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Original Paper

Evaluation of Turkish maize landraces through observing their yield and agro-morphological traits for genetic improvement of new maize cultivars

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For protection of diverse genetic resources of local landraces and to get the benefit for next generation, research works should be continuing through screening of local landraces by using with local germplasm; which will be very useful to conserve the genetic variability and will provide to economic profits to the farmers by improving their uses. In the context, One hundred twenty-five maize landraces with two commercial maize hybrids ('Kalumet' and 'Katone') were evaluated for yield and agro-morphological performance for genetic improvement of future maize varieties. The landraces were collected from the Black Sea Region of Turkey and were evaluated under the augmented complete design under Diyarbakir agro-ecological conditions during 2015. After observation, it was observed that all genotypes showed significant variations for all traits especially for yield and yield attributes. Considering the overall performance of all landraces, the days to tasseling and silking were varied from 39.5 to 64.5 and from 49.5 to 70.5 days; while the SPAD meter were varied from 37.8 to 70 unit, the plant height from 165 to 315.5 cm, the ear height from 55.8 to 190 cm, the stalk thickness from 11.3–26 mm, the ear length from 6.21 to 25.38, the ear diameter 14.13 to 48.92 mm, the ears per row from 2.33 to16.3, seeds per row, the ear weight from 10.2 to 285.26 g, the rachis diameter from 11.58 to 39.51mm and the grain yield from 63.68 to 1,498.13 kg ha^{-1}. Where, the range for all traits in landraces varied huge and exceeded commercial check genotypes. Therefore, it was determined that the genotypic distinction of the landraces may be used as pre-breeding material for developing the suitable maize varieties for sustainable maize production in diverse agro-ecological conditions of Mediterranean region including Turkey.

Keywords: maize, landraces, yield, morphological traits, phenotyping, *Zea mays* L.

1 Introduction

Maize is one of the world's most significant crops for food security, cultivated for human consumption as well as animal feeding and also in recent years, is progressively playing an essential role as a source of biofuel (Lana et al., 2017). Maize is cultivated in a wide range of environmental conditions, due to its wider range adaptibility. However, in recently from selection schemes of commercial breeding is extremely decreased the number of genetic diverse cultivars of the crop.

The concepts of genetic erosion and the maintenance of plant genetic resources are rooted in the first decade of the twentieth century (Palumbo et al., 2017). A landrace is an ancient population of a cultivated crop that has become adaptedto the local conditions and to the agronomic practices of farmers (Palumbo et al., 2017). Most frequently, landraces are characterized by high diversity and thus provide a valuable source for potentially useful traits and an irreplaceable bank of co-adapted genotypes (Brush,1995). The evaluation of genetic diversity and genetic structure of landraces could provide to prevent genetic erosion as well as to sustain landraces (Shanbao et al., 2009).

Genus of Zea has the five species of large grasses under the family Poaceae and their native is Mexico and Mesoamerica. Among them, four species namely, *Zea mays*, *Zea luxurians*, *Zea perennis* and *Zea diploperennis*. Where, the best-known species is corn, or maize (*Zea*

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mays L.) and the species has the highest importance, which was derived from one of the Mexican teosintes (likely *Z. mays* parviglumis) in pre-Columbian times more than 6,000 years ago (Editors of Encyclopaedia Britannica, 2016). Because of its divergent types, maize cultivation has been distributed over a wide range of climatic conditions. The major portion of corn is produced between 30° latitude to 55° latitude (from tropical and sub-tropical to temperate regions), with little portion of corn is grown at 47° latitudes anywhere in the world (Shaw, 1988). While, several wild species are considered to be endangered or endangered.

In Turkey, maize occupies around 680,000 hectares with annual production of 6.4 million tones with an average yield 940 kg ha^{-1} (Turkish Statistical, 2017). While to meet the food demand of the people of the twenty-first century, maize crop will become a strategic product in Turkey as well as in the world. Maize area and production is increasing day by day in all over the world and also in Turkey due to its high yield potential of commercial hybrids.

Among the corn growing countries, Turkey is the foremost countries, where corn has been cultivating since prehistoric period of the world. As a result, many corn varieties derived from different sub-species are found in almost every region of Turkey; mainly in coastal regions of Turkey. According to initial findings on maize material collected in Turkey during the years 1925 to 1927, 'flint corn' (*Zea mays* indurate Sturt) was spread everywhere in Turkey (Zhukovsky, 1951). However, due to farmers in the black sea region are still cultivating/popular local landraces cultivars as a traditional manner and use for household consumption, hybrid maize in the region is not very much popular. Generally, landraces cultivars are genetically diverse and have been under farmer's selection for many years in terms of adaptation, plant characteristics, yield, biotic and abiotic stress tolerance or resistance (Wasala et al., 2013).

There is great growing trend in different countries for adaptability of maize cultivars to achieve the requirement of market demand. At the same time, due to the restriction of genetic diversity in modern varieties, it should be emphasized for maintaining the diverse genetic traits for future plant breeding program. While to protect the diverse genetic resources of local landrace and to benefit the use of next generation, research works should be continuing through screening of local landraces by using with local germplasm; which will be very useful to conserve the genetic variability, improve their uses and will provide economic profits to the farmers (Kumar et al., 2015). Considering the vital issue, the present study was undertaken to evaluate the agro-morphologic performance of maize landraces and to find out their potentiality in maize breeding for developing the suitable maize varieties for different agro-ecological conditions.

2 Materials and methods

2.1 Experimental site, design treatments and experimental procedure

The field study was conducted at research area of the Faculty of Agriculture, Dicle University, Turkey during the maize growing season of 2015. One hundred twenty-five maize landraces with two commercial maize hybrids as check genotypes ('Kalumet' and 'Katone') were used as plant material, collected from various locations in the Black Sea Region of Turkey (Table 1). The experiment was laid out in augmented complete design with two rows and each row was 5 m long with intra row plant spacing of 0.70 × 0.25 m. Seedbed was prepared using a cultivator and later disked for a proper seedbed. All maize genotypes were sown with sowing machine on 28th June 2015. Fertilizer, diammonium-phosphate (DAP, containing 46% total phosphorus and 18% nitrogen) was applied at the rate of 100 kg ha^{-1}, and ammonium nitrate (33%) was applied at 150 kg ha^{-1}.

