% of \bar{X})
M_4 generation						
Control	31.50 \pm 0.20	–	6.14	3.12	20.12	4.20
0.1% MMS	33.20 \pm 0.18	+1.70	10.20	7.25	28.96	9.25
0.2% MMS	34.10 \pm 0.26	+2.60	12.16	8.16	43.20	12.66
0.3% MMS	35.07 \pm 0.21	+3.57	14.18	8.97	44.70	14.20
0.4% MMS	33.60 \pm 0.27	+2.10	9.76	6.20	32.15	10.15
CD ($p = 0.05$)	–	1.37	–	–	–	–
0.01% SA	32.60 \pm 0.32	+1.10	9.25	6.36	34.10	10.66
0.02% SA	33.45 \pm 0.34	+1.95	10.96	7.61	38.76	11.50
0.03% SA	34.30 \pm 0.26	+2.80	11.92	7.92	36.96	12.10
0.04% SA	33.30 \pm 0.22	+1.80	8.86	5.36	30.15	9.76
CD ($p = 0.05$)	–	1.05	–	–	–	–
0.01% HZ	32.40 \pm 0.28	+0.90	8.74	6.26	30.98	10.15
0.02% HZ	33.30 \pm 0.31	+1.80	10.32	6.91	35.70	10.97
0.03% HZ	34.15 \pm 0.22	+2.65	10.76	7.15	32.89	11.23
0.04% HZ	32.70 \pm 0.27	+1.20	7.96	5.22	28.20	8.64
CD ($p = 0.05$)	–	1.12	–	–	–	–
M_5 generation						
Control	31.65 \pm 0.18	–	5.50	2.90	21.07	3.98
0.1% MMS	33.60 \pm 0.26	+1.95	12.20	8.34	60.12	15.12
0.2% MMS	35.15 \pm 0.31	+3.50	14.11	9.16	64.23	17.11
0.3% MMS	35.95 \pm 0.34	+4.30	16.22	11.11	66.11	18.22
0.4% MMS	34.70 \pm 0.22	+3.05	10.13	7.86	55.14	16.25
CD ($p = 0.05$)	–	1.63	–	–	–	–
0.01% SA	33.15 \pm 0.27	+1.50	11.25	7.32	56.20	15.95
0.02% SA	34.55 \pm 0.30	+2.90	13.26	9.92	58.11	16.62
0.03% SA	35.60 \pm 0.34	+3.95	14.92	10.07	61.50	17.02
0.04% SA	33.95 \pm 0.26	+2.30	9.98	7.12	53.52	13.92
CD ($p = 0.05$)	–	1.33	–	–	–	–
0.01% HZ	33.25 \pm 0.24	+1.60	10.72	6.62	54.70	14.07
0.02% HZ	34.41 \pm 0.29	+2.76	11.90	8.84	56.90	14.98
0.03% HZ	35.05 \pm 0.35	+3.40	12.61	8.98	59.26	15.86
0.04% HZ	33.60 \pm 0.23	+1.95	8.84	5.58	51.55	11.68
CD ($p = 0.05$)	–	1.12	–	–	–	–

*CD ($p = 0.05$) mean Critical Difference at 5% level of significance

contributing traits under study. Higher values of mean for number of fertile branches, number of pods and total plant yield were recorded at 0.3% MMS treatment in both M_4 and M_5 generations. The comparative analysis of different mutagen treatment conditions for total plant yield showed that MMS concentrations 0.3% followed by 0.2% provided highest increase in both M_4 and M_5 generations (Figure 1 and Figure 2). Overall, in

the present investigation MMS followed by SA and HZ generated maximum positive shift in mean plant yield with respect to control value of black gram var. PU-19. In mutagenesis programmes, substantial changes in mean values indicate random occurrence of mutations in quantitative traits. However, the shift in mean values in the positive direction indicates that more positive mutations have occurred for these traits. The increase in

Table 3 Estimates of mean values (\bar{X}), shift in \bar{X} and genetic parameters for total plant yield (g) in M_4 and M_5 generations of urdbean var. PU-19

Treatment	Mean \pm S.E.	Shift in \bar{X}	PCV (%)	GCV (%)	h^2 (%)	GA(% of \bar{X})
M_4 generation						
Control	7.86 \pm 0.10	–	6.88	2.98	25.52	5.38
0.1% MMS	8.56 \pm 0.14	+0.70	10.32	6.36	42.20	16.11
0.2% MMS	9.54 \pm 0.21	+1.68	14.66	9.25	46.70	18.92
0.3% MMS	10.34 \pm 0.24	+2.48	16.25	9.67	52.11	20.12
0.4% MMS	8.76 \pm 0.16	+0.90	9.20	6.20	38.86	16.27
CD ($p = 0.05$)	–	0.58	–	–	–	–
0.01% SA	8.81 \pm 0.22	+0.95	9.37	6.53	30.67	15.50
0.02% SA	9.21 \pm 0.25	+1.35	11.15	7.15	38.92	16.73
0.03% SA	9.76 \pm 0.17	+1.90	12.11	7.66	39.25	17.91
0.04% SA	8.66 \pm 0.20	+0.80	8.86	6.33	36.66	15.07
CD ($p = 0.05$)	–	0.44	–	–	–	–
0.01% HZ	8.41 \pm 0.13	+0.55	8.29	5.92	34.23	13.81
0.02% HZ	9.06 \pm 0.16	+1.20	10.92	6.71	36.12	15.36
0.03% HZ	9.56 \pm 0.20	+1.70	11.09	6.92	35.34	16.02
0.04% HZ	8.48 \pm 0.12	+0.62	7.98	5.23	28.96	11.61
CD ($p = 0.05$)	–	0.38	–	–	–	–
M_5 generation						
Control	7.80 \pm 0.15	–	5.90	2.70	26.11	6.12
0.1% MMS	9.06 \pm 0.20	+1.26	12.12	7.20	63.12	20.34
0.2% MMS	10.60 \pm 0.18	+2.80	15.27	10.11	66.44	22.11
0.3% MMS	11.60 \pm 0.29	+3.80	17.21	11.19	68.70	23.54
0.4% MMS	9.30 \pm 0.31	+1.50	12.12	7.85	59.53	21.90
CD ($p = 0.05$)	–	0.98	–	–	–	–
0.01% SA	9.40 \pm 0.26	+1.60	10.52	7.71	60.44	19.76
0.02% SA	10.10 \pm 0.30	+2.30	12.34	8.12	63.77	21.34
0.03% SA	10.50 \pm 0.22	+2.70	13.31	8.66	66.55	22.55
0.04% SA	9.40 \pm 0.27	+1.60	10.32	7.54	58.77	19.13
CD ($p = 0.05$)	–	0.88	–	–	–	–
0.01% HZ	8.95 \pm 0.18	+1.15	10.15	6.12	59.33	18.55
0.02% HZ	9.70 \pm 0.23	+1.90	11.26	7.23	60.11	20.07
0.03% HZ	10.15 \pm 0.31	+2.35	12.15	7.92	63.07	20.96
0.04% HZ	9.02 \pm 0.25	+1.22	8.11	6.61	53.55	18.82
CD ($p = 0.05$)	–	0.95	–	–	–	–

*CD ($p = 0.05$) mean Critical Difference at 5% level of significance

mean plant yield, in the present study, may be attributed to the increased number of flowers, fertile branches and pods. A high degree of correlation between these traits was earlier reported by Khan et al. (2004) in mungbean and Khan and Wani (2005) in chickpea. Increase in mean was much prominent in M_5 as compared to M_4 generation. The increased mean seed yield in M_5 over M_4 and control population could be attributed to effective

selection adopted for various yield contributing traits in M_4 generation. Bhatia and Swaminathan (1962) while working with wheat concluded that the mean of the irradiated population shows relatively decreasing trend compared to control if selection was not applied with regard to a specific character. The selection of progenies on the basis of superior mean and greater variance in M_4 was found highly useful in this study which had led to

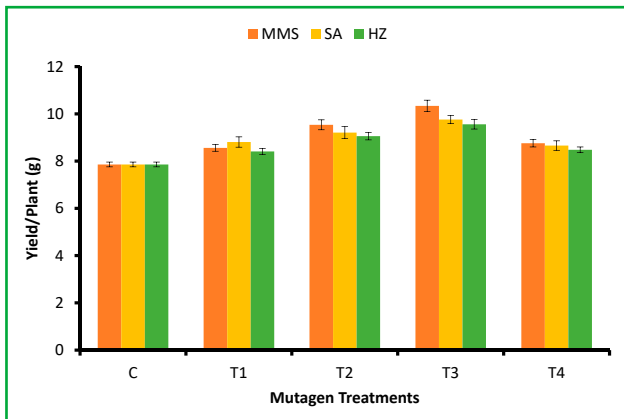


Figure 1 Comparative representation of yield/plant (g) at different mutagen treatment conditions in M₄ generation of urdbean var. PU-19
 MMS – 0.1% (T1), 0.2% (T2), 0.3% (T3), 0.4% (T4) and SA; HZ – 0.01% (T1), 0.02% (T2), 0.03% (T3), 0.04% (T4)

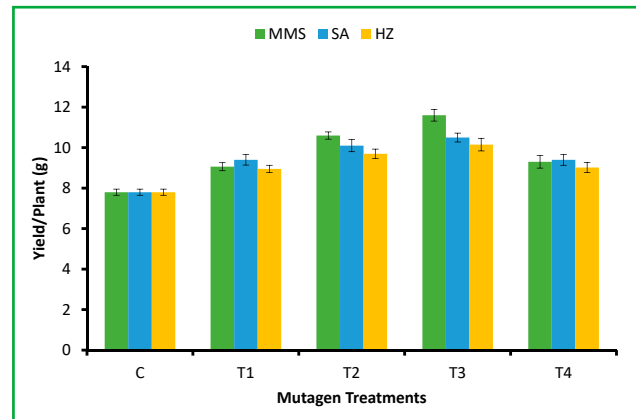


Figure 2 Comparative representation of yield/plant (g) at different mutagen treatment conditions in M₅ generation of urdbean var. PU-19
 MMS – 0.1% (T1), 0.2% (T2), 0.3% (T3), 0.4% (T4) and SA; HZ – 0.01% (T1), 0.02% (T2), 0.03% (T3), 0.04% (T4).

increased mean seed yield in M₅ generation. Waghmare and Mehra (2000) achieved considerably increased mean seed yield in M₃ generation after gamma rays and EMS treatments in *Lathyrus sativus*.

Estimates of genetic parameters like genotypic coefficient of variation, heritability and genetic advance are indispensable in indicating the degree of stability to environmental impacts and the probable trait transmission from parent to offspring (Wani, 2007). In the present study, the genetic parameters like genotypic coefficient of variation, heritability and genetic advance showed an increase over the control in all the treatments. The PCV estimates were higher than the GCV estimates for all the traits of yield, indicating the fair influence of environmental fluctuations on these traits. High heritability estimates for different traits have been found useful for selecting suitable types based on their phenotypic performance. The data, in general, indicates a relatively higher heritability estimates for yield and yield attributes in M₅ generation. Increased heritability values in M₅ in comparison to M₄ generation may be due increased homozygosity of the genes involved. Johnson et al. (1955) advocated that heritability estimates along with genetic advance are usually more helpful than the heritability value alone in predicting the effect of selection. Genetic advance is indicative of genetic progress for a particular trait under suitable selection procedure (Kaul and Garg, 1982), thus carries much significance in self-pollinated crops. The results are in conformity with the reports of Singh et al. (2001) and Raut et al. (2004) in mungbean and chickpea. The higher values of heritability and genetic advance suggest that the variability so evolved could be effectively exploited for the improvement of urdbean crop.

4 Conclusion

The findings of the study suggest that preliminary assessment on induced diversity in genetically complex trait like yield could effectively be done by applying statistical tools on quantitative traits of the crop. The high GCV together with high heritability and genetic advance observed in the selected mutant lines compared to parent genotype confirms the substantial potential for improving yield trait through further selection in the urdbean var. PU-19. Therefore, the high yielding mutant lines screened from the mutagenized populations could be used directly in urdbean breeding program for generating elite mutant cultivars or through hybridization for obtaining desirable segregants in the subsequent generations.

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Fatty acid profile analysis of grape by-products from Slovakia and Austria

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The objective of the present study was to determine the fatty acid profile of grape pomace, grape stem and grape bunch of three different cultivars of *Vitis vinifera* sp. (Green Veltliner, Pinot Blanc and Zweigelt) from two countries as a possible sources for animal nutrition. Fatty acid profile analysis was performed using the Agilent 6890 A GC machine. Significant differences ($P < 0.05$) in fatty acid content of analyzed samples were detected between the countries, as well as between the cultivars within countries. Grape pomaces and grape bunches were rich in polyunsaturated fatty acids (70.91–71.86%), represented mainly by linoleic acid (69.79–70.32%), and low in saturated fatty acids (12.42–12.96%). Grape stems were characterized by a high saturated fatty acids content (24.46–30.85%), but on the other hand, these samples had the highest α -linoleic acid concentration (9.98–14.52%). Oleic acid (12.24–15.17%) was the most abundant from monounsaturated fatty acids (12.69–15.33%) in all the analyzed samples. These results indicate a strong impact of the grape variety and location on the fatty acid profile of grape by-products and their potential to be evaluated as feed additives with high polyunsaturated fatty acids concentration in animal nutrition.

Keywords: grape pomace, grape stalk, fatty acids, PUFA, SFA

1 Introduction

Grape industry generates a large amount of by-products with problematic disposal which can cause serious environmental issues (Botella et al., 2005, Rondeau et al., 2013, Bekhit et al., 2016). The two most abundant by-products of grape processing are pomace and stalks (Makris et al., 2007). Grape pomace represents about 20–25% of the weight of wine grapes (Yu and Ahmedna, 2013), the amount of stems can vary between 1.4–7% (Souquet et al., 2000). The nutritional value and the digestibility of these by-products is, due to high fiber content, generally low, but many experiments showed, that these products can be used a substantial source of certain nutrients and biologically active compounds in animal nutrition (Viveros et al., 2011, Teixeira et al., 2014, Chamorro et al., 2015, Domínguez et al., 2016, Kerasiotti

et al., 2017). They can also help to reduce production costs and to create innovative feed mixtures in order to increase the quality of animal products (Tangolar et al., 2009, Fontana et al., 2013, Guerra-Rivas et al., 2016, Kafantaris et al., 2018). According to Botella et al. (2005) the incorporation of winery by-products in livestock feeds may also positively affect the environment by reducing the toxic impact of their inappropriate disposal by leaving on open spaces or burning. Fatty acids of grape by-products, particularly those of grape pomace, are characterized with high concentrations of linoleic and oleic acids (Yi et al., 2009). Due to this fact, by-products of wine industry could positively influence the fatty acid profile of milk and meat, with a perspective of obtaining less saturated and healthier animal products (Nistor et al., 2014, Guerra-Rivas et al., 2016, Chedea et al., 2018). On this regard the objective

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of this study was to determine and compare the fatty acid profile of grape pomace, grape stems and grape bunches from two countries as possible sources of these nutrients for animal nutrition.

2 Material and methods

The pomace, as a by-product of juice pressing in wine industry, mainly contained of residual grape skin, seeds and pulps. Grape stems were only rachis, peduncle and pedicels after removing grape berries. In total, 54 samples from 3 varieties from 6 different locations were analysed. Laboratory samples were processed in the Laboratory of Quality and Nutritive Value of Feeds (Department of Animal Nutrition, Slovak University of Agriculture in Nitra) using standard laboratory procedures and principles (EC No 152/2009). Prior to evaluating the fatty acid profile of analyzed samples, triglycerides in their lipid fraction to glycerol and free fatty acids were hydrolyzed. Free fatty acids were then converted to methylesters (FAMES) according to the following procedure. Solution was diluted by hexane (10 ml) and 2 N potassium hydroxide in methanol (1 ml). Analytic tube was heated in water bath (30 seconds at 60 °C). After 1 minute 1 N hydrochloric acid (2 ml) was added. The top layer was transmitted (2 ml) to autosampler vial containing ninhydrin (Na_2SO_4). On a specialized analytical column (Supelco 47885-U) the separation of FAMES, based on the carbon number and level of saturation, took place. FAMES with the shortest carbon chain (the lowest boiling point) were separated first. Subsequently, the individual fatty acids were identified by a flame ionization detector (FID). Analysis were performed on gas chromatograph Agilent 6890A GC (Agilent Technologies, USA). The fatty acids profile of grape by-products was determined as percentage of crude fat. Results were statistically evaluated with IBM SPSS v. 20.0. Descriptive statistics using one-way ANOVA were generated. Then, statistical significance of results were separated using Tukey test.

3 Results and discussion

The analyzed grape by-products were characterized by their specific fatty acid (FA) profiles (Table 1 and Table 2). Despite the significant ($P < 0.05$) differences between the countries, as well as between the cultivars within countries, some similarities in the fatty acid composition of grape pomace, stems and bunches were detected. The samples mainly composed of polyunsaturated fatty acids (PUFA), mostly represented by linoleic acid, especially in grape pomace and grape bunches. This result is consistent with the grape seed content of these products as a source of linoleic acid rich grape oil (Fernandes et al., 2013, Yousefi et al., 2013, Hussein and Abdrabba, 2015, Ovcharova et al., 2016). In grape stems

interesting content of α -linoleic acid was detected. Oleic acid, as a monounsaturated fatty acid (MUFA), was the most abundant in all the studied by-products. Grape stems contained the highest amount of saturated fatty acids (SFA), mainly palmitic and stearic acid. The high content of palmitic acid in pomaces may be due to surplus saturated compounds in their waxy structure (Gülcü et al., 2019). Arachidonic and behenic acid were present in pomaces below 1%, whereas in bunches these fatty acids, except two samples (Pinot Blanc and Zweigelt from Slovakia), were not found. This corresponds with low levels of SFA in grape seeds (Tangolar et al., 2009; Gül et al., 2013, Mironeasa et al., 2016, García-Lomillo and González-San José, 2017).

The FA profile of grape pomace is well documented in the literature, but only a limited number of papers has been published on the content of FA in grape stem and grape bunch. In red grape pomace Yi et al. (2009) found average values of 21.2% SFA, 14.4% MUFA and 62.7% PUFA. Ribeiro et al. (2015) reported an average PUFA concentration in grape pomaces around 72.86% with the predominance of linoleic (60.04%) and α -linolenic (13.64%) acid, followed by oleic (12.97%) and palmitic (6.72%) acid. Stearic acid was present in the analyzed pomaces below 5%. In comparison with Guerra-Rivas et al. (2016) lower amounts of all the FA were detected for grape pomaces. On the other hand, Tsiplakou and Zervas (2008) and Gülcü et al. (2019) measured higher content of the same FA, except for linoleic acid. Russo et al. (2017) studied the FA profile of six grape pomaces with very similar results as obtained in this experiment. These authors also reported that grape stalk contained 21% palmitic, 4.6% stearic, 10.7% oleic, 35.4% linoleic, 13.4% α -linoleic and 11.3% behenic acid.

The total comparison of FA profile of grape by-products from Slovakia and Austria is shown in Table 3. The grape pomace samples from both countries had significantly different ($P < 0.05$) content of all the studied FA. In the case of grape stems significant differences ($P < 0.05$) for oleic, α -linoleic, arachidic and behenic acids concentration, as well as overall MUFA content, were found. The grape bunches from two counties significantly differed ($P < 0.05$) in stearic, oleic, linoleic and α -linoleic acids content. A justification for this differences between the FA content of grape-by products could be related to different agro-climatic conditions of the growing regions (García-Lomillo and González-San José, 2017). Bennemann et al. (2016) state, that the quality of grapes is greatly influenced by factors such as soil, weather, temperature, humidity and solar radiation.

Table 1 Fatty acid profile of grape by-products from Slovakia (% fat⁻¹)

		Green Veltliner	Pinot Blanc	Zweigelt
		Mean ±Standard Deviation		
Palmitic acid	pomace	8.64 ±0.11 ^a	8.13 ±0.01 ^b	7.69 ±0.02 ^c
	stems	15.80 ±0.45 ^a	10.68 ±0.49 ^b	13.14 ±0.68 ^c
	bunch	8.85 ±0.02 ^a	8.57 ±0.10 ^b	7.47 ±0.05 ^c
Stearic acid	pomace	3.56 ±0.05 ^a	3.95 ±0.00 ^b	4.03 ±0.00 ^c
	stems	3.52 ±0.21 ^a	4.03 ±0.18 ^b	3.86 ±0.05 ^{ab}
	bunch	3.42 ±0.02 ^a	4.06 ±0.03 ^b	4.17 ±0.01 ^c
Oleic acid	pomace	10.91 ±0.07 ^a	17.52 ±0.02 ^b	16.34 ±0.02 ^c
	stems	14.34 ±0.92 ^a	16.12 ±0.12 ^b	15.04 ±0.31 ^{ab}
	bunch	10.21 ±0.01 ^a	17.09 ±0.15 ^b	17.03 ±0.06 ^c
Linoleic acid	pomace	73.08 ±0.23 ^a	67.59 ±0.02 ^b	68.75 ±0.01 ^c
	stems	36.86 ±1.14 ^a	57.19 ±1.21 ^b	45.47 ±1.05 ^c
	bunch	74.40 ±0.03 ^a	67.66 ±0.25 ^b	68.90 ±0.04 ^c
α-linoleic acid	pomace	1.75 ±0.02 ^a	0.78 ±0.00 ^b	0.77 ±0.01 ^b
	stems	15.17 ±0.62 ^a	5.74 ±0.13 ^b	9.03 ±0.62 ^c
	bunch	2.38 ±0.03 ^a	1.22 ±0.06 ^b	1.01 ±0.05 ^c
Arachidic acid	pomace	0.28 ±0.01 ^a	0.24 ±0.00 ^b	0.24 ±0.00 ^b
	stems	3.21 ±0.07 ^a	1.21 ±0.03 ^b	2.89 ±0.09 ^c
	bunch	ND ^a	0.24 ±0.00 ^b	0.25 ±0.00 ^c
Behenic acid	pomace	0.19 ±0.01 ^a	0.11 ±0.00 ^b	0.11 ±0.00 ^b
	stems	3.62 ±0.11 ^a	1.95 ±0.08 ^b	4.76 ±0.15 ^c
	bunch	ND	ND	ND
PUFA	pomace	74.83 ±0.25 ^a	68.37 ±0.02 ^b	69.52 ±0.02 ^c
	stems	54.26 ±1.81 ^a	62.93 ±1.15 ^b	54.51 ±0.46 ^a
	bunch	76.78 ±0.03 ^a	68.88 ±0.18 ^b	69.91 ±0.08 ^c
MUFA	pomace	11.32 ±0.08 ^a	17.95 ±0.02 ^b	16.72 ±0.02 ^c
	stems	14.34 ±0.92 ^a	16.31 ±0.43 ^b	15.04 ±0.31 ^{ab}
	bunch	10.21 ±0.01 ^a	17.39 ±0.15 ^b	17.34 ±0.06 ^b
SFA	pomace	12.93 ±0.18 ^a	12.57 ±0.02 ^b	12.30 ±0.01 ^c
	stems	28.87 ±0.78 ^a	18.45 ±0.73 ^b	26.06 ±0.71 ^c
	bunch	12.28 ±0.02 ^a	12.98 ±0.29 ^b	11.99 ±0.15 ^a
Ratio Σn3/n6	pomace	0.02 ±0.00 ^a	0.01 ±0.00 ^b	0.01 ±0.00 ^b
	stems	0.43 ±0.00 ^a	0.10 ±0.00 ^b	0.20 ±0.02 ^c
	bunch	0.03 ±0.00 ^a	0.02 ±0.00 ^b	0.01 ±0.00 ^b
Ratio Σn3/n6	pomace	41.72 ±0.47 ^a	86.60 ±0.13 ^b	89.59 ±0.75 ^c
	stems	2.34 ±0.03 ^a	9.96 ±0.39 ^b	5.05 ±0.47 ^c
	bunch	31.31 ±0.37 ^a	55.34 ±2.88 ^b	68.26 ±3.48 ^c

ND – value below detection limit, MUFA – monounsaturated fatty acids, PUFA – polyunsaturated fatty acids, SFA – saturated fatty acids. Values followed by different letters within a row are significant at the level 0.05

Table 2 Fatty acid profile of grape by-products from Austria (% fat⁻¹)

		Green Veltliner	Pinot Blanc	Zweigelt
		Mean ±Standard Deviation		
Palmitic acid	pomace	8.85 ±0.01 ^a	7.96 ±0.03 ^b	8.70 ±0.03 ^c
	stems	19.13 ±0.15	16.79 ±0.31	19.62 ±5.85
	bunch	9.89 ±0.28	7.69 ±0.03	9.86 ±1.56
Stearic acid	pomace	3.25 ±0.01 ^a	3.44 ±0.01 ^b	3.77 ±0.01 ^c
	stems	3.92 ±0.11	4.35 ±0.11	5.75 ±2.05
	bunch	3.60 ±0.07 ^a	3.36 ±0.02 ^a	4.42 ±0.51 ^b
Oleic acid	pomace	9.80 ±0.04 ^a	15.98 ±0.03 ^b	15.86 ±0.01 ^c
	stems	9.43 ±0.74 ^a	12.04 ±0.23 ^{ab}	15.26 ±2.69 ^b
	bunch	10.96 ±0.10 ^a	16.39 ±0.05 ^b	16.86 ±0.51 ^b
Linoleic acid	pomace	73.85 ±0.09 ^a	68.89 ±0.10 ^b	66.61 ±0.04 ^c
	stems	38.92 ±1.07	36.06 ±0.29	38.40 ±6.70
	bunch	72.98 ±0.39 ^a	70.63 ±0.11 ^a	66.82 ±2.41 ^b
α-linoleic acid	pomace	1.81 ±0.01 ^a	1.15 ±0.03 ^b	1.21 ±0.02 ^c
	stems	18.73 ±1.05 ^a	15.65 ±0.21 ^a	9.17 ±2.47 ^b
	bunch	1.84 ±0.02 ^a	1.09 ±0.07 ^b	1.13 ±0.08 ^b
Arachidic acid	pomace	0.23 ±0.01 ^a	0.34 ±0.01 ^b	0.27 ±0.01 ^c
	stems	2.41 ±0.09 ^a	3.52 ±0.07 ^b	2.30 ±0.24 ^a
	bunch	ND	ND	ND
Behenic acid	pomace	0.17 ±0.00 ^a	0.24 ±0.00 ^b	0.17 ±0.00 ^a
	stems	3.81 ±0.20 ^a	5.73 ±0.16 ^b	3.71 ±0.48 ^a
	bunch	ND	ND	ND
PUFA	pomace	75.66 ±0.10 ^a	70.04 ±0.10 ^b	67.82 ±0.04 ^c
	stems	57.66 ±0.39	51.71 ±0.42	47.57 ±9.10
	bunch	74.83 ±0.39 ^a	71.72 ±0.09 ^a	67.95 ±2.48 ^b
MUFA	pomace	10.09 ±0.05 ^a	16.50 ±0.03 ^b	16.60 ±0.01 ^c
	stems	9.43 ±0.74 ^a	12.04 ±0.23 ^a	16.61 ±2.48 ^b
	bunch	10.96 ±0.10 ^a	16.39 ±0.05 ^b	17.00 ±0.39 ^c
SFA	pomace	12.89 ±0.01 ^a	12.38 ±0.04 ^b	13.37 ±0.04 ^c
	stems	29.27 ±0.34	30.39 ±0.29	32.88 ±7.35
	bunch	13.49 ±0.31 ^{ab}	11.06 ±0.04 ^a	14.28 ±2.07 ^b
Ratio Σn3/n6	pomace	0.02 ±0.00 ^a	0.02 ±0.00 ^b	0.02 ±0.00 ^c
	stems	0.48 ±0.04 ^a	0.43 ±0.01 ^a	0.24 ±0.03 ^b
	bunch	0.03 ±0.00 ^a	0.02 ±0.00 ^b	0.02 ±0.00 ^b
Ratio Σn3/n6	pomace	40.778 ±0.27 ^a	60.16 ±1.34 ^b	55.25 ±0.77 ^c
	stems	2.08 ±0.18 ^a	2.30 ±0.03 ^a	4.28 ±0.56 ^b
	bunch	39.56 ±0.59 ^a	65.07 ±4.09 ^b	59.20 ±2.07 ^b

ND – value below detection limit, MUFA – monounsaturated fatty acids, PUFA – polyunsaturated fatty acids, SFA – saturated fatty acids. Values followed by different letters within a row are significant at the level 0.05

Table 3 Comparison of fatty acid profile of grape by-products from Slovakia and Austria