Table 1 Maize landraces collected from the Black Sea Region of Turkey.

Accession number	Province -District	Accession number	Province -District
DZ-M-1	Artvin-Murgul-Küre	DZ-M-64	Artvin-Borçka-Çtdüzköy
DZ-M-2	Rize- Çayeli- Çınartepe	DZ-M-65	Artvin-Borçka-Çatdüzköy
DZ-M-3	Rize- Çayeli- Sefalı	DZ-M-66	Artvin-Borçka-Çatdüzköy
DZ-M-4	Artvin -Arhavi-Zeytinlik- Güngören	DZ-M-67	Artvin-Borçka-Çatdüzköy
DZ-M-5	Trabzon -Akçaabat-Dörtyol	DZ-M-68	Artvin-Hopa-Çamurlu
DZ-M-6	Trabzon -Akçaabat-Dörtyol	DZ-M-69	Artvin-Merkez
DZ-M-7	Trabzon -Akçaabat-Dörtyol	DZ-M-70	Artvin-Merkez
DZ-M-8	Trabzon -Akçaabat-Dörtyol	DZ-M-71	Karabük-Eskipazar- Ova
DZ-M-9	Trabzon- Akçaabat-Dörtyol	DZ-M-72	Giresun-Merkez

Table 1 Continued 1

Accession number	Province -District	Accession number	Province -District
DZ-M-10	Trabzon- Sürmene	DZ-M-73	Zonguldak-Ereğli-Yazıcılar
DZ-M-11	Trabzon -Sürmene	DZ-M-74	Ordu-Fatsa-Ilıca
DZ-M-12	Trabzon -Sürmene	DZ-M-75	Samsun-Merkez
DZ-M-13	Rize -Fındıklı-Ihlamurlu	DZ-M-76	Giresun-Görele-Hürriyet
DZ-M-14	Rize -Fındıklı-Yeniköy	DZ-M-77	Giresun-Merkez-Mesudiye
DZ-M-15	Trabzon -Of-Yenimahalle	DZ-M-78	Tokat- Erbaa
DZ-M-16	Trabzon -Of-Yenimahalle	DZ-M-79	Samsun-Merkez-Hacınaipli
DZ-M-17	Trabzon- Of-Yenimahalle	DZ-M-80	Amasya-Göynücek-Ulusu
DZ-M-18	Trabzon- Of-Yenimahalle	DZ-M-81	Samsun-Atakum-engiz
DZ-M-19	Trabzon Of-Çayırbağ	DZ-M-82	Tokat-Atakum-Ataköy
DZ-M-20	Trabzon Of-Çamlıyurt	DZ-M-83	Tokat -Turhal-Sarıçiçek
DZ-M-21	Trabzon -Yomra-Çamlıyurt	DZ-M-84	Karabük-Safranbolu-Düzce
DZ-M-22	Trabzon -Yomra-Çamlıyurt	DZ-M-85	Samsun-Bafra-Dededağ
DZ-M-23	Rize- Çayeli-Buzlupınar	DZ-M-86	Karabük-Safranbolu-Düzce
DZ-M-24	Rize- Fındıklı-Sulak	DZ-M-87	Karabük-Ovacuma
DZ-M-25	Rize- Çayeli-Haytebeşikçiler	DZ-M-88	Karabük-Safranbolu-Yukarıçiftlik
DZ-M-26	Artvin -Murgul-Küre	DZ-M-89	Ordu-Fatsa-YukarıMah.
DZ-M-27	Rize-Fındıklı-Gültepe-Sulak	DZ-M-90	Tokat -Turhal
DZ-M-28	Rize-Merkez-Emekçiler	DZ-M-91	Samsun-Bafra-Dededağ
DZ-M-29	Artvin-Arhavi-Zeytinlik-Güngören	DZ-M-92	Amasya-Merkez-Kovabayır
DZ-M-30	Rize-Güneysu-Ortaköy	DZ-M-93	Tokat -Erbaa-Yenimahalle
DZ-M-31	Rize-Güneysu-Ortaköy	DZ-M-94	Ordu-Fatsa-Ilıcakavaklar
DZ-M-32	Rize-Fındıklı	DZ-M-95	Trabzon -Merkez
DZ-M-33	Rize-Güneysu-Ortaköy	DZ-M-96	Karabük-Ovacuma
DZ-M-34	Trabzon-Merkez	DZ-M-97	Zonguldak-Merkez
DZ-M-35	Trabzon-ŞalpazarıÜzümözü	DZ-M-98	Samsun-Merkez
DZ-M-36	Rize-Merkez-Alipaşa	DZ-M-99	Samsun-Merkez-Sarayköy
DZ-M-37	Rize-Hemşin-Hilal	DZ-M-100	Ordu-Fatsa-Ilıca
DZ-M-38	Rize-Hemşin-Hilal	DZ-M-101	Tokat- Niksar
DZ-M-39	Rize-Güneysu-Ortaköy	DZ-M-102	Amasya-Merkez-Takuncak
DZ-M-40	Artvin-Arhavi-Zeytinlik-Güngören	DZ-M-103	Giresun-Bulancak-Kışla
DZ-M-41	Artvin-Arhavi-Zeytinlik-Güngören	DZ-M-104	Samsun-Tekkeköy
DZ-M-42	Artvin-Borçka-Caniti-Düzköy	DZ-M-105	Giresun-Bulancak-Kışla
DZ-M-43	Rize-Fındıklı-Gültepe-Sulak	DZ-M-106	Karabük-Eskipazar- Ova
DZ-M-44	Artvin-Hopa-Madenli-Çamlıköy	DZ-M-107	Sinop-Gerze-Bolalı
DZ-M-45	Artvin-Hopa-Madenli-Çamlıköy	DZ-M-108	Artvin-Arhavi-Kireçli
DZ-M-46	Artvin-Arhavi-Zeytinlik-Güngören	DZ-M-109	Tokat- Zile
DZ-M-47	Artvin-Borçka-Caniti-Düzköy	DZ-M-110	Amasya-Merkez-Kovabayır
DZ-M-48	Rize-Ardeşen-Kurtuluş	DZ-M-111	Trabzon-Of-Bölümlü
DZ-M-49	Rize-Ardeşen-Kurtuluş	DZ-M-112	Giresun-Bulancak-Kışla
DZ-M-50	Artvin-Arhavi-Zeytinlik-Güngören	DZ-M-113	Samsun-Kavak-Alaçam