		Slovakia	Austria	Significance
		Mean ±Standard deviation (% fat ⁻¹)		
Palmitic acid	pomace	8.15 ±0.41	8.50 ±0.41	0.000
	stems	13.21 ±2.27	18.51 ±3.21	0.578
	bunch	8.30 ±0.64	9.15 ±1.35	0.041
Stearic acid	pomace	3.84 ±0.22	3.49 ±0.23	0.000
	stems	3.80 ±0.26	4.67 ±1.32	0.223
	bunch	3.88 ±0.35	3.79 ±0.54	0.012
Oleic acid	pomace	14.92 ±3.06	13.88 ±3.06	0.000
	stems	15.17 ±0.92	12.24 ±2.89	0.013
	bunch	14.77 ±3.43	14.74 ±2.85	0.000
Linoleic acid	pomace	69.81 ±2.51	69.79 ±3.21	0.000
	stems	46.51 ±8.89	37.80 ±3.64	0.656
	bunch	70.32 ±3.11	70.14 ±2.96	0.005
α-linoleic acid	pomace	1.10 ±0.49	1.39 ±0.32	0.000
	stems	9.98 ±4.17	14.52 ±4.44	0.001
	bunch	1.54 ±0.64	1.35 ±0.37	0.000
Arachidic acid	pomace	0.25 ±0.02	0.28 ±0.05	0.000
	stems	2.44 ±0.93	2.74 ±0.60	0.000
	bunch	0.16 ±0.12	ND	ND
Behenic acid	pomace	0.14 ±0.04	0.20 ±0.03	0.000
	stems	3.44 ±1.23	4.41 ±1.02	0.000
	bunch	ND	ND	ND
PUFA	pomace	70.91 ±2.99	71.17 ±3.50	0.000
	stems	57.23 ±4.41	52.31 ±6.33	0.140
	bunch	71.86 ±3.72	71.50 ±3.24	0.003
MUFA	pomace	15.33 ±3.06	14.40 ±3.23	0.000
	stems	15.23 ±1.01	12.69 ±3.40	0.003
	bunch	14.98 ±3.58	14.78 ±2.89	0.000
SFA	pomace	12.60 ±0.29	12.88 ±0.43	0.000
	stems	24.46 ±4.71	30.85 ±4.02	0.594
	bunch	12.42 ±0.47	12.94 ±1.79	0.039
Ratio Σn3/n6	pomace	0.02 ±0.01	0.02 ±0.00	0.000
	stems	0.24 ±0.15	0.38 ±0.12	0.000
	bunch	0.02 ±0.01	0.19 ±0.00	0.000
Ratio Σn3/n6	pomace	72.64 ±23.23	52.06 ±8.76	0.000
	stems	5.78 ±3.36	2.89 ±1.09	0.000
	bunch	51.64 ±16.40	54.61 ±11.80	0.000

ND – value below detection limit, MUFA – monounsaturated fatty acids, PUFA – polyunsaturated fatty acids, SFA – saturated fatty acids. The level of significance was set at $P < 0.05$

4 Conclusions

The results of this experiment indicate a significant impact of the grape variety and location on the FA profile of grape by-products. But despite these differences some similarities can be found. Grape pomaces and grape bunches were rich in PUFA, especially linoleic acid, and low in SFA. Grape stems were characterized by a high SFA content, but on the other hand, these samples had the highest *H*-linoleic acid concentration. Overall it can be concluded that the by-products of wine industry, primarily grape pomace, could find application in animal nutrition as feed additives with high PUFA content. However, further research in the future is needed.

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Studies on rearing performances of mulberry silkworm (*Bombyx mori* Linnaeus, 1758) in hooghly district of West Bengal (India): A newly explored area

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This study is aimed at proposing mulberry sericulture as an alternative to strengthen the agricultural economy of Hooghly district of West Bengal, India. Exploration of four breeds of mulberry silkworm, *Bombyx mori* (Linnaeus, 1758), popular in West Bengal (viz., two multivoltine breeds – Nistari Plain and Nistari Marked, One Bivoltine breed – SK6 X SK7 hybrid and one F1 Hybrid from cross between Nistari Plain and SK6 X SK7 hybrid) have been conducted in the Sericulture Research Laboratory, Post Graduate department of Zoology, Hooghly Mohsin College, Chinsurah, Hooghly, West Bengal, India, for three consecutive years (2016–2019) for various rearing parameters (viz., larval duration, matured larval weight, cocoon weight, shell weight, shell ratio, effective rate of rearing, absolute silk content and yield) along with common meteorological data (viz., average temperature, average relative humidity, photoperiod). The study reveals Hooghly district to be very conducive for mulberry sericulture with the multivoltine breeds to be most suitable, although rearing of F1 hybrid and bivoltine breeds should also be promoted for their better economic value. Among the seasons, late Autumn (Oct. – Nov.) and early Spring (Feb. – Mar.) are found to be most suitable commercial rearing seasons for almost all the breeds, whereas the extended summer months including Spring, Summer and rainy season (Mar. – Aug.) are unfavourable, indicating deleterious effect of temperature and humidity on the rearing performance of these breeds, for which remedies such as sub-lethal heat shock can be explored.

Keywords: Silkworm rearing, cocoon weight, absolute silk content, yield, effective rate of rearing, commercial rearing season

1 Introduction:

Hooghly, with its assemblage of numerous factories fringing the banks of Bhagirathi (river Ganga), has become model for industrially developed districts, but in spite of all these years of industrialization, the basic rural characteristics remain the same. Still over 70% of its inhabitants practice agriculture. A mixture of highly fertile Gangetic and Vindhya alluvium soils coupled with well-developed irrigation infrastructure have earned Hooghly the reputation of an agriculturally advanced district as well. The average household income of this Gangetic alluvial zone is highly dependent up on crops like cereals and potato and hence the significantly depressed prices of potato for the past few years has compelled the authorities to consider intensive

agricultural practice while drafting the state agricultural plan for the district. In recent years only few selected crops are considered beneficial for intensive culture, but with increasing local demand, other allied agricultural produce are fast gaining popularity, as the demand far exceeds the production. The whole development plan is much in need of a thorough reorientation in the direction of balanced expansion of both the agriculture and its allied sectors. In this context introduction of sericulture which has long since practiced in the neighbouring districts like Malda (Taufique and Hoque, 2019) and Murshidabad, can be considered as momentous, keeping in mind its remunerative employment generation capacity (Chandan Roy et al., 2012). The introduction of sericulture which is a labour-intensive industry (Chandan

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Roy et al., 2012) will also be a boon for the district as with almost 1 : 1 male-female ratio, female workers will also be able to contribute equally (Dewangan, 2017). Bearing in mind, the fertile nature of the soil and conducive climatic condition of this district, an attempt for introduction of mulberry sericulture should be considered as need of the hour. The present study aims at identifying the commercial crop rearing seasons for successful mulberry silk culture in this region.

2 Materials and methods

Hooghly, one of the central districts of West Bengal, extends between 20° 30' 32" and 23° 1' 20" of North latitude and between 87°30' 20" and 88°30' 15" East longitude. Flanked by river Rupnarayan on its left, Hooghly extends from Guptipara Char to Bhabanipur Char on the river Bhagirathi and share its border with districts like Burdwan and Bankura in the north, and Howrah in the south, while the 24 Parganas (N) and Nadia lie in the east and Medinipur (W) limit its western most edge.

Most of the meteorological data related to average temperature, average relative humidity and photoperiod were recorded in the laboratory, while data like average monthly rainfall was collected from online weather data providers.

In the present study four breeds of mulberry silkworm, *Bombyx mori*, popular in West Bengal (viz., two multivoltine breeds – Nistari Plain and Nistari Marked, One Bivoltine breed – SK6 X SK7 hybrid and one F1 Hybrid from cross between Nistari Plain and SK6 X SK7 hybrid) were used. Eggs of disease free layings (DFLs) of each breed were procured as Egg-cards from Ranaghat and Shibnibas extension farms of State Sericulture Directorate and reared in the rearing room of the Sericulture Research Laboratory, Post Graduate department of Zoology, Hooghly Mohsin College,

Chinsurah, Hooghly. Larvae were fed with fresh mulberry leaves of improved variety-S_{1635'} procured from mulberry plantation, Hooghly Mohsin College. Saplings for the plantation were provided by Dhubulia extension farm, State Sericulture Directorate. Rearing was done throughout the year, for three consecutive years between 2016 and 2019. During this time eight commercial rearing parameters were recorded in triplicates for each breed for each season. Almost 300 larvae from a single disease free laying were used for each replicate. Data collected from each replicate were then pooled and subjected to suitable statistical analysis.

3 Result and discussion

Climatic condition

As photoperiod is known to have effect on larval weight (Hirasaka and Koyama, 1970; Rajanna, 1986) it was recorded throughout the year. Photoperiod of almost (Light : Dark) 1 : 1 was observed during Spring, while the ratio increased during the extended Summer months (May – Aug.) and Autumn (Sep. – Oct.). From late Autumn (Oct. – Nov.) to early Spring (Feb. – Mar.) the ratio was lower than 1 : 1. Average temperature also has massive impact on sericulture (Rahmathulla, 2012). During late Autumn and early Spring average temperature was recorded to be most suitable while it was marginally above the optimum (23–28 °C) during Spring. The extended Summer months (May – Aug.) accounted for the most unfavourable average temperature. Moreover photoperiod and average temperature were found to be significantly correlated. Relative humidity (RH) is another important deciding factor (Sisodia and Gaherwal, 2017) and found to be in significantly strong positive correlation with average Rainfall. Optimum combination of average temperature (23–28 °C) and RH (75–80%) (Sisodia and Gaherwal, 2017) was recorded during late Autumn and early Spring (Table 1).

Table 1 Mean meteorological data (2016–2019) of Hooghly district, West Bengal

Seasons	Average Photoperiod (hr.)		Average temperature (°C)	Average relative humidity (%)	Average rainfall (mm)
	light	dark			
Feb. – Mar. (early spring)	11:23	12:36	26.4	65.0	0.17
Mar. – Apr. (spring)	12:00	11:59	29.6	67.7	8.43
May – June (summer)	13:14	10:46	31.1	75.9	11.74
July – Aug. (rainy season)	13:24	10:36	29.8	84.5	32.06
Sep. – Oct. (autumn)	12:17	11:43	29.2	79.8	28.65
Oct. – Nov. (late autumn)	11:38	12:22	27.0	75.1	8.79
Dec. – Jan. (winter)	11:02	12:58	20.2	68.4	2.06

Seasonal influence on rearing performance

Effect of various seasonal parameters on commercial rearing performances (viz., larval duration, matured larval weight, cocoon weight, shell weight, shell ratio, effective rate of rearing, absolute silk content, yield etc.) of different breeds of mulberry silkworm is depicted in Table 2.

Larval duration

In case of Nistari plain breed, longest larval duration was observed during early Spring (Feb. – Mar.) and Winter (Dec. – Jan.) (23 days), followed non-significantly by that of Spring (Mar. – Apr.), rainy season (July – Aug.), Autumn (Sep. – Oct.) and late Autumn (Oct. – Nov.) (22 days). Significantly shortest larval duration was observed during Summer (May – June) (21 days).

Observation in Nistari marked breed revealed that Winter was longest (24 days) in terms of larval duration, followed non-significantly by late Autumn (23.03 days), and significantly by Autumn (22.03 days) and others, with Summer being the shortest (20.10 days), having non-significant difference with Spring (20.5 days). Larval duration during early Spring, rainy season (22 days) and Autumn (22.03 days) differed non-significantly.

Larval duration in F1 hybrid showed the following trend: Winter being the longest (25.33 days), followed non-significantly by late Autumn (25 days). Larval duration in remaining seasons varied within the range of 21.50 days and 23.04 days, it was shortest during Summer (21.5 days) and varied non-significantly from that of Spring (22.33 days) and rainy season (22.5 days).

In the selected bivoltine breed, longest larval duration was observed during late Autumn (27 days), followed non-significantly by that during Winter (26.5 days). Shortest larval duration was recorded during Summer (22 days), having non-significant difference with that during Spring (23 days).

As a whole hybrid and bivoltine breeds showed longer larval duration than multivoltine breeds. Irrespective of breeds, rainy months and Summer shortened the duration, while Winter prolonged the duration to the maximum extent (Table 1).

Larval weight

Average weight of randomly selected 10 matured larvae was recorded during all the seasons for all four breeds. In Nistari Plain breed, maximum larval weight was observed in Winter (29 g), followed non-significantly by that during early Spring (28.2 g) and significantly by late Autumn (27 g). For rest of the seasons larval weight in Nistari Plain was recorded to be within the range of 23.6 g and 26 g, with least larval weight (23.6 g) during

the Summer, having significant difference with that during Spring (25.1 g), rainy season (25.1 g) and Autumn (26 g).

Maximum larval weight in the Nistari marked breed was also recorded during Winter (29.9 g), followed significantly by late Autumn (27.8 g) and early Spring (27.2 g) having non-significant variation between the two. Larval weights during the remaining seasons were within the range of 23 g and 25.2 g, with minimum larval weight of 23 g during the Summer differed non-significantly with that during the rainy season (23.9 g).

In case of F1 hybrids, late Autumn (41.6 g) was the season with highest larval weight, followed significantly by early Spring (39 g), larval weight for rest of the seasons varied between 32.10 g and 35.11 g Least larval weight of 32.10 g during Summer, varied non-significantly with that during rainy season (33.20 g) and Autumn (33.21 g).

Larval weight in the selected Bivoltine breed was at its highest during late Autumn (40.1 g), followed non-significantly by early Spring (40 g), for rest of the seasons, larval weight was recorded within the range of 32 g and 37.50 g

Bivoltine breeds and hybrids showed higher larval weight than multivoltine breed.

Cocoon weight

Single cocoon weight of Nistari Plain breed was found to be highest during late Autumn (0.96 g), followed non-significantly by that during Autumn (0.95 g) and significantly by early Spring (0.92 g). Variation of cocoon weight for rest of the seasons was within the range of 0.72 g and 0.86 g Lowest cocoon weight of 0.72 g was recorded during Summer. Cocoon weight observed during rainy season (0.83 g) Spring (0.85 g) and Winter (0.86 g) differed non-significantly.

Maximum single cocoon weight in Nistari marked breed was observed during late Autumn (1.01 g), followed significantly by that during Winter (0.92 g) and others, varied from 0.53 g to 0.88 g Least cocoon weight recorded during Summer (0.53 g) did not vary significantly from that during Rainy season (0.55 g).