Table 1 Continued 2

Accession number	Province -District	Accession number	Province -District
DZ-M-51	Artvin-Arhavi-Lome-Kavak	DZ-M-114	Zonguldak-Devrek-Yazıcık
DZ-M-52	Rize-Ardeşen-Seslikaya	DZ-M-115	Samsun-Bafra-Dededağ
DZ-M-53	Rize-Ardeşen-Seslikaya	DZ-M-116	Samsun-Tekkeköy
DZ-M-54	Rize-Ardeşen-Seslikaya	DZ-M-117	Samsun-Merkez-Hacıismail
DZ-M-55	Trabzon-Çaykara	DZ-M-118	Zonguldak-Devrek-Yazıcık
DZ-M-56	Trabzon-Çaykara	DZ-M-119	Tokat- Turhal-Sarıçiçek
DZ-M-57	Trabzon-Çaykara	DZ-M-120	Samsun-Merkez-Daracak
DZ-M-58	Rize-Fındıklı-Aksu	DZ-M-121	Zonguldak-Merkez
DZ-M-59	Artvin-Arhavi-Lome-Kavak	DZ-M-122	Giresun-Merkez-Esentepe
DZ-M-60	Rize-Fındıklı-Sümer	DZ-M-123	Samsun-Kavak-Ahırlı
DZ-M-61	Artvin-Borçka-Tepe-Düzköy	DZ-M-124	Çorum-Laçin-Gökgözler
DZ-M-62	Artvin-Borçka-Tepe-Düzköy	DZ-M-125	Rize-Güneysu-Kıbledağı
DZ-M-63	Artvin-Borçka-Çat-Düzköy		

2.2 Data, their recording procedure and analysis

Data on SPAD value, plant height (cm), ear height (cm), tasseling period (days), ear-silking period (days), stalk thickness (mm), ear length (cm), ear diameter (mm), row number ear^{-1}, the number of kernels row^{-1}, ear weight, rachis diameter (mm) and grain yield (kg ha^{-1}) were recorded during the study period.

2.2.1 Data collection procedure

SPAD readings were measured with a 'SPAD 502' chlorophyll meter (Minolta Osaka, Japan). During harvesting data on ear length, ear diameter, row number ear^{-1}, the number of seeds row^{-1} and ear weight were assessed from 10 randomly selected apical ears in each experimental plot by using standard procedure.

The height of randomly selected ten plants was measured (cm) and then averaged. Number of cobs was counted from ten plants selected at random from each plot and average was calculated. Total grains of the ten cobs were counted and grain weight of all the cobs selected from each plot was taken by using triple beam balance and averaged and thousand grain weights (gm) were done. For grain yield, cobs of each plot after removing were shelled with the help of an electric Sheller and were weighed to have grain yield plot^{-1}. Then yield was converted from kg plot^{-1} into t ha^{-1}. Biological yield was calculated in kilograms by deducting seed yield from the total biomass of each plot and converted into tonnes per hectare. Collected data were then analyzed using the computer program JMP 10 and Excel (SAS Institute Inc., 1989).

3 Results and discussion

Maize is both phenotypically and genetically diverse. Genetic variability among individuals in population should follow the effective selection to get desirable characters of a specific genotype (Rather et al., 2003). Phenology such as days to 50% anthesis, days to 50% silk emergence, days to maturity; yield traits such as grain weight and grain yield, ear height, % tryptophan content, cob length and 1000-kernel weight; ear length and diameter, ear aspects, plant height, and number of diseased cobs (Hoque et al., 2008; Kadir, 2010; Muchie and Fentie, 2016), can contribute to genetic diversity assessment. Whereas, these characters are variables due to different genetic makeup of the specific variety and their growing environment. However, under the changing environmental, the performance of maize genotypes vary according to their adaptability in a specific environment. Therefore, to get desirable genotypes for a specific environment, a rigorous breeding program is important to take into account the consequences of environment and exploring and developing more competitive maize genotypes (Ferdoush et al., 2017). In the present study, for genetic improvement of new maize cultivars for sustainability of maize production under changing climate of Mediterranean region including Turkey, one hundred twenty-five maize landraces with two commercial maize hybrids ('Kalumet' and 'Katone') were evaluated through observing their tasseling period (days), ear-silking period (days), SPAD value, plant height (cm), ear height (cm), stalk thickness (mm), ear length (cm), ear diameter (mm), row number ear^{-1}, the number of kernels row^{-1}, ear weight, rachis diameter (mm) and grain yield (kg ha^{-1}), which are described as follows (Table 2).

Table 2 Mean performance of phenology, growth and yield attributes of maize landraces

Accessions	TP	ESP	SPAD	PH	EH	ST	EL	ED	RPE	NKRE	KWE	GY	RD
DZ-M-1	59.0	62.0	54.5	230.0	111.6	18.4	15.37	30.19	15.0	37.3	85.16	312.6	19.19
DZ-M-2	58.5	65.5	52.7	310.8	170.8	21.9	16.63	32.79	7.4	17.7	74.50	371.0	22.89
DZ-M-3	55.5	62.5	52.3	260.8	127.5	18.2	13.00	29.25	9.0	17.5	40.57	77.7	20.70
DZ-M-4	58.5	66.5	57.7	299.1	185.8	23.2	19.80	38.15	11.5	32.1	133.46	700.4	24.65
DZ-M-5	58.0	66.0	59.0	269.1	117.5	16.9	19.65	44.86	14.4	44.9	217.19	1028.7	24.98
DZ-M-6	55.5	64.5	68.9	205.3	108.3	18.5	14.84	40.74	12.9	27.2	127.01	729.36	24.24
DZ-M-7	58.0	63.5	54.7	246.6	125.8	19.9	16.65	43.51	14.3	36.6	184.45	1498.1	25.11
DZ-M-8	57.5	65.5	62.1	244.1	120.8	20.9	15.75	39.21	11.9	29.7	131.93	735.9	23.24
DZ-M-9	58.0	66.0	63.8	270.8	112.5	24.5	17.77	45.55	14.9	33.9	187.33	1190.8	25.48
DZ-M-10	55.5	64.0	64.3	270.8	144.1	20.7	14.58	33.39	8.4	17.4	91.95	723.0	26.42
DZ-M-11	58.5	64.5	70.0	258.3	86.6	20.9	20.16	48.92	14.6	41.0	285.26	940.0	22.82
DZ-M-12	58.5	67.0	52.8	308.3	190.0	22.3	17.85	35.45	9.6	30.7	102.87	615.4	21.27
DZ-M-13	56.0	63.5	41.0	257.5	124.1	20.1	16.58	34.05	10.3	36.0	120.56	697.8	20.46
DZ-M-14	61.0	67.0	46.9	282.5	159.1	19.5	15.35	33.72	8.5	26.0	93.95	544.6	21.96
DZ-M-15	57.5	64.0	52.3	255.8	133.3	17.9	15.64	31.5	8.4	24.5	83.60	427.7	20.63
DZ-M-16	55.5	61.0	58.8	284.1	146.6	22.2	13.31	39.33	11.5	23.3	118.41	758.7	23.10
DZ-M-17	52.0	63.0	61.5	274.1	137.5	19.2	16.28	36.00	7.2	21.6	103.20	693.9	23.40
DZ-M-18	57.0	64.0	61.7	295.0	165.0	21.0	16.71	37.20	10.0	29.0	128.44	828.9	23.04
DZ-M-19	39.5	50.0	53.4	225.0	96.6	20.7	12.25	34.25	9.3	28.8	82.92	508.4	18.51
DZ-M-20	49.0	57.0	57.1	243.3	100.0	16.8	14.06	34.90	9.0	17.4	120.60	418.9	24.40
DZ-M-21	43.0	50.0	43.7	244.1	88.3	20.4	12.31	32.97	9.7	17.6	68.33	414.2	21.77
DZ-M-22	45.0	53.5	52.3	254.1	104.1	16.2	12.22	36.54	7.2	19.4	69.39	378.4	22.04
DZ-M-23	55.5	62.5	50.9	271.6	138.3	19.8	14.63	37.14	10.3	21.5	115.66	767.5	23.34
DZ-M-24	60.0	67.0	43.4	293.3	180.0	18.5	18.63	34.23	8.6	24.5	116.23	542.7	22.06
DZ-M-25	55.5	61.5	59.1	252.5	129.1	16.1	15.40	35.76	8.4	28.1	97.59	510.6	21.07
DZ-M-26	54.5	61.5	55.1	272.5	135.0	20.0	15.46	40.43	9.7	29.4	139.21	1028.0	23.82
DZ-M-27	49.0	56.0	54.6	281.6	132.5	21.9	18.15	40.32	8.0	38.8	183.54	956.4	20.96
DZ-M-28	56.5	64.0	52.3	274.1	133.3	19.3	13.99	32.93	10.1	27.2	90.15	517.1	21.32
DZ-M-29	56.0	63.5	63.0	241.6	159.1	20.9	10.38	32.67	9.0	17.2	45.53	315.6	20.16
DZ-M-30	54.0	61.0	43.2	295.8	163.3	22.2	13.08	35.84	9.4	21.4	96.94	614.8	23.86
DZ-M-31	58.0	62.0	53.9	275.0	145.8	12.0	18.00	22.71	13.6	34.8	10.2	848.2	25.12
DZ-M-32	57.5	63.5	50.5	314.1	174.1	21.9	13.30	30.38	5.8	13.2	67.49	590.1	21.80
DZ-M-33	59.0	65.0	37.9	265.0	140.8	18.3	12.86	32.46	8.4	19.4	77.61	414.7	17.82
DZ-M-34	58.5	65.0	47.0	294.1	167.5	21.1	16.76	33.07	7.4	20.1	91.45	589.3	22.70
DZ-M-35	58.0	64.0	53.1	280.0	145.0	17.1	14.72	30.15	8.1	20.9	78.95	509.3	21.34
DZ-M-36	57.0	66.0	47.8	257.5	128.3	17.6	21.97	35.63	8.0	16.4	77.36	845.4	24.63
DZ-M-37	56.5	62.5	42.6	290.8	168.3	18.3	16.55	36.27	9.4	35.5	118.60	668.9	22.97
DZ-M-38	58.5	66.5	54.6	299.1	179.1	20.8	15.65	39.37	9.3	26.0	113.49	704.4	29.20
DZ-M-39	56.5	61.0	48.1	268.3	148.3	17.0	15.66	34.66	10.4	32.8	122.19	549.0	20.11
DZ-M-40	52.5	60.0	54.2	272.5	154.0	17.8	14.82	34.94	9.3	27.2	104.28	718.2	21.49
DZ-M-41	61.0	67.0	46.7	211.6	126.6	17.0	18.97	34.00	10.0	30.1	122.57	682.8	20.27