In case of F1 hybrid, late Autumn (1.90 g) was the season, in which highest cocoon weight was recorded, followed significantly by early Spring (1.70 g), for rest of the seasons the recordings were within the range of 1.44 g and 1.65 g, whereas least cocoon weight during Summer (1.44 g) did not vary significantly with that during rainy season (1.47 g).

The selected Bivoltine breed produced maximum cocoon weight during late Autumn (1.80 g), followed non-significantly by early Spring (1.75 g). Single cocoon

Table 2 Effect of seasons on rearing performance of mulberry silkworm in Hooghly district of West Bengal

Rearing parameter	Larval duration (days)				Weight of 10 matured larvae (g)				Single cocoon weight (g)				Single shell weight (g)				
	NP	NM	F1	BV	NP	NM	F1	BV	NP	NM	F1	BV	NP	NM	F1	BV	
Season																	
Feb. – Mar.	23.00	22.00	23.00	24.00	28.20	27.20	39.00	40.00	0.92	0.75	1.70	1.75	0.10	0.10	0.10	0.28	0.30
Mar. – Apr.	22.00	20.50	22.33	23.00	25.10	24.30	35.04	37.50	0.85	0.64	1.50	1.65	0.10	0.09	0.22	0.31	
May – June	21.00	20.10	21.50	22.00	23.60	23.00	32.10	34.00	0.72	0.53	1.44	1.56	0.08	0.07	0.18	0.25	
July – Aug.	22.00	22.00	22.50	24.00	25.10	23.90	33.20	35.00	0.83	0.55	1.47	1.60	0.09	0.08	0.18	0.26	
Sep. – Oct.	22.00	22.03	23.04	25.00	26.00	25.20	33.21	36.00	0.95	0.88	1.55	1.66	0.12	0.10	0.20	0.29	
Oct. – Nov.	22.00	23.03	25.00	27.00	27.00	27.80	41.60	40.10	0.96	1.01	1.90	1.80	0.14	0.14	0.36	0.32	
Dec. – Jan.	23.00	24.00	25.33	26.50	29.00	29.90	35.11	32.00	0.86	0.92	1.65	1.50	0.11	0.11	0.30	0.24	
CD at 5%	1.7	1.20	1.04	1.08	1.3	1.13	1.30	1.29	0.04	0.03	0.04	0.06	0.02	0.01	0.03	0.01	0.01

Rearing parameter	Shell ratio (%)				ERR (%)				Absolute silk content (kg)				Yield/10,000 larvae brushed (kg)				
	NP	NM	F1	BV	NP	NM	F1	BV	NP	NM	F1	BV	NP	NM	F1	BV	
Season																	
Feb. – Mar.	10.87	13.33	16.47	17.14	79.67	78.00	98.33	75.81	0.80	0.78	2.75	2.27	7.33	5.85	16.72	13.27	
Mar. – Apr.	11.76	14.06	14.67	18.79	76.38	77.10	88.89	66.67	0.76	0.69	1.96	2.07	6.49	4.93	13.33	11.00	
May – June	11.11	13.21	12.50	16.03	54.20	52.00	40.67	49.33	0.43	0.36	0.73	1.23	3.90	2.76	5.86	7.70	
July – Aug.	10.84	14.55	12.24	16.25	58.55	57.02	51.64	53.22	0.53	0.46	0.93	1.38	4.86	3.14	7.59	8.52	
Sep. – Oct.	12.63	11.36	12.90	17.47	57.00	62.03	51.55	56.78	0.68	0.62	1.03	1.65	5.42	5.46	7.99	9.43	
Oct. – Nov.	14.58	13.86	18.95	17.78	81.40	81.00	98.33	73.33	1.14	1.13	3.54	2.35	7.81	8.18	18.68	13.20	
Dec. – Jan.	12.79	11.96	18.18	16.00	66.50	69.44	75.64	70.80	0.73	0.76	2.27	1.70	5.72	6.39	12.48	10.62	
CD at 5%	1.8	1.09	1.40	1.02	8.61	8.96	9.36	7.95	0.04	0.03	0.02	0.04	0.35	0.34	0.37	0.46	

**ERR – effective rate of rearing, NP – nistari plain breed, NM – nistari marked breed, F1 – F1 hybrid, BV – selected Bivoltine breed, CD at 5% – critical difference at 5%

weight in this breed varied within the range of 1.50 g and 1.66 g for the remaining seasons. Lowest cocoon weight in Winter (1.50 g) found not to vary significantly with that during Summer (1.56 g).

As a whole bivoltines performed better than hybrids and multivoltines. From the seasonal point of view, Spring, Summer and Rainy months were found adverse.

Shell weight

Observations on Nistari Plain breed revealed that, single shell weight reached its maximum during late Autumn (0.14 g), followed non-significantly by Autumn (0.12 g) and significantly by Winter (0.11 g), while rest of the shell weights were in the range of 0.08 g and 0.10 g. Lowest shell weight recorded during Summer (0.08 g) did not differ significantly from that during rainy season (0.09 g), Spring (0.10 g) and early Spring (0.10 g).

In Nistari marked breed, highest single shell weight was observed during late Autumn (0.14 g), followed significantly by Winter (0.11 g), and remaining seasons (0.07 g to 0.10 g). Shell weight during Summer (0.07 g) found to be the lowest, which did not differ significantly from that during rainy season (0.08 g).

Highest shell weight in F1 hybrid was recorded during late Autumn (0.36 g), followed significantly by that during Winter (0.30 g), for rest of the seasons, shell weight varied within the range of 0.18 g and 0.28 g, lowest being recorded during Summer and rainy season (0.18 g).

In case of the Bivoltine breed, shell weight was recorded to be maximum during late Autumn (0.32 g), followed non-significantly by Spring (0.31 g) and significantly by early Spring (0.30 g) and rest of the seasons (0.24 gm to 0.29 g) with lowest shell weight being recorded during Winter (0.24 g), which differed non-significantly with that during Summer (0.25 g).

Like single cocoon weight, shell weight also found to be highest in bivoltine breeds, followed by hybrids and multivoltines. Average single shell weight was found to be in significantly strong positive correlation with average single cocoon weight (0.961), average SR% (0.921), average ERR (0.839), average ASC (0.962) and average Yield (0.942). March to August were seasonally adverse for shell weight.

Shell ratio (%)

In Nistari Plain breed SR% was computed to be maximum during late Autumn (14.58) having significant variation with the rest of the observations (10.84 to 12.79). Lowest SR% computed during rainy season (10.84) and early Spring (10.87) were found not to differ significantly with those during Summer (11.11) and Spring (11.76).

SR% reached its maximum in Nistari marked breed during rainy season (14.55), followed non-significantly by that during Spring (14.06). Recordings in the rest of the seasons varied from 11.36 to 13.86. Lowest SR% during Autumn (11.36) did not differ significantly with that during Winter (11.96).

In case of F1 hybrid, highest SR% was computed during late Autumn (18.95), followed non-significantly by that during Winter (18.18) and significantly by the rest of the season (12.24 to 16.47). Lowest SR% computed during rainy season (12.24) did not vary significantly from those during Summer (12.5) and Autumn (12.9).

The selected Bivoltine breed exhibited maximum SR% during Spring (18.79), followed significantly by that during late Autumn (17.78) and non-significantly by that during early Spring (17.14) and Autumn (17.47). For the remaining seasons the SR% were computed within the range of 16 and 16.25. Lowest SR% was observed during Winter (16).

As a whole F1 hybrid and bivoltine breed exhibited better SR% than the multivoltine breeds. Average SR% showed significantly strong positive correlation with average ERR (0.866), average ASC (0.944) and average Yield (0.908). Summer was found to be seasonally adverse for SR% in almost all the breeds.

Effective rate of rearing (%)

Maximum Effective rate of rearing (ERR%) in Nistari Plain breed was computed during late Autumn (81.4), followed non-significantly by that during early Spring and Spring and significantly by the rest of the seasons (54.20 to 66.50). Lowest ERR observed during Summer (54.20) found not to vary significantly with that during rainy season (58.55) and Autumn (57).

In Nistari marked breed highest ERR was recorded during late Autumn, followed non-significantly by that during early Spring (78) and Spring (77.10), and significantly by the remaining seasons (52 to 69.44) and no significant difference was found between the lowest ERR during Summer (52) and that during rainy season (57.02).

In F1 hybrid ERR was observed to be maximum during late Autumn and early Spring (98.33) followed significantly by Spring (88.89) and others. Significantly lowest ERR in F1 hybrid was observed during Summer (40.67). No significant difference was observed between ERR during rainy season (51.64) and Autumn (51.55).

ERR of Bivoltine breed reached its maximum during early Spring (75.81), followed non-significantly by that during late Autumn (73.33) and Winter (70.80) and significantly by the rest of the seasons (49.33 to 70.80). Lowest ERR recorded during Summer (49.33) was found not to vary

Table 3 Correlation matrix: Average weather parameters vs Average rearing parameters

	Photoperiod-L	Photoperiod-D	Temperature	Humidity	Rainfall	Larval duration	MLW	CW	SW	SR	ERR	ASC	Yield
Photoperiod-L	1												
Photoperiod-D	-1.000**	1											
Temperature	0.812*	-0.812*	1										
Humidity	0.769*	-0.769*	0.503	1									
Rainfall	0.734	-0.734	0.557	0.925**	1								
Larval duration	-0.733	0.733	-0.840*	-0.171	-0.256	1							
MLW	-0.789*	0.789*	-0.516	-0.516	-0.566	0.689	1						
CW	-0.700	0.700	-0.427	-0.241	-0.302	0.751	0.901**	1					
SW	-0.754	0.754	-0.449	-0.406	-0.498	0.699	0.931**	0.961**	1				
SR	-0.694	0.694	-0.441	-0.435	-0.540	0.638	0.836*	0.807*	0.921**	1			
ERR	-0.802*	0.802*	-0.452	-0.709	-0.683	0.514	0.910**	0.731	0.839*	0.866*	1		
ASC	-0.786*	0.786*	-0.469	-0.550	-0.609	0.643	0.963**	0.882**	0.962**	0.944**	0.950**	1	
Yield	-0.807*	0.807*	-0.473	-0.586	-0.611	0.630	0.975**	0.872*	0.942**	0.908**	0.968**	0.994**	1

* correlation is significant at the 0.05 level (2-tailed), ** correlation is significant at the 0.01 level (2-tailed)

significantly with that during Autumn (56.78) and rainy season (53.22).

ERR % was observed to be best in hybrids and strikingly lowest in bivoltines. ERR% was affected during Summer, Rainy and Autumn months. Correlation studies showed significantly positive correlation with average single shell weight (0.839), average mature larval weight (0.910), average SR% (0.866), average ASC (0.950) and average Yield (0.968).

Absolute Silk content

In case of Nistari Plain breed, Absolute Silk content (ASC) was calculated to be highest during late Autumn (1.14 kg), for rest of the seasons ASC was calculated to be in the range of 0.43 kg and 0.80 kg Lowest ASC recorded during Summer (0.43 kg) differed significantly with that during rainy season (0.53 kg).

Highest ASC in Nistari marked breed was calculated during late Autumn (1.13 kg). ASC in remaining seasons was calculated to be within the range of 0.36 kg and 0.78 kg Least ASC calculated during Summer (0.36 kg) was found to differ significantly with that during rainy season (0.46 kg).

Observations regarding ASC in F1 hybrid was calculated to be maximum during late Autumn (3.54 kg), followed significantly by that during early Spring (2.75 kg) and Winter (2.27 kg). ASC in rest of the seasons was calculated to be within the range of 0.73 kg and 1.96 kg Least ASC calculated during Summer (0.73 kg) differed significantly from that during rainy season (0.93 kg).

ASC in bivoltine breed was calculated to be maximum during late Autumn (2.35 kg), followed significantly by that during early Spring (2.27). For the remaining seasons, ASC varied between 1.23 kg and 2.07 kg Least ASC was calculated during Summer (1.23 kg).

ASC was calculated to be best in hybrids and worst in multivoltines, late Autumn being the best season and Summer and rainy season being the worst. Average ASC was found to be in significantly strong positive correlation with average ERR (0.950) and average Yield (0.994).

Yield

In Nistari Plain breed maximum yield was calculated during late Autumn (7.81 kg), followed significantly by early Spring (7.33 kg). Yield in remaining seasons varied between 3.90 kg and 6.49 kg Lowest yield calculated during Summer (3.90 kg) differed significantly from that during rainy season (4.86 kg).

Highest yield in Nistari marked breed was calculated during late Autumn (8.18 kg), significantly followed by rest of the seasons where yield was calculated to be

within the range of 2.76 kg and 6.39 kg Yield during Summer (2.76 kg) was calculated to be lowest and differed significantly with that during rainy season (3.14 kg).

In case of F1 hybrid, maximum yield was calculated during late Autumn (18.68 kg), followed significantly by that during early Spring (16.72 kg). Yield for rest of the seasons was calculated to be within the range of 5.86 kg and 13.33 kg Least yield was calculated during Summer (5.86 kg) and differed significantly from that during rainy season (7.59 kg).

In Bivoltine breed highest yield was calculated during early Spring (13.27 kg), followed non-significantly by that during late Autumn (13.20 kg). Remaining seasons exhibited yield within the range of 7.7 kg and 11 kg Lowest of the yield was calculated during Summer (7.7 kg) and differed significantly from that during rainy season (8.52 kg).

It was observed that Yield was best in F1 hybrid followed by Bivoltines and lowest in Multivoltines. Irrespective of breeds, late Autumn was found to be most suitable followed by early Spring, but affected during Summer and Rainy months. Correlation studies showed that Yield had significant positive correlation with single cocoon weight (0.872), single shell weight (0.942), ERR % (0.968) and ASC (0.994).

From the above discussion it became clear that almost all the rearing parameters were observed to be better during late Autumn (Oct. – Nov.) and worse during both Summer (May – June) and Rainy season (July – Aug.), and most of the observations in these two seasons found not to differ significantly as well.