Table 2 Continued 1

Accessions	TP	ESP	SPAD	PH	EH	ST	EL	ED	RPE	NKRE	KWE	GY	RD
DZ-M-42	56.5	63.0	44.4	265.8	130.0	17.6	16.97	35.29	8.4	24.8	100.33	451.5	24.41
DZ-M-43	57.0	63.5	51.7	295.8	155.0	20.7	14.35	30.45	6.25	10.0	48.34	176.4	23.12
DZ-M-44	56.5	64.5	47.2	290.0	175.0	15.1	17.15	35.88	10.6	30.7	127.70	814.7	21.13
DZ-M-45	57.5	66.5	46.7	290.0	175.8	19.0	14.04	33.58	9.9	21.0	84.82	671.1	22.05
DZ-M-46	56.5	65.5	56.0	296.6	155.8	17.1	15.47	32.65	10.3	28.3	103.38	541.8	18.56
DZ-M-47	54.5	61.5	54.7	262.5	142.5	18.9	16.47	34.98	9.6	32.8	112.46	629.9	20.30
DZ-M-48	44.0	51.5	63.3	256.6	110.0	16.8	17.64	38.30	10.6	30.4	136.10	494.7	16.64
DZ-M-49	55.0	62.0	37.8	310.0	130.0	22.2	21.50	41.56	9.2	42.6	192.24	1189.0	23.52
DZ-M-50	57.5	63.5	58.4	218.3	90.8	20.1	16.91	29.56	16.3	34.0	98.40	736.5	20.90
DZ-M-51	54.0	59.5	51.9	261.6	136.6	16.2	16.94	37.47	10.0	29.2	114.54	620.2	22.40
DZ-M-52	64.5	70.5	53.1	270.8	157.5	16.3	12.63	34.24	7.6	16.3	78.11	489.4	25.68
DZ-M-53	58.0	66.0	47.8	289.1	154.1	17.3	12.00	31.26	6.4	14.2	57.61	317.4	19.94
DZ-M-54	59.5	65.5	53.8	298.3	188.3	20.4	14.12	37.32	14.0	28.2	107.26	807.9	23.58
DZ-M-55	54.0	60.5	51.8	251.6	125.8	20.9	16.71	38.68	11.7	33.2	151.95	1235.4	23.91
DZ-M-56	56.0	62.0	51.8	260.0	135.0	19.6	14.85	34.24	12.1	23.0	92.95	990.6	22.58
DZ-M-57	53.5	59.5	53.8	269.1	111.6	23.5	17.69	38.64	10.7	30.4	144.3	1006.5	23.05
DZ-M-58	57.5	65.5	47.3	308.3	181.6	19.7	14.50	33.27	9.4	21.8	88.26	567.5	19.90
DZ-M-59	56.0	64.0	43.2	280.0	155.0	21.9	17.40	39.28	12.0	40.8	139.92	848.5	24.04
DZ-M-60	55.0	62.0	44.0	180.0	140.0	21.5	14.60	39.70	15.0	34.0	142.78	150.7	22.10
DZ-M-61	51.5	60.0	55.9	253.8	116.6	19.3	16.80	31.92	8.2	36.4	88.98	524.8	18.98
DZ-M-62	46.5	54.0	59.1	239.1	122.5	20.0	13.92	30.78	7.7	22.7	75.35	538.7	19.29
DZ-M-63	55.0	63.0	51.1	273.3	145.8	20.6	19.34	38.49	10.8	38.2	156.29	1000.6	23.36
DZ-M-64	56.0	63.5	57.2	264.1	118.3	17.1	16.18	32.65	10.3	26.7	78.37	590.6	20.31
DZ-M-65	57.5	66.5	43.1	276.6	131.0	15.6	17.00	31.78	8.6	35.1	93.73	531.1	18.09
DZ-M-66	53.0	62.0	47.8	285.0	61.6	16.8	25.38	33.54	11.8	45.6	156.53	777.7	17.36
DZ-M-67	57.5	64.0	52.6	265.0	105.8	17.9	21.00	33.70	9.4	37.4	122.90	450.9	19.74
DZ-M-68	59.5	66.0	44.8	295.0	148.3	20.3	17.47	33.40	7.7	17.4	100.32	173.1	23.32
DZ-M-69	50.5	57.0	53.9	225.8	77.9	19.1	13.37	29.95	8.0	19.0	54.46	100.3	19.35
DZ-M-70	45.5	54.0	51.2	250.8	100.83	17.9	12.32	31.39	8.8	13.9	59.08	264.9	21.81
DZ-M-71	47.5	55.5	49.1	263.3	117.5	20.1	14.90	30.53	10.2	26.7	85.56	590.4	18.55
DZ-M-72	44.0	51.0	55.9	230.8	95.5	17.3	16.04	32.76	9.2	26.4	107.60	580.5	18.31
DZ-M-73	56.0	63.0	62.8	287.5	137.5	21.4	15.28	33.34	8.0	18.4	98.01	429.0	22.36
DZ-M-74	46.0	54.5	49.6	214.1	90.0	18.3	10.12	34.70	11.2	18.5	45.81	177.0	23.775
DZ-M-75	57.0	66.0	56.9	297.5	149.17	21.6	11.99	36.54	7.7	14.1	85.05	473.1	24.22
DZ-M-76	60.0	70.0	54.2	286.6	176.7	26.0	17.60	29.60	9.6	43.8	73.80	312.0	17.96
DZ-M-77	43.0	50.0	55.9	165.0	60.0	15.4	10.72	26.97	6.25	11.2	35.60	562.5	17.85
DZ-M-78	56.0	62.0	48.5	255.8	141.7	15.1	8.89	31.54	10.8	17.2	52.18	335.9	19.57
DZ-M-79	55.0	61.0	62.8	282.5	102.5	24.8	15.30	36.16	12.0	28.0	96.58	534.7	23.68
DZ-M-80	41.0	50.0	55.4	255.8	108.3	18.0	14.90	31.22	9.6	22.8	65.32	263.5	19.12
DZ-M-81	49.0	59.0	44.1	216.6	80.0	17.6	12.30	33.18	10.0	20.8	59.35	348.6	21.24
DZ-M-82	52.0	61.0	48.6	276.6	144.1	24.8	15.79	32.96	9.4	24.4	90.10	279.6	18.95

Table 2 Continued 2

Accessions	TP	ESP	SPAD	PH	EH	ST	EL	ED	RPE	NKRE	KWE	GY	RD
DZ-M-83	50.5	69.0	44.7	285.8	137.5	21.5	14.76	28.29	8.3	17.9	70.78	324.4	18.60
DZ-M-84	63.0	70.0	52.6	230.0	135.0	18.6	15.56	28.76	11.3	29.6	89.11	219.1	16.86
DZ-M-85	44.5	53.0	54.5	254.1	102.5	18.7	12.48	28.14	5.2	14.6	50.30	267.6	20.64
DZ-M-86	59.0	65.0	54.2	270.0	120.0	18.9	17.90	26.20	4.0	6.0	41.77	504.0	21.60
DZ-M-87	56.0	63.5	54.9	286.6	138.3	17.9	15.96	31.60	8.6	20.2	87.91	946.1	22.88
DZ-M-88	52.0	59.0	56.1	243.3	136.7	18.6	13.95	30.68	10.3	26.1	67.66	495.5	19.28
DZ-M-89	40.5	50.0	49.9	194.1	55.8	14.8	12.24	25.95	4.3	11.2	38.97	201.9	19.25
DZ-M-90	56.0	62.5	53.7	305.0	147.5	21.3	14.37	34.27	9.9	22.0	94.25	513.0	20.17
DZ-M-91	47.5	54.0	51.5	224.1	74.1	19.3	12.67	28.29	6.7	16.1	44.76	153.3	19.36
DZ-M-92	46.0	53.5	55.8	241.6	95.0	18.1	20.12	35.70	11.6	30.0	103.32	463.3	23.9
DZ-M-93	61.0	69.0	54.1	315.5	160.0	23.2	13.58	25.73	6.6	16.4	65.99	104.1	24.47
DZ-M-94	45.5	53.5	53.2	200.0	76.6	18.1	14.68	24.48	4.2	10.4	43.49	1217.7	26.12
DZ-M-95	50.0	59.0	47.1	260.0	129.1	21.3	11.93	31.49	8.5	18.6	75.72	636.3	22.86
DZ-M-96	60.5	67.0	52.8	282.5	179.1	18.6	18.64	30.12	7.4	29.0	91.31	410.0	20.22
DZ-M-97	50.0	58.0	57.0	240.0	97.5	19.1	14.50	29.63	8.3	22.3	68.84	200.4	18.73
DZ-M-98	56.5	64.5	52.7	267.5	124.1	23.0	15.20	34.09	8.9	21.5	90.97	495.0	25.09
DZ-M-99	43.0	53.5	55.8	232.5	92.5	18.6	15.62	28.84	7.8	22.0	74.84	421.7	19.02
DZ-M-100	40.5	49.5	50.9	245.8	86.6	16.1	10.96	33.00	7.2	14.4	58.56	342.1	20.56
DZ-M-101	58.5	65.5	54.2	275.0	180.0	16.1	13.91	30.06	7.1	19.5	67.68	226.1	18.19
DZ-M-102	50.5	58.0	45.4	200.8	97.5	16.3	15.00	32.72	10.5	27.3	82.28	502.9	20.81
DZ-M-103	61.5	69.5	41.5	286.6	148.0	18.9	14.08	33.02	10.2	23.0	97.93	610.3	20.20
DZ-M-104	46.5	54.5	59.9	204.1	82.9	20.4	12.50	14.13	2.3	5.0	10.57	528.0	39.51
DZ-M-105	58.0	66.0	51.7	281.6	140.8	25.8	17.07	31.39	11.6	27.3	89.01	301.0	20.81
DZ-M-106	59.0	66.0	53.4	270.0	112.5	20.2	8.90	25.85	5.0	7.0	32.28	73.8	17.30
DZ-M-107	59.0	68.0	51.9	296.6	164.1	20.9	15.64	35.28	7.4	13.8	95.97	531.1	23.32
DZ-M-108	61.0	67.0	50.2	260.0	125.0	22.9	14.56	34.13	8.6	19.0	85.32	255.9	21.40
DZ-M-109	47.5	55.0	57.1	224.1	87.8	20.8	12.52	32.96	8.0	23.6	76.75	554.7	21.58
DZ-M-110	47.5	54.5	54.5	250.8	125.8	16.8	14.09	34.67	10.0	21.9	84.14	519.9	22.41
DZ-M-111	54.5	61.0	55.9	269.1	141.6	15.0	16.20	33.00	10.6	29.6	92.61	594.1	21.36
DZ-M-112	47.0	54.0	46.2	259.1	113.3	18.4	17.35	32.83	9.3	33.5	102.52	451.9	19.15
DZ-M-113	46.5	55.5	58.7	252.5	131.2	19.9	9.55	33.15	6.5	10.0	45.41	63.6	20.50
DZ-M-114	49.0	56.5	57.6	247.5	87.5	19.4	14.09	30.58	9.3	18.8	80.78	232.8	18.99
DZ-M-115	41.5	49.5	53.2	214.1	80.8	15.8	13.38	28.51	5.4	12.6	61.18	258.9	18.49
DZ-M-116	43.0	50.5	57.7	185.8	79.1	15.7	12.93	25.75	6.1	16.5	48.31	149.4	16.07
DZ-M-117	56.0	60.0	49.4	307.5	115.0	22.4	11.30	32.90	8.5	16.0	63.18	122.1	20.05
DZ-M-118	55.5	64.0	43.9	299.1	160.0	19.1	17.30	30.84	10.8	34.2	91.49	507.1	18.32
DZ-M-119	58.5	66.5	54.2	283.3	135.8	18.6	17.32	32.67	10.7	30.6	93.96	522.6	22.75
DZ-M-120	59.0	65.0	49.0	292.5	140.8	19.6	15.70	31.49	8.5	23.1	84.01	423.7	18.18
DZ-M-121	50.0	59.0	57.7	205.0	71.6	19.8	16.24	33.78	7.6	25.8	84.69	506.2	21.76
DZ-M-122	45.0	53.0	56.3	258.3	100.8	22.2	13.84	31.19	8.0	19.8	71.00	433.4	19.46
DZ-M-123	58.5	66.0	48.0	277.5	165.8	17.1	17.55	29.43	7.8	24.5	78.63	417.3	17.70

Table 2 Continued 3

Accessions	TP	ESP	SPAD	PH	EH	ST	EL	ED	RPE	NKRE	KWE	GY	RD
DZ-M-124	60.5	67.0	53.9	167.5	105.8	11.3	6.21	24.43	12.7	21.0	20.93	134.7	11.58
DZ-M-125	55.5	62.0	59.1	280.8	136.6	19.2	11.44	32.84	11.4	19.0	72.03	821.4	22.06
KALUMET	60.0	61.7	54.2	268.3	102.5	19.6	20.22	48.48	16.1	45.1	264.28	1451.9	27.13
KATONE	59.3	61.8	57.9	252.2	98.4	19.6	20.82	47.83	15.5	43.2	272.93	1467.4	26.28
Mean ML	53.8	61.3	52.7	261.9	128.8	19.2	15.18	33.29	9.3	24.4	92.75	537.6	21.44
Mean HM	59.6	61.7	56.0	260.2	100.4	19.6	20.52	48.15	15.8	44.1	268.60	1459.65	26.70
Std Dev	5.71	5.32	5.92	31.67	30.82	2.54	2.87	4.95	2.51	8.84	45.37	295.62	3.08