4 Conclusion

Effective rate of rearing, absolute silk content and yield are three of the most important rearing parameters for the farmers. Thorough observations and statistical analysis of the recorded data (Table 2) reveal that during late Autumn and early Spring these three parameters are found to be at their best, because of optimum larval duration, followed by better matured larval weight, which reflects an increase in cocoon and shell weight, having strong positive correlation (0.961). Therefore, late Autumn and early Spring can be considered as two of the best seasons for commercial rearing in Hooghly district of West Bengal; November to April has also been observed as favourable season for mulberry silkworm rearing in traditional silkworm rearing belt of West Bengal by Majumdar et. al., and these two seasons are also found to be favourable in other parts of India in studies conducted by Kumar et. al. (2013) and Rahmathulla (2012). These findings are also supported

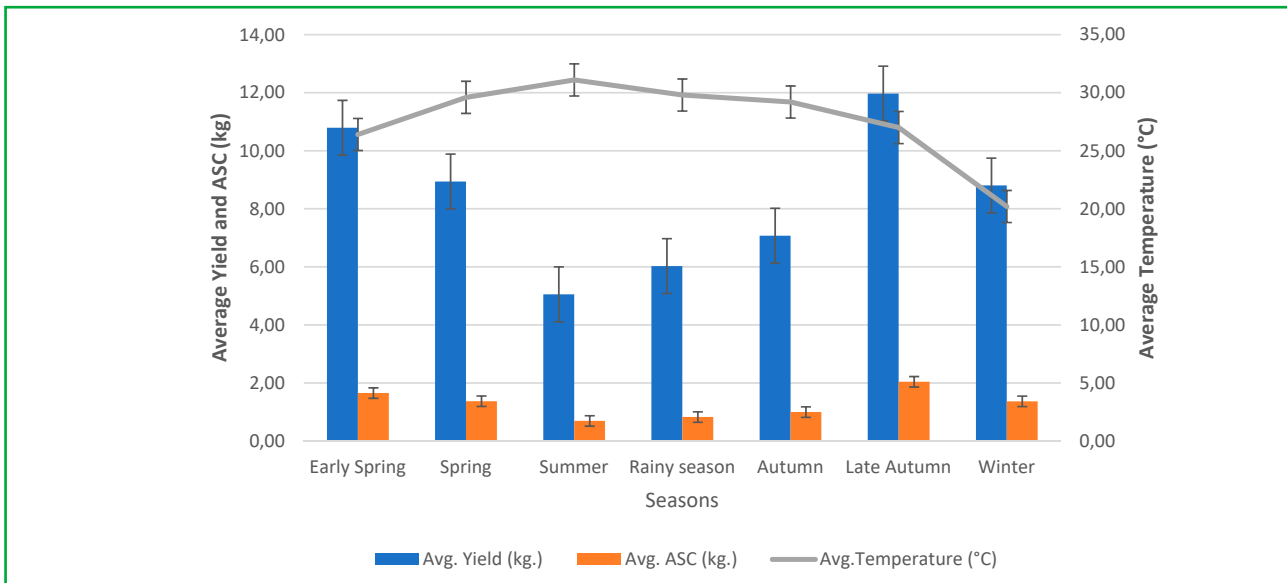


Figure 1 Clustered combo bar chart of avg. yield and ASC with avg. temperature as line chart on secondary axis

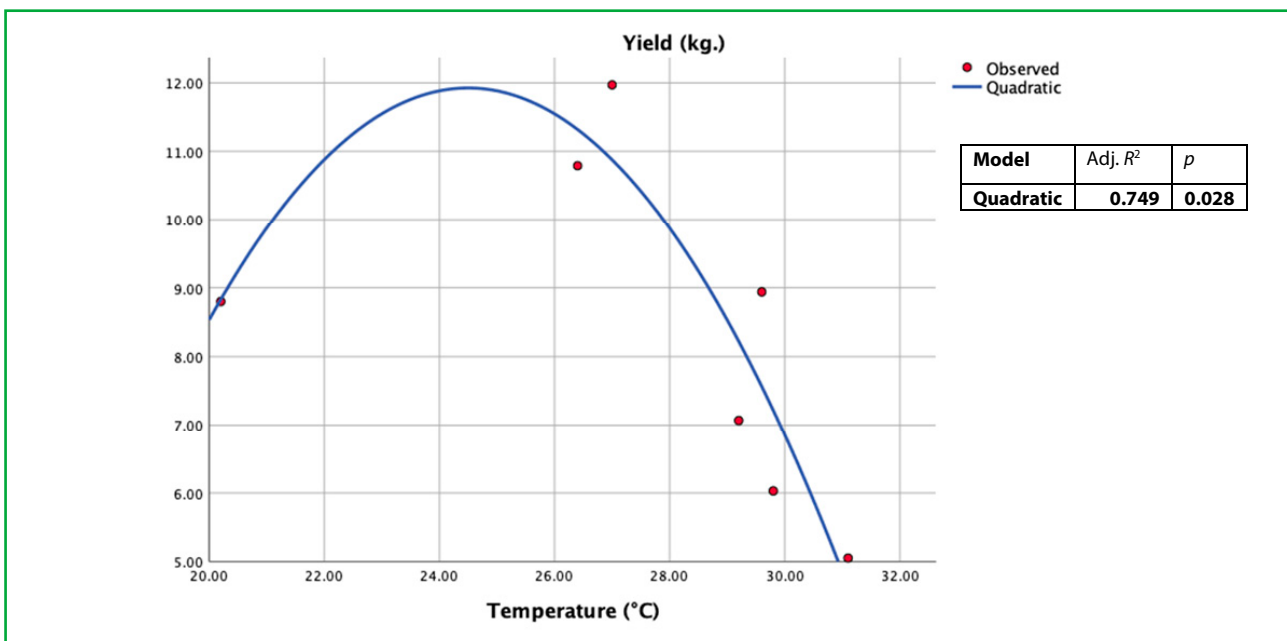


Figure 2 Quadratic regression between average temperature (°C) and average yield (kg)

by the statistical tests comparing average rearing performance data with the mean meteorological data. When average yield and average ASC are plotted against average temperature in a combo clustered-column and line (on secondary axis) chart (Figure 1), highest average yield and highest average ASC are observed during late Autumn (Oct. – Nov.), followed closely by that during early Spring (Feb.-Mar.). From the quadratic regression analysis it is evident that best average yield (Figure 2: Adj. $R^2 = 0.749$ and Sig. = 0.028) and average ASC (Figure 3: Adj. $R^2 = 0.066$ and Sig. = 0.051) is achieved when the average temperature ranges between 25–26.5 °C.

Effect of Temperature and Humidity on average rearing parameters is also evident from the Correlation matrix shown in Table 3, where average ERR, average ASC and average Yield are negatively correlated with Temperature and Humidity, indicating high temperature and relative humidity affecting rearing performance.

Climatic conditions especially temperature and humidity play pivotal role in silkworm rearing (Sisodia and Gaherwal, 2017), this is in clear conformity with the findings of the present study regarding exploration of mulberry sericulture in Hooghly district of West

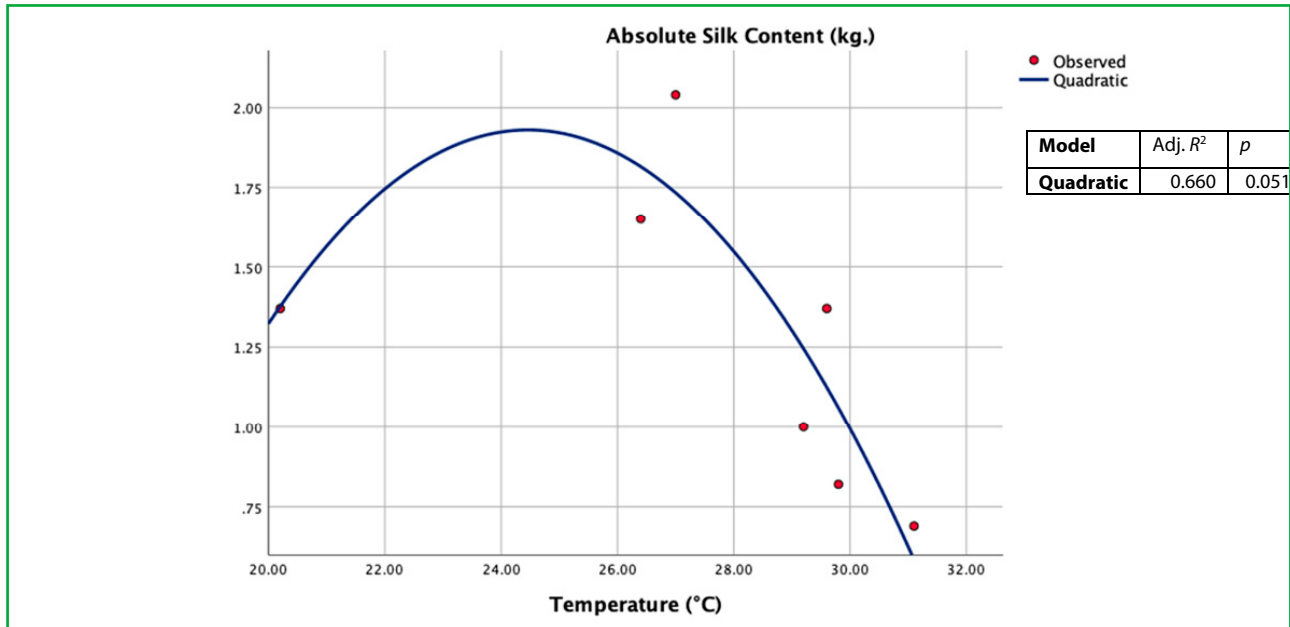


Figure 3 Quadratic regression between average temperature (°C) and average ASC (kg)

Bengal and also in studies conducted in other districts of the state (Hoque and Taufique, 2018). Late Autumn is the most suitable season for silkworm rearing having similarity with adjoining districts, where sericulture is in practice (Majumdar et al., 2017). Early Spring (Feb. – Mar.) can also be exploited as 2nd commercial crop rearing season. Still, Summer, Rainy season and Spring, i.e. the extended summer months (March to August) are found to be unfavourable for rearing of any kind of breeds, other researchers have also confirmed the wet Summer months (June to September) as unfavourable for mulberry sericulture in West Bengal (Majumdar et al., 2017). Further investigations are needed to find out, how this situation can be handled; heat shock (sublethal) (Rahmathulla, 2012) can be searched of. And so far as silkworm breeds are concerned, in Hooghly district of West Bengal, multivoltine breeds are more suitable to handle but hybrids are best to rear. Bivoltines can also be explored for its quality and high price as and when DFLs are available during late Autumn and early Spring.

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Influence of plants on soil mites (Acari, Oribatida) in gardens

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The research of the influence of crops on the species diversity of oribatid mites in garden within the small farmer conditions was carried out in four different microhabitats – carrots, beans (broad beans), onions and tomatoes. Soil samples on each crop were analysed and compared – determined the soil nutrient content and specific elements, continuously recorded the soil temperature, humidity, climate and microclimate relations were also followed within the research, as well as X-ray spectrometric analysis of the soil was done. Chemical elements have no significant impact on soil fauna. Annual average of 563 ind. per square metres and was reported in the microhabitat of beans, while 167 ind. per square metres in carrots, only. Under the broad bean, the highest abundance and species richness and highest species diversity of oribatids was confirmed and seemed to closely connect with the microclimate conditions of this crop. *Tectocephus velatus sarekensis* Trägårdh, 1910, *Zetomimus furcatus* Pearce and Warburton, 1905, *Steganacarus striculus* C.L.Koch, 1836 and *Protoribates capucinus* Berlese, 1908 seem to be tolerant oribatids, which were confirmed in every microhabitats of the garden. On the contrary, some oribatids occurred in one crop only and specify it. Differences in soil temperature and humidity under plants as well as the type of crops affected occurrence of species spectrum and their abundance. In farm garden, we found two rare species of oribatids; the second records for the fauna of Slovakia – *Corynoppia kosarovi* (Jeleva, 1962) and *Mesoplophora pulchra* Sellnick, 1928.

Keywords: agricultural crops, analysis, oribatids, urban area

1 Introduction

Urban areas are typical with the variety of heterogeneous environment as well a wide range of habitats that have a great impact on the animal species diversity. Gardens represent a type of intensively maintained places, mainly the traditional farm garden with an orchard and crops (Krumpálová et al., 2020). The soil edaphon is an important component of biocoenosis, reflects the burden on biotopes and is an important bio-indicator of environmental quality (Porhajášová-Ivanič et al., 2016). Soil contains, in addition to inanimate ingredients, soil organisms that are essential for most soil functions. Without organisms, soil ceases to be soil and becomes inanimate substrate. Every soil of at least average quality

contains a huge number of organisms (Angst et al., 2017). Soil organisms are sensitive to environmental contamination, in which they occur and also called stress as bio-indicators. Their reaction may result to the environmental load in different ways – in a change in behaviour; a change in habitat; a quantitative change and composition of species spectrum; and into physiological or morphological deformation of the individual or of the whole community (Baranová et al., 2015). Human activity alters the physico-chemical properties of the soil, resulting in a negative impact on soil viability, activity and microbial biomass (Zhao et al., 2013).

The soil mites together with springtails are the largest groups of soil arthropods. Soil mites of the Oribatida

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group are the most numerous at depths up to 15 cm from the soil surface (Walter and Proctor, 1999). The importance of saprophagous species is unquestionable in nature – decomposition of organic substances, ensuring the return of nutrients to the soil, which are necessary for growth plants. Their next task is the spread of microorganisms in the soil habitat, which also they affect plant growth either directly or indirectly (Setälä, 1995). Oribatids are rich in terms of species, but are also functionally important soil organisms; therefore often they are bio indicators to indicate the environment (Klimek et al., 2016). Oribatida can be considered a micro fauna sensitive to agricultural activity, such as crop selection, use pesticides and fertilization (Behan-Pelletier, 1999). They can be used in quality assurance, or soil degradation. In demonstrating the quality of soil, we can apply the species alone composition, but also the chemical or physical properties of the soil (Behan-Pelletier, 1999).

The main aim of this research was to find similarity and differences in soil mite assemblages (Acari, Oribatida) under four types of crops. The hypothesis was, based on different type of crops, to detect variance in species diversity, abundance, equitability and predominance some oribatids.

2 Material and methods

Study site represent a typical garden at a family house in Hlohovec town (Western Slovakia, Danube lowland). The area belongs to the Pannonia area (with eu-pannonic xerothermal flora). Soil, which is represented in the monitored area, is cambisol – gleyic cambisols (rich on the minerals). Four garden microhabitats with different crops were selected for soil sampling. Microhabitats were approximately the same size 200 × 600 cm; the first sampling plot was with sown beans (*Vicia faba* L.), root vegetables – carrot (*Daucus carota* L.) was second plot, the third microhabitat was with planted onion (*Allium cepa* L.) and fourth study plot was with tomatoes (*Solanum lycopersicum* L.).

We pick up the samples three times during one growing season – at the beginning of vegetation season (May), in the middle of the season (July) when plants already had fruits, the third collection was carried out after the end of the growing season (October) when plants have already been removed from the soil. From each study site we took six soil samples from a representative part of the crops with soil metal collector with a total volume of 200 cm³ (measuring 4 × 5 × 10 cm). Totally 72 soil samples were extracted in a high-gradient Tullgren type photo thermal collector modified according to Crossley and Blair (1991). The soil samples were dried as the temperature gradually increased from 15 to 45 °C continuously for

seven days. The oribatids were mounted in temporary preparations filled with 40% lactic acid – translucent medium. To identify individuals into genera we used the determination key (Kunst, 1971), subsequently, we identify specimens into species level following the works of Weigmann and Miko (2006), Olszanowski (1996), Subias and Arillo (2001). The differences between ecological groups were tested by PAST software (Hammer et al., 2001); Kruskal-Wallis nonparametric test to determine if there are statistically significant differences between groups of an independent variable on a continuous dependent variable H (chi squared) = 7.87; $p = 0.048$.

We used the DELTA CLASSIC XRF (U.S.) spectrometer to determine the soil nutrient content and specific elements – Pb, S, Fe, Mn, K, Cu, Cr, Ca, Zn, Mo, Ba, Sr, Rb, Ti, Zr, Cl, I, As, Cd, Zb, Co. For the possibility of comparing the soil moisture on the observed plots was determined soil water content by gravimetric method. We are also continuously every 4 hours (from March to November) record the soil temperature at a depth of approximately 10 cm using miniature samples iButton DS1921G meters with a measuring range of -40 to +85 °C, with a resolution of 0.5 °C, and measuring accuracy ±1 °C.

3 Results and discussion

Soil properties in the garden

We measured the values of chemical elements, for each study site separately. The stationary with the bean crop had higher measured values only for the elements – potassium, calcium and iron. The highest measured values were just calcium, which ranged from approximately 30,000 to 48,000 ppm (note: ppm – parts per million) (Figure 1). Potassium values ranged from approximately 10,000 to 20,000 ppm and iron ranged from 22,000 to 26,000 ppm.

A microhabitat with a tomato had, similar to a stationary with a bean, measured above values only for potassium, calcium (ranged from about 40,000 to 46,000 ppm) and iron elements (below 24,000 ppm) (Figure 1). The study plot with onion reached the lowest measured values of soils elements with higher readings were calcium (from 29,000 to 33,000 ppm), potassium (15,000–17,000 ppm) and iron (26,000–28,000 ppm). In the microhabitat with the carrot crop were similar elements; a higher values – calcium (46,000–50,000 ppm), iron was approximately at 19,000 ppm and potassium had a low values (17,000 ppm). The other elements varied at the zero measurement limits (Figure 2). Based on these measurement results, we assumed the contained chemical elements have no significant impact on soil fauna.

Soil temperature was varied from 16 to 24 °C, while the air temperature was from 10 to 22 °C (monthly average).

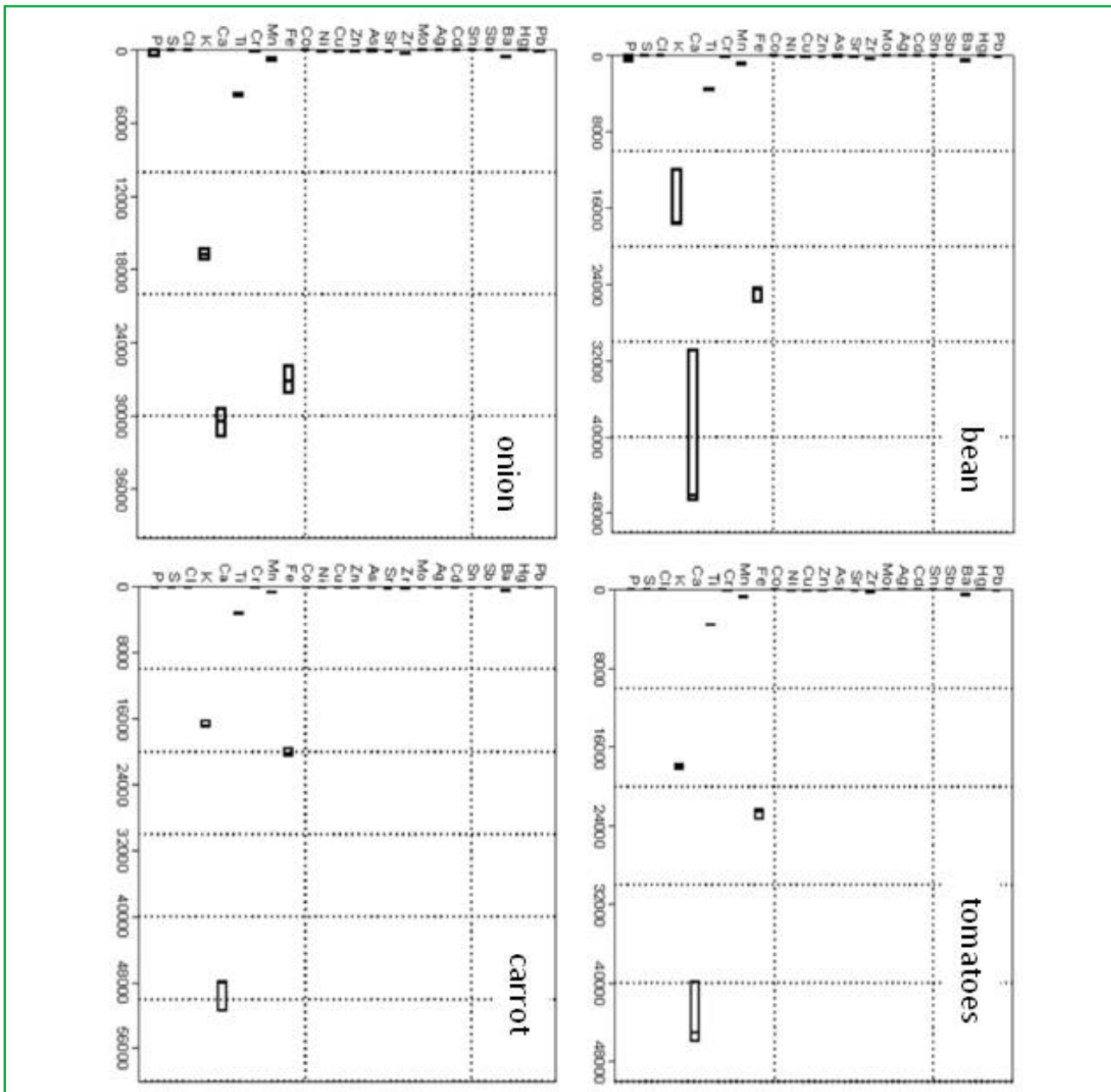


Figure 1 Box plot values of chemical elements (95% data reliability) of study habitats

The most striking temperature difference between two periods was May and June (4 °C). More marked temperature differences of soil between microhabitats can be seen mainly between tomatoes and beans. The stationary with tomatoes had the highest average soil temperatures. On the contrary, beans measured the lowest average soil temperatures (Figure 2) during the whole season.

The average soil moisture conditions were in the spring for all crops in the interval 11–13%. Significant changes in soil moisture occurred in the second sampling, during summer, when the lowest humidity (4.7%) was recorded under the tomato. On the contrary, the highest humidity

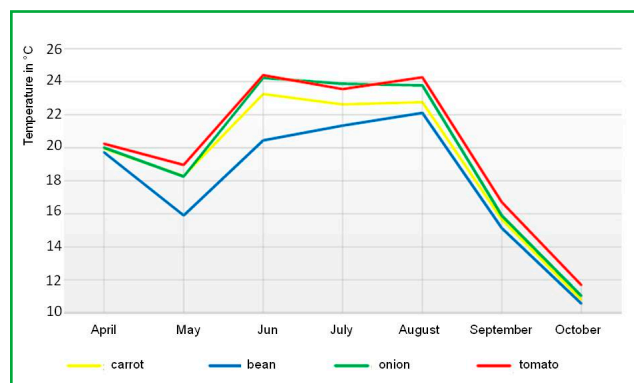


Figure 1 The course of monthly average soil temperatures at individual study plots

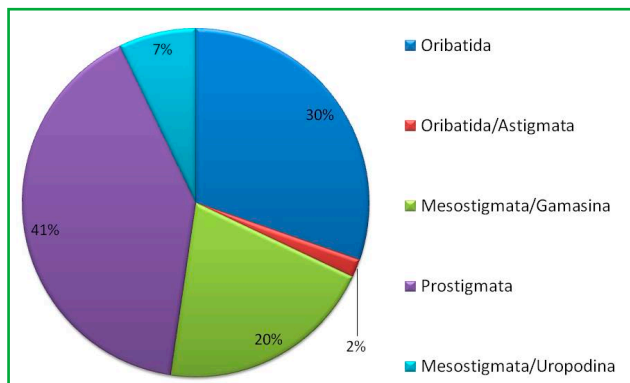


Figure 3 Acari structure in the soil of the garden

in summer was in beans microhabitat (11.8%) and in the onion (11.4%). In the autumn we noticed the highest soil moisture in the whole monitored season. The tomato station has the highest soil moisture overall (21.3%) (Table 1).

Table 1 Soil moisture at individual study plots determined by gravimetric method (%)

	May	July	October
Tomato	11.3	4.72	21.3
Onion	13.2	11.4	19.3
Beans	11.6	11.8	17.7
Carrot	13.1	8.7	18.8

Invertebrate assemblages in the garden

During the research, we picked out the soil samples in four study sites with different crops and collected all together, 1,272 individuals of soil arthropods. In the garden soil

predominated Acari cohort, which had a total of 74.21% (944 individuals); the second highest group was of the Collembola – 268 individuals (21.07%). Springtails were most abundant in the summer and autumn collection, especially in July; the low number was recorded in May. The Hymenoptera – Formicoidea was subdominant group having 38 individuals (2.99%). The other groups in garden did not even have one percent representation: Hemiptera – Aphidoidea, Isopoda, Araneae, Chilopoda – *Scolopendra* gen. and Coleoptera – Curculionidae family.

Acari was formed by eudominant groups of Prostigmata, which had 382 individuals (40.7%), Oribatida had 286 individuals (30.3%) and Mesostigmata (Gamasina) with 192 individuals (20.3%). 68 individuals (7.2%) belong to the Mesostigmata (Uropodina) and 16 individuals (1.7%) to the group Oribatida (Astigmata) (Figure 3).

Analysis of Oribatida assemblages in the garden

The priority of our research was to determine the impact of crops on the soil mites (Acari – Oribatida). We collected 193 adult individuals throughout the garden during the monitored season belonging to 24 species (Table 2); nymph-development stadium were not included into evaluation. Most species found in the garden were less than 6% of dominance. Except of two species – *Tectocephus velatus sarekensis* and *Zetomimus furcatus*, which of all 24 specimens they had eudominant proportion in the garden soil.

Abundance, species spectrum and diversity of some oribatids coenoses in garden differed. A total of 81 adults (18 species) were sampled at the soil under beans. The highest species spectrum was recorded in summer and autumn; in the summer we recorded the highest

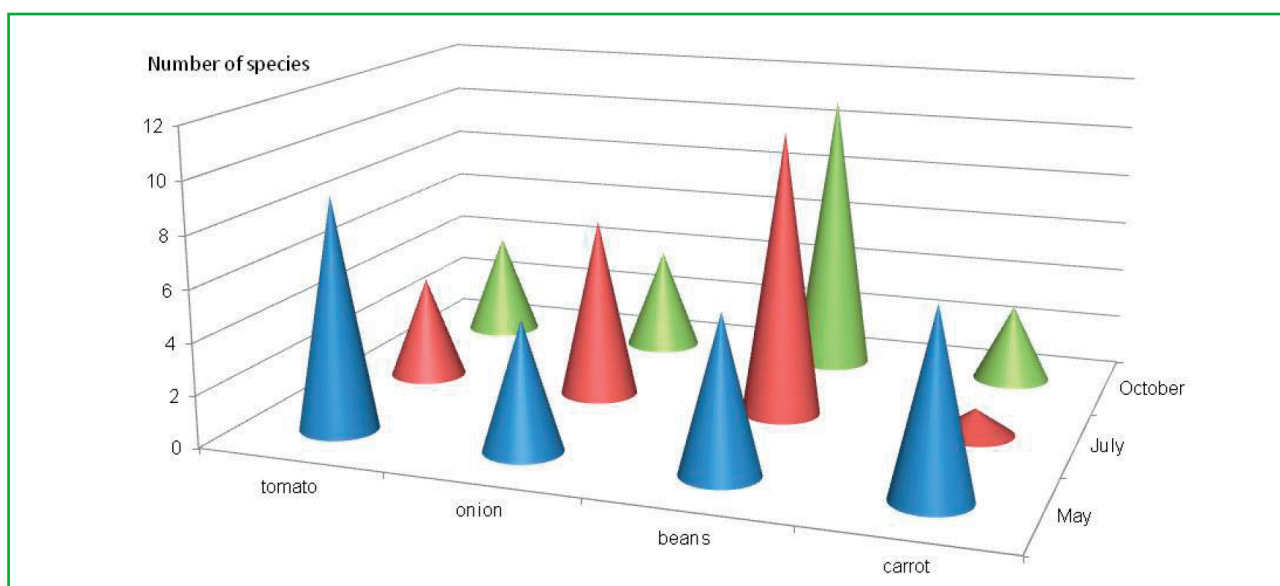


Figure 4 Seasonal differences in the abundance of oribatids found in different crops in the garden

Table 2 Abundance of Oribatida mites in the soil of some crops, indices diversity and total dominance of oribatids in the garden