ML – Mean of Maize landraces; HM – Mean of hybrid maize; Std Dev. – Standard Deviation; TP – Tasseling period; ESP, Ear Silking period; PH – Plant height (cm); EH – Ear height (cm); ST – Stalk thickness (mm); EL – Ear length (cm); ED – Ear diameter (mm); RPE – Rows ear^{-1}; NKRE – kernel rows ear^{-1}; KWE – Kernel weight ear^{-1} (g ear^{-1}); GY – grain yield (kg ha^{-1}); RD – Rachis diameter (mm)

3.1 Phenological variation (days)

Phenological variation of tasseling and ear-silking stage of all genotypes were varied significantly due to different genetic makeup of the specific genotype. Among the landraces, cultivar 'DZ-M-52' took the longest time (64.5 and 72.5 days) for tasseling and ear-silking, while, cultivar 'DZ-M-19' took the shortest period (39.5 days) for tasseling and cultivar 'DZ-M-100' took 49.5 days for ear-silking. Variation of tasseling and ear-silking period of all landraces were due to the different genetic makeup of the tested genotypes that ultimately influenced under different environmental conditions (Table 2). The assumption of the result related to phenological variation also supported by Idikut and Kara (2011), who reported that tasseling period varied according to genotype and environmental conditions. Similarly, Gokmen et al. (2001) also reported that tasseling period decreased with increasing sowing density and nitrogen dose.

3.2 Variation of SPAD value

Chlorophyll (the green pigment of the leaf) in plants is considered one of the most important compounds, which can transform light energy into chemical energy through a process known as photosynthesis. Whereas, photosynthetic rate in plants is directly depended on leaf chlorophyll content as well as environmental factors such as light intensity. Chlorophyll meter (SPAD meter) is a decision making tools and good indicator for determining the photosynthetic activity in plant (Akhter et al., 2016). In the present study, cultivar 'DZ-M-011' recorded the maximum SPAD value (70 unit), and while cultivar 'DZ-M-049' showed the lowest unit of SPAD value (37.8). However, mean SPAD value of check cultivar was 56.1 (Table 2). Indicated that some landraces have the high rate of photosynthesis capacity than check cultivars.

3.3 Variation of plant height (cm)

Plant height is a heritable trait in maize and is closely associated with plant density and lodging resistance. Exceeding plant height is an undesirable feature in maize for grain yield causes lodging (Peiffer et al., 2014). However, varieties/cultivars cultivate for silage are a desirable feature. In the present study, cultivar 'DZ-M-093' (315.5 cm) was found the tallest and cultivar 'DZ-M-77' was found the shortest among the all genotypes. Whereas, mean plant height of check cultivars was 260.3 cm. Indicating that the cultivar 'DZ-M-77' was lodging tolerant, while cultivar 'DZ-M-093' may be susceptible to lodging (Table 2). Although, plant height of a cultivar/species is depend on genetic makeup, while environmental condition can also influence the plant height, which is confirmed by many studies in earlier. Oner and Gulumser (2014) and Oner (2015) reported that plant height of maize varied within the range of 102 to 374 cm in Turkey; whereas 102 to 324 cm in Spain (de Galarreta and Alvarez, 2001), 215.5 to 274.8 cm in America (Azar et al., 1997), 180 to 300 cm in Brazil (Goodman and Paterniani, 1969).

3.4 Variation of yield traits

After observation, it was observed that all genotypes showed a significant variations for all characters especially for yield and yield attributes (Table 2).

3.4.1 Ear height (cm)

In the present study, the maximum ear height was recorded in 'DZ-M-012' (190 cm) and the shortest was found in 'DZ-M-89' (55.8 cm) (Table 2). Generally, landraces had higher ear height then check cultivars. Ear height is highly influenced by genetic factors and varies according to the varieties and significantly affected by growing environment during ear elongation. Similar to plant height and ear height is also a very important characters for describing new varieties of maize, as well as green and dry matter production, finally for grain yield (Zsuzsanna et al., 2002). While, ear height feature is important for machine harvesting and should not below a meter (Tuten et al., 1984; Erden, 1991; Santos et al., 1993; Gokmen, 1995).

3.4.2 Stalk thickness/stem diameter (mm)

Stem diameter is strongly influenced by environmental conditions during stem elongation (Yilmaz et al., 2007), while declined due to genotypic variations in stem diameter of corn (Konuskan, 2000; Gozubenli et al., 2001, 2003; Turgut et al., 2005). Some researchers reported that stem diameters of corn is higher in hybrids maize as compared with local varieties, and influences by growing environment (Gozubenli et al., 2001, 2003; Turgut et al., 2005; Yilmaz et al., 2007). In the present study, the highest stalk thickness was determinate from cultivar 'DZ-M-076' with 26 mm and the lowest at 'DZ-M-124' with 11.3 mm. Stalk thickness was significantly affected by environmental conditions during stem elongation. In the study, all landraces were generally narrow stalk thickness then check cultivar (Table 2). Sharifi et al. (2009) reported that stalk thickness decreased with the increasing plant density. Stem diameter and plant height could also be considered for selection in forage corn breeding (Ahmadi et al., 2014).

3.4.3 Ear length (cm)

Some researchers indicated that ear length was influenced by the genotypes, plant density, location, year and nitrogen fertilizer (Goodman and Paterniani, 1969). In the present study, in terms of ear length, considerable variation was observed among the landraces. Among the genotypes, the maximum ear length (25.38 cm) was recorded for landrace 'DZ-M-066' and the minimum value (6.21 cm) was recorded for 'DZ-M-124'. However, ear length of 53 landraces had higher than check cultivar. Similar results in same location (Black Sea Region) related to ear length for landraces also was confirmed by Oner and Gulumser (2014).

3.4.4 Ear diameter (cm)

Carvalho et al. (2017), found the phenotypic, genetic and environmental linear positive correlation between the grain yield and ear diameter as well as grains mass ear^{-1} with. They also identified the genotype × environment interaction, and heritability in a broad sense for the grain yield, ear diameter, grains row^{-1} and also stem diameter (Carvalho et al., 2017). The results of the previous study, indicated that ear diameter has a positive correlation with the final grain weight of maize. In the present research, the maximum ear diameter (48.92) was determined at 'DZ-M-011' landrace and while the minimum (14.13) was from 'DZ-M-104'. It was also noted that ear diameter of landraces was generally thiny than check cultivar.