Species	Abundance on separate study sites				Oribatida's dominance (%)
	tomato	beans	carrot	onion	
<i>Achipteria coleoprata</i> (Linné, 1758)	1	3		1	2.59
<i>Achipteria nitens</i> (Nicolet, 1855)	1				0.52
<i>Corynoppia kosarovi</i> (Jeleva, 1962)		1			0.52
<i>Cosmochthonius lanatus</i> (Michael, 1885)	1				0.52
<i>Ctenobelba pectinigera</i> (Berlese, 1908)	1	2			1.55
<i>Epilohmannia cylindrica</i> (Berlese, 1904)				1	0.52
<i>Epilohmannia minima</i> Schuster, 1960	2		3	1	3.11
<i>Galumna lanceata</i> (Oudemans, 1900)		2			1.4
<i>Mesoplophora pulchra</i> Sellnick, 1928		1			0.52
<i>Metabelba pulverosa</i> Strenzke, 1953		3			1.55
<i>Microppia minus</i> (Paoli, 1908)		1			0.52
<i>Nothrus anauniensis</i> Canestrini & Fanzago, 1876		3		1	2.7
<i>Protoribates capucinus</i> Berlese, 1908	3	3	3	1	5.18
<i>Ramusella cf. elliptica</i> (Berlese, 1908)		4			2.7
<i>Ramusella furcata</i> (Willmann, 1928)				2	1.4
<i>Ramusella insculpta</i> (Paoli, 1908)		7			3.63
<i>Rhysotritia ardua</i> (C.L. Koch, 1841)		1	2	1	2.7
<i>Schelorbates laevigatus</i> (C.L. Koch, 1835)		2			1.4
<i>Scutovertex</i> sp.	7		3	1	5.70
<i>Sphaerochthonius splendidus</i> (Berlese, 1904)		1			0.52
<i>Steganacarus carinatus</i> f. <i>pulcherrima</i> (Berlese, 1887)		1			0.52
<i>Steganacarus</i> (<i>Atropacarus</i>) <i>striculus</i> (C.L.Koch, 1835)	3	2	2	1	4.15
<i>Tectocephus velatus sarekensis</i> Trägårdh, 1910	23	33	5	10	36.79
<i>Zetomimus furcatus</i> (Pearce & Warburton, 1906)	16	11	6	10	22.28
Species diversity (Shannon's index)	1.68	2.18	1.87	1.82	
Species richness (Margalef's index)	2.22	3.87	1.89	2.94	
Species equitability (Pielou's index)	0.72	0.75	0.96	0.76	

abundance (32 individuals), in the end of the season this number decreased (to two individuals). Microhabitat with tomatoes throughout the season obtained 58 adults. The highest species spectrum was in spring (36 individuals, nine species). Subsequent samplings showed not only a lower number of species (only four species were found), but also the abundance dropped to 11 individuals (both summer and autumn) (Figure 4, Table 2). At the soil under carrot, we found seven species (only 24 individuals) for the whole season; the highest number of individuals was in the spring (18 individuals), in summer the abundance dropped (one specimen), in autumn slightly increased to five individuals (3 species). The microhabitat with the onion had a total of 11 species

(30 individuals) in the whole season. The number of individuals in this site was during the year relatively balanced; the richest species spectrum we found in summer (Figure 4, Table 2).

The highest species diversity (Shannon index) was recorded in soil below a bean crop of 2.18, lowest under the tomato ($H' = 1.68$). On the contrary, the highest species equitability (Pielou index) achieved the oribatids in carrot ($E = 0.96$); the species richness (Margalef index) varied from 1.89 to 3.87; highest value was on the stationary with onions (Table 2).

The number of individuals ranged from zero to approximately five individuals. In the first sampling

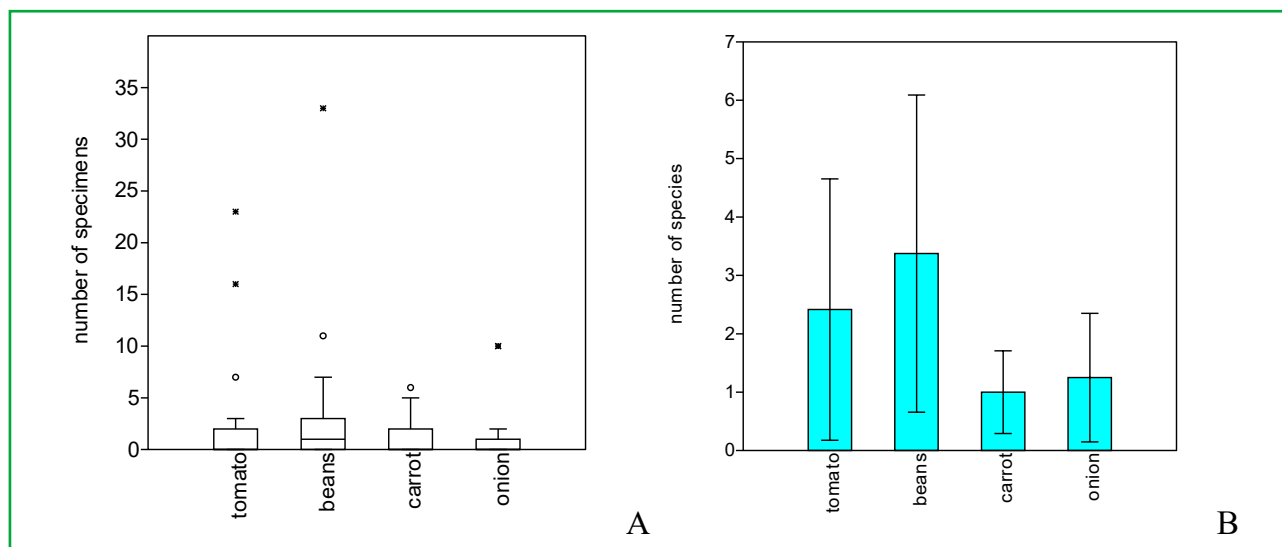


Figure 5 A – box plot of the number of oribatid individuals in samplings during season; B – box chart of the number of oribatids in the whole season (max., min., median; 95 percent data confidence)

under the tomato crop, 16 individuals (outlier) of species *Tectocephus velatus sarekensis* reached, in the third sampling under the bean crop we registered higher occurrence of the same species (13 individuals). Other crops had significant richness only one sampling per season (Figure 5A). The greatest number of oribatid species within the garden we found under the bean crop that reached its maximum of six species. A higher number of species was also recorded under the beans (Figure 5B).

Four species occurred in all four habitats of garden – *Tectocephus velatus sarekensis*, *Zetomimus furcatus*, *Steganacarus striculus* and *Protoribates capucinus*. *Tectocephus velatus sarekensis* and *Zetomimus furcatus* achieved the highest frequency of occurrence in individual crops overall and were also the eudominant species in each collection (except for the spring field collection in carrots, where we didn't even notice it). On the contrary, during the research we found the oribatids

that occurred in soil under only one crop and specify the assemblages (Figure 6, Table 2). Species *Achipteria nitens* and *Cosmochthonius lanatus* occurred under tomatoes only; soil microhabitat with onion also had two specific species – *Ramusella furcata* and *Epilohmannia cylindrica*. No specific species under the carrot were recorded that would be unique to this crop. On the contrary, the soil under bean had several specific species including – *Corynoppia kosarovi*, *Galumna lanceata*, *Mesoplophora pulchra*, *Metabelba pulverosa*, *Micropopia minus*, *Ramusella cf. elliptica*, *Ramusella insculpta*, *Schelorbates laevigatus*, *Sphaerochthonius splendidus* and *Steganacarus carinatus f. pulcherrima*.

Among the oribatids in garden we also noted exceptional findings of species that were not until recently confirmed in our country, respectively, they are not typical for central European territory. The species *Corynoppia kosarovi* (family Oppiidae) is the second confirmed finding for Slovakia. It is a very interesting species that has been recorded only once during the entire research period. Its occurrence was recorded in summer in the bean crop (July 18, 2017). The first finding was recorded in the same year in Bratislava at the cemetery (Mangová et Krumpál, 2017). Another extremely interesting finding is the species *Mesoplophora pulchra*, the third finding for Slovakia. We found it in the autumn sampling in bean crop (October 27, 2017). It is a subtropical – tropical species that prefers the south of European and the Mediterranean environment (Starý, 2008; Miko, 1987; Miko, 1995).

Oribatida (Astigmatina), Mesostigmata (Gamasina), Prostigmata, Mesostigmata (Uropodina) and Acari (Oribatida) were represented in all chosen study sites. Two

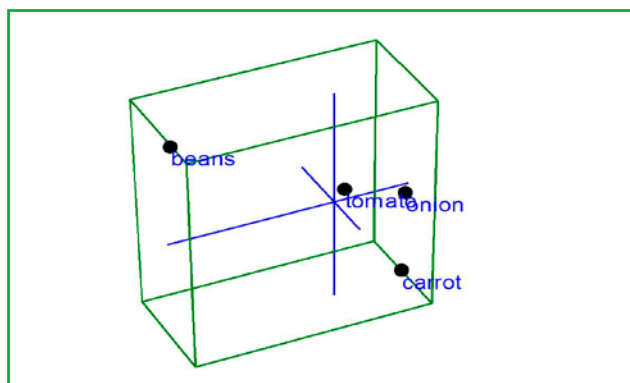


Figure 6 Landmark chart specifying the oribatid assemblages according the type of crops

groups of arthropods, which occurred in all four habitats, were Collembola and Hymenoptera. Collembola was most abundant in the summer and autumn collection, especially in July; the smallest number was recorded in May. These results also partially correspond to paper of Bandyopadhyaya et al. (2002), which recorded the highest frequency in spring and autumn. The reduction in the abundance of Collembola was recorded immediately after soil cultivation. In our case it was in the spring, when the soil was prepared for planting. In autumn, after the crops have been removed from the soil, the soil was not being so disturbed that it would be caused the fall of springtails.

The nitrogen and carbon content of the soil positively affect the abundance of individuals; these elements create suitable living conditions in the soil (Horwood and Butt, 2000; Maraun and Scheu, 2000; Petersen and Luxton, 1982). Therefore, we assumed that under beans would be higher species diversity most diverse as leguminous plants using symbiotic bacteria bind atmospheric nitrogen. Obtained results of oribato-coenosis confirm this assumption (diversity index, species spectrum). Equally, differences in soil temperature and humidity may have affected occurrence of species and their abundance in monitored habitats (Štipčáková, 2018; Krumpálová et al., 2020).

The cosmopolitan species of *Tectocepheus velatus sarekensis* was observed in every soil sampling from selected microhabitats, but also in each collection (except for one summer under the carrot crop). The occurrence of this species was to be expected during our research, as *T. velatus* often dominates on cultivated soils (Luptáček et al., 2012; Maribie et al., 2011).

The dominant species in all habitats was also *Zetomimus furcatus*, its dominance was quite unexpected. So far it has only been known from three locations in Slovakia (Starý, 2008). According to Weigmann and Miko (2006), its occurrence is focused on oligotrophic peat lands and forests; this species seems to tolerate a low proportion of nutrients in the soil than other species. *Micropoppia minus* was also found in oribatocoenoses during our study. It is a species with small body dimensions of 170–215 µm (Weigmann and Miko 2006), which is also abundant in agrosystems, preferring habitats with increased humidity (Luptáček et al., 2012). The preference of humid habitats explains its low abundance in dry habitats. Most of the species that we found have occurred in very low abundance. Probably the cause of this state was the habitat requirements of species, which according to the garden failed, as well the garden disturbance.

Starý (2008) declares that the number of individuals is affected by several factors and changes in Oribatida

communities can be observed from several aspects. First there are periodic variations in coenoses, which are affected by environmental changes caused by anthropogenic activity and natural changes. Fertility, mortality, length life cycle, number of generations per year and migratory capacity of dominant species affect the seasonal dynamics of oribatids the most. Agro ecosystem soils have the lowest species diversity and mite equitability compared to forests and soils used as pastures (Arroyo and Iturrondobeitia, 2006). To increase community richness and abundance not only soil mites but overall edaphone, it would be advisable to replace crops within the garden. This agricultural practice has confirmed stimulating mite regeneration (Neher, 1999).

Results of this research, exactly the research of the oribatids in the garden of the family house under different crops are unique and first of its kind not only in Slovakia but also in Central Europe.

4 Conclusions

The abundance of individuals under crops varied. Highest abundance, similarly and species diversity was under the bean crop, a yearly average of 563 ind m⁻² and lowest one was noticed in micro habitat with carrot, only 167 ind m⁻². By comparison, forest soil habitats achieve abundance as well several 100,000 individuals per square meter (Norton, 1994). We have confirmed the assumption that under the beans was the abundance and species diversity of oribatids highest, which can also be closely connected with the microclimatic conditions of the crop. Species richness and abundance of oribatids in the soil of different crops closely related to the microclimatic conditions of the crop. The temperature of the soil under the bean was significantly lower (Figure 2) than below other crops; as well as the markedly higher soil moisture was there. The leguminous plants create a compact vegetation cover that provides shading, reduce the effects of extreme temperatures and water vaporisation from soil; and this can positively affect soil mites (Figure 2, Table 1) – more stable microclimate conditions could be the cause of minor fluctuations of species at the oribatid assemblages.

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Genetic diversity and production potential of animal food resources

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The submission aims to present results of the five-year research project, oriented on the evaluation of genetic diversity of selected populations of economically important animal species in Slovakia, their sustainable adaptation and production potential in the context of preservation of genetic resources and food safety. Under the supervision of Department of Animal Genetics and Breeding Biology, Faculty of Agrobiolgy and Food Resources of the Slovak University of Agriculture in Nitra run between 2015-2019 project called Molecular-genetic diversity and production potential of animal genetic resources in Slovakia (APVV-14-0054). Considering the difficulty and complexity of studied issues was research realized in close collaboration with the University of Natural Resources and Life Sciences Vienna (BOKU) and Zagreb University. Erosion of genetic diversity represents the main threat for food safety of mankind. Individuals of economically important animal species groups accumulate risks and threats of loss of sustainable adaptation as a reaction to the environment due to intense selective breeding. It is therefore important and needed to focus on permanent monitoring and evaluation of diversity of economically important breeds based on the diverse parameter and suitable methods.

Keywords: Genetic diversity, economically important breeds, Animal genetic resources, Slovakia

1 Introduction

In the context of transformation and globalisation processes are ever often discussed questions of food safety and tools for its maintenance. Already in 2007, Commission for genetic resources for nutrition and agriculture FAO, resp. International technical conference in Swiss city Interlaken approved Global Plan for Animal Genetic Resources and Interlaken Declaration. It contains 23 strategical priorities, which shall beside others help erase extreme poverty and ensure sustainable development. No poverty is the No. 1 within the Sustainable Development Goals to transform our World under gesture of UN and protection of biodiversity is part of that Global Plan fo Action represented under Goal No. 15: Life on Land. Needed is, therefore, global collaboration, as well as intense development of programmes and politics of sustainable utilisation and development, protection and description of Animal genetic resources at the national level.

This submission represents the results of the research project aimed at the evaluation of animal genetic diversity of economically important species in Slovakia, which was realised at the Faculty of Agrobiolgy and Food Resources of the Slovak University of Agriculture in Nitra, under gesture of Department of Animal *Genetics* and Breeding Biology: Molecular-genetic diversity and production potential of animal genetic resources in Slovakia (APVV-14-0054).

2 Research of genetic diversity as a base for the protection of animal food resources

With the effort to stop the erosion of animal genetic diversity it is important to give attention to the genetic diversity of economically important animal species. Intense genetic interventions and systematic breeding are sources of the high risk of inbreeding and production of inbred progeny, which has a negative impact especially on the genetic diversity of small populations.

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Individuals within such groups accumulate risks and threats of loss of adaptation mechanisms as a reaction to the environment. It is therefore important and needed to focus on permanent monitoring and evaluation of diversity of economically important breeds based on the diverse parameter and suitable evaluation methods. Estimation of genetic diversity loss and monitoring of changes of each population from the point of view of all elements entering the breeding process is therefore essential. It is a simple action, but even complex of the network of supporting each other.

A project called Molecular-genetic diversity and production potential of animal genetic resources in Slovakia (APVV-14-0054) was carried under the supervision of Department of Animal Genetics and Breeding Biology, Faculty of Agrobiolgy and Food Resources of the Slovak University of Agriculture in Nitra between 2015–2019. The project team consisted of 9 researchers, from which two new workplaces have been generated by the project and early career scientists employed.

Core themes of the project were:

- a) Knowledge and preservation of the diversity of breeds and the preservation of genetically important animals with high production potential.
- b) Identification of population-specific variants of SNP markers as a condition of long-time survival.
- c) Establishment of SNP panels for milk and meat production in cattle as well as performance in horses.

Populations for research included dairy (Jersey), beef (Limousine, Charolais, Hereford, Angus, Piedmontese and Romagnola) and dual-purpose (Slovak Pinzgau, Slovak Spotted, Carpathian Brown) in case of cattle and warm-blood (Furioso, Nonius, Lipican, Slovak warm-blood) as well as cold-blood (Norik) horses. During the whole project period were established complex methodologies for determination of identifiers of “higher-order” not only in cattle and horses but even in other livestock and wild species (Kasarda et al., 2016a; Kadlečik et al., 2017a; Moravčíková et al., 2018a). An integral component of complex methodology was the determination of genetic diversity or uniqueness of genetically close populations and sub-populations (Kasarda et al., 2016b; Moravčíková et al., 2017a; Kukučková et al., 2018a; Moravčíková et al., 2018b).

Different methods for determination of admixture level of populations were used based on Bayesian approach and discriminant analysis of principal components (DAPC) (Moravčíková et al., 2017b, c; Lehocká et al., 2020) evaluation of genetic differentiation of populations

based on linkage disequilibrium (LD) with simultaneous identification of genomic regions showing selection signatures linked on their genetic differentiation (Kukučková et al., 2016a,b), determination of the level of genetic differentiation of populations based on Wright's F_{ST} index and determination of genomic regions characteristic for particular breeds and local populations, respectively (Lehocká et al., 2019a). As part of the methodology for evaluation of the effect of selection on genome structure and identification of genomic regions significantly affected by the specific selection type, were evaluated parameters as extended haplotype homozygosity (EHH) integrated haplotype score (iHS) (Kasarda et al., 2015), Wright's F_{ST} on the whole genome level (Moravčíková et al., 2018c), level of autozygosity based on the distribution of homozygous segments (ROH) (Kasarda et al., 2018a; Moravčíková et al., 2018d; Moravčíková et al., 2019a) and the variability of linkage disequilibrium (Kasarda et al., 2016c; Moravčíková et al., 2019b). An integral part of the genetic diversity monitoring of cattle and horse breeds was the evaluation of observed heterozygosity (H_o) and gene diversity (H_e), the average number of alleles (MNA), effective number of Alleles (A_{ne}), Shannon information index (I) in case of STR markers solely (Kasarda et al., 2016d; Vostrá Vydrová et al., 2018). Wright's FIS index as the molecular equivalent of inbreeding coefficient calculated based on pedigree information, genomic inbreeding coefficient based on the distribution of homozygous segments (ROH) in the genome, inbreeding coefficient (F) and its increase (ΔF) based on pedigree analysis (Kadlečik et al., 2016, Kadlečik et al., 2017b; Kasarda et al., 2017a; Kasarda et al., 2019), level of population fragmentation based on F -statistics and analysis of molecular variance (AMOVA), Nei genetic distances at intra- and inter-population level (D_a), migration level and genetic drift intensity (Kukučková et al., 2016c; Kukučková et al., 2018b; Miluchová et al., 2018; Moravčíková et al., 2020), present and historical effective population size (N_e) based on molecular and pedigree data (Kadlečik et al., 2016; Kukučková et al., 2016b; Moravčíková et al., 2017d). In cattle, the genotype-phenotype associations were tested concerning milk production (Trakovická et al., 2017) and beef quality (Miluchová et al., 2018; Trakovická et al., 2018a, b, c; Trakovická et al., 2019). In pigs, the effect of candidate genes responsible for carcass traits and meat quality were analysed (Trakovická et al., 2016a, b). In terms of the well-being and functional traits of dairy cattle, the optimal methodology for reliable claw traits measurement were suggested (Vlček et al., 2016a, b; Vlček et al., 2017a, b, c). The genetic parameters for the claw traits and metabolic diseases were estimated based on the Bayesian and REML approaches (Kasarda et al., 2018c, Kasarda et al., 2019a).

Considering the difficulty and complexity of studied issues was research realized in close collaboration with the University of Natural Resources and Life Sciences Vienna (BOKU) and Zagreb University for comparison analyses, in particular. The national platform constituted of breeding associations and organizations in Slovakia. Results of each research tasks were presented on national, as well as on international level in form of in total 73 publications (Where 9 in CC, 21 indexed in WOS and SCOPUS, 1 scientific monograph with the renowned foreign publisher, 3 scientific monographs in Slovakia).

3 Obtained results

The results of the project show, that Slovak Pinzgau and Slovak Spotted are significantly endangered. In case of Slovak Pinzgau it is based on analyses of homozygous runs longer than 16 Mb, reflecting present inbreeding, which present 0.81% of the genome (Kukučková et al., 2017a, b; Kasarda et al., 2019b). Similar results were obtained in case of Slovak Spotted, whereas 0.43% of the genome was covered by homozygous segments

(ROH) (Kasarda et al., 2018a, Kasarda et al., 2019c). Those observations clearly point a relatively large proportion of inbred animals in the present generation in case of both breeds. Level of endangerment was further evaluated by effective population size. In the case of Slovak Pinzgau was estimated effective population size cca. 30 individuals with loss of 7.01 animal per generation (Kukučková et al., 2017b; Moravčíková et al., 2017d). Effective population size in Slovak Spotted was 38.69 with loss of 7.64 animal per generation (Lehocká et al., 2019b). Both values are below the recommended minimum effective population size limit (50 animals) for the preservation of genetic diversity and could be considered as alarming. Even in the case of beef breeds was observed also the linear decrease of effective population size, however, the values were between (20–120 animals). The decrease was more rapid in the case of Limousin cattle. In the case of observed horse populations, results show a relatively stable level of genetic diversity (Kasarda et al., 2020).

Relatively high heterozygosity was observed in the case of Slovak Spotted ($H_o = 0.69$; $H_e = 0.70$) based on STR

Table 1 SNP marker panel significantly associated with milk performance in Pinzgau cattle (Kasarda et al., 2017a)

CHR	Illumina ID	Position (bp)	-log (p-value)	QTL trait
1	ARS-BFGL-NGS-18066	111357945	4.77e-06	Milk Yield, Dressing percentage
7	BTB-00955523	105621232	4.74e-06	Milk Yield, Protein Yield, SCS score
8	Hapmap48090-BTA-81304	60269047	4.47e-06	
9	Hapmap60949-rs29020404	52283151	7.68e-06	Marbling Score, Milk, Protein and Fat yield
15	ARS-BFGL-NGS-12339	20018872	1.05e-06	
	ARS-BFGL-NGS-118767	24021537	2.93e-06	
16	BTA-38204-no-rs	3075859	2.52e-06	
18	ARS-BFGL-NGS-15438	53224638	5.18e-06	

Table 2 SNP marker panel significantly associated with milk performance in Slovak Spotted (Moravčíková et al., 2018e)

CHR	Illumina ID	Position (bp)	-log (p-value)	QTL trait
2	ARS-BFGL-NGS-102253	62853776	2.66E-05	Fat content
4	ARS-BFGL-NGS-76618	104909380	2.85E-05	Fat content
6	Hapmap58359-rs29011329	7514029	2.37E-05	Fat content
8	BTB-01415809	47479350	1.50E-06	Protein content
11	ARS-BFGL-NGS-39507	74139241	7.63E-06	Milk production, Protein content
12	BTB-00500829	68846890	3.72E-06	Protein content
14	Hapmap50356-BTA-42148	17401030	1.91E-05	Fat content
15	ARS-BFGL-NGS-44706	76438547	2.50E-05	Protein content
15	ARS-BFGL-NGS-25994	76466667	2.50E-05	Protein content
17	ARS-BFGL-NGS-96040	12766838	2.89E-05	Fat content
21	ARS-BFGL-NGS-103866	5690539	3.03E-05	Fat content

markers, whereas FIS index didn't show any significant effect of inbreeding on genetic diversity. Further analysis showed a higher level of heterozygosity and gene diversity of Slovak Pinzgau cattle ($H_o = 0.75$; $H_e = 0.73$) in comparison to Slovak Spotted, confirmed by negative FIS (-0.02) index. In total, a higher level of genetic diversity in comparison to other local cattle (Holstein, Simmental, Montbeliard, Austrian Pinzgau) was also indicated by other parameters, including (MNA = 7.82) and Shannon information index ($I = 1.55$) (Kasarda et al., 2019b; Kasarda et al., 2019d). In case of horses were analysed 3 warmblood breeds (Lipizzan, Furioso, Nonius). For the analyses were used animals representing gene-pool of those breeds in Slovakia. The observed level of genetic diversity and heterozygosity (0.89) within populations show the dominance of heterozygous animals and therefore a good level of genetic (Moravčíková et al., 2016; Kasarda et al., 2016d; Kasarda et al., 2018d; Kasarda et al., 2019e).

Genomic information was used to characterize the structure of observed populations and evaluation of admixture level in Slovak Pinzgau and Slovak Spotted in

connection to other European cattle and subsequently identified genomic regions, which could be specific especially for local populations of Pinzgau or other endangered breeds. The analyses identified clear differentiation among 15 populations, as well as an expected higher level of genomic similarity between Slovak Pinzgau and Austrian Pinzgau cattle (Kukučková et al., 2017b; Kasarda et al., 2019b). According to a more general view, Cika and Pinzgau were closest breeds. Regarding the relatively low value of F_{ST} index, resulting from a high level of genetic similarity between individuals, identification of regions representing differences between Slovak and Austrian population was possible. Strongest signals were observed on Chr. 6. Regions with high homozygosity were detected on Chr 2, 4 and 11 (Kasarda et al., 2015; Kukučková et al., 2017b; Kasarda et al., 2018a; Moravčíková et al., 2018c).

Subsequent GWAS analysis was realized in Slovak Pinzgau and Slovak Spotted. In both cases was the aim to identify genomic positions associated with milk performance and proportion of fat and protein in milk. In the case of Pinzgau cattle data consisted of information about 7729

Table 3 SNP marker panel for milk performance and reproduction (Moravčíková et al., 2017a)

CHR	SNP ID	Position (bp)	Gene	Trait
1	rs109007595	35014129	POU1F1	Production and composition of milk, urea, SCS
1	rs41608610	81589478	DGKG	Milk fatty acids, UFA, Production of milk, SCS
2	rs43706906	79923716	STAT1	Production and composition of milk, urea, SCS
4	rs110559656	93257549	LEP	Milk fatty acids, SFA, Production of milk, SCS
5	rs41604573	70471512	RIC8B	α -LA
6	rs29024684	87396306	Casein family	α S1-CN, α -LA, milk protein content
6	rs41577868	37983812	ABCG2	Milk fatty acids, UFA, Production of milk, SCS
6	rs41653166	95988438	CSN2	β -CN
6	rs41654958	90730485	Casein family	Protein content
6	rs42225005	67643584	Casein family	β -LG
9	rs29018912	17726910	MEI4	α -LA
13	rs110270855	1278678	PLCB1	Production and composition of milk, urea, SCS
13	rs41624761	1655502	PLCB1	Production and composition of milk, urea, SCS
13	rs41630716	60242262	MC3R	κ -CN
18	rs41572288	54450227	GRLF1	Production of milk, SCS, urea, acidosis
19	rs109686238	14673538	CCL3	Milk fatty acids, SFA
19	rs110562092	13887927	ACACA	Milk fatty acids, SFA, Production of milk, SCS
20	rs41640170	36097136	EGFLAM	Milk protein content
22	rs29020976	39491373	PTPRG	α S1-CN
24	rs29016076	34928812	ABHD3	β -LG
26	rs41606739	33003665	GPAM	α -LA
26	rs41624917	15383866	PLCE1	Production and composition of milk, urea, SCS

daughters (130087 milk records) 35 sires, with genomic information available. Identification of genomic regions associated with milk performance and its content was made using a linear mixed model. Results of analyses confirmed signals especially in regions of QTLs associated with milk performance, protein and fat content as well as somatic cell count or marbling (Table 1), confirming dual-purpose character of Pinzgau cattle (Kasarda et al., 2017a). Similarly as in the case of Pinzgau population were observed associations between SNP markers and breeding values for milk, fat and protein (in kg and %) in Slovak Spotted. A linear model with random regression was used to analyse data. SNP markers with significant effect were located predominantly in genomic regions of Chr 8, 11 a 12. In table 2 is the list of SNP markers with the most important effect on the variability of observed traits (Moravčíková et al., 2018e).

4 Conclusions

The results of the five-year research project, oriented on the evaluation of genetic diversity of selected populations of economically important animal species in Slovakia, their sustainable adaptation and production potential in the context of preservation of genetic resources and food safety served as the background for this review. Erosion of genetic diversity represents the main threat for food safety of mankind. It is therefore important and needed to focus on permanent monitoring and evaluation of diversity of economically important breeds based on the diverse parameter and suitable methods. Expected results of such research will be the identification of unique genomic regions for the particular populations with national importance and identification of regions affected by selection on the genome-wide level and application of comprehensive methodologies of the genomic data utilisation in animal production and protection of animal genetic resources.

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