3.4.5 Row/ear, kernels/row(no)

The highest row number per ear was observed in DZ-M-050 with 16.3 mm, while the lowest row number per ear was in DZ-M-104 with 2.33 mm. 'DZ-M-050' was unique landrace which was superior to check cultivars. In case of number of kernels row^{-1}, the maximum (45.6) was observed in cultivar 'DZ-M-066', while the minimum number of kernels row^{-1} (5) was observed in 'DZ-M-104'. Therefore, cultivar 'DZ-M-066' and 'DZ-M-05' were superior in respect of rows ear^{-1} and kernels row^{-1} to check cultivars. Boćanski et al. (2009). Found a significant correlation between grain yield, on one side and number of kernels per row, ear length, kernel row number and ear height. Similar result also confirmed by Avlov et al. (2012), in their study they found strong phenotypic correlation between grain yield and cob weight, plant height, ear height, ear length, kernel number row^{-1} and 100-kernel weight.

3.4.6 Ear weight (g)

Ear weight of maize has positive correlation with the final grain yield of maize (Pavan et al., 2011) and vary from genotype to genotype of maize (Fetahu et al., 2015). In the present research, the maximum ear weight (285.26) was recorded from 'DZ-M-111' and the minimum ear weight (10.2) was observed for 'DZ-M-031'. Indicating that landrace 'DZ-M-111' was unique landrace to superior check cultivar. Path analysis revealed that ear weight could be used as a selection criterion because of its highly positive direct effects on forage yield (Ahmadi et al., 2014).

3.4.7 Grain yield (kg ha^{-1})

Stable performance of maize cultivars for a specific growing region is critical for obtaining the high and stable yield (Boshev et al., 2013; Nzuve et al., 2013). In the context, one hundred twenty-five maize landraces with two commercial maize hybrids ('Kalumet' and 'Katone') were evaluated for yield and agro-morphological performance for genetic improvement of future maize varieties. After observation, the maximum grain yield (1498.1 kg ha^{-1}) was recorded from the landrace 'DZ-M-007, and while the lowest grain yield (63.6 kg ha^{-1}) was recorded from 'DZ-M-113'. In the present study, grain yield of all genotypes showed a wide range of variation, due to genotypic and phenotypic variability of the tested landraces that ultimately influenced under environmental condition. Grain yield was affected by climatic factors as reported by Galarreta and Alvarez (2001). Similar results have been also reported by Oner and Gulumser (2014) in landrace maize.

3.4.8 Rachis diameter (mm)

Rachis Diameter was measured with calipers on the lower half of the broken ear. It was measured from the base of an upper glume on one side of the cob to the base of an upper glume directly opposite. Since the base of the glume is usually somewhat below the rim of the cupule,

this measurement does not represent the maximum diameter of the rachis but rather its diameter to the points at which the upper glume arises (Ulysses, 1963). Maize ear architecture is significant and positive correlated with ear fasciation, defined as abnormal flattened ears with high kernel row number. Mendes-Moreira et al. (2015) found a highly significant correlation between ear fasciation and some ear (rachis diameter) and cob diameters and row number traits. They also reported that the quantitative abnormal character is widely present in most of maize landraces. In the present study, rachis diameter was varied from 11.58 to 39.51 mm and it is closely related to the grain yield. Because, if the rachis diameter is large, and therefore the ear diameter will be large. There will be an increase in the yield of the grain, since there will be more kernels and number of kernels in the large cob. Knowledge of the genes affecting maize ear architecture lead to improve the grain yield. Therefore, future studies should focus on a valuable source of genes or allelic variants for yield improvement and elucidation of the genetic basis of ear fasciation traits.

3.5 Correlation analysis

The measurement of relationship coefficient is essential in plant breeding because it measures the degree of correlation between two or more traits (Dewey and Lu, 1959). Ferdoush et al. (2017) noticed that correlation co-efficient analysis had positive and significant association with yield plant^{-1} (g) and other traits such as ear girth (cm), 1000-kernel weight (g), yield plot^{-1} (g), grain yield (tha^{-1}) with dry weight. In the presence of great relationship between two traits, selection in one trait will cause a change in its mean through additive gene influence of selected individuals and simultaneously cause an indirect modify in the mean of the other trait (Kumar et al., 2015). Results show that there are strongly positive correlations between the TP with all traits except SPAD and RD. The ESP shows positive correlation with the PH, FEH, ST, EL, ED, RPE, NKRE and KW. A very strong positive correlation appeared between the PH with the FEH, ST, EL and KW. It showed that strong positive effect between the SD with the FEH, KW and RD. The EL has strong positive correlations with the ED, RPE, NKRE, KW, GY, and RD. It found very strong positive correlations between the ED with the RPE, NKER, KW, GY, and RD. The RPE showed a significant positive correlation between with NKRE, KW, and GY. The NKRE was significantly and positively correlated with the KW and GY. KW was significantly correlated with GY and RD. There was a strong positive relationship between the GY with the RD (Table 3). The results of the present study, related to significant and positive correlation between grain yield and other traits also confirmed by Khodarahmpour (2012) and Ferdoush et al. (2017), who reported that grain yield, grains row^{-1}, grains ear^{-1}, ear height, ear-down leaves, total leaves, grain depth, grain dry matter weight and 1000-grain weight had significant and positive correlation. Therefore, correlation between yield and other characters can be used as basis of suitable characters selection for future breeding program to develop desirable variety in future. Similarly, Ahmadi et al. (2014) found a significant and positive correlation between forage yield with stem diameter, ear weight, kernels row^{-1}, ear length, days to silk emergence and days to physiological maturity. While, in

Table 3 Correlation coefficients between investigation features

	TP		ESP		SPAD		PH		FEH		ST		EL		ED		RPE		NKRE		KW		GY	
TP	1																							
ESP	0,95	***	1																					
SPAD	-0,11		-0,14		1																			
PH	0,52	***	0,55	***	-0,19	*	1																	
FEH	0,65	***	0,68	***	-0,22	*	0,74	***	1															
ST	0,18	*	0,23	*	0,08		0,40	***	0,24	**	1													
EL	0,30	**	0,28	**	-0,04		0,31	**	0,13		0,13		1											
ED	0,24	**	0,18	*	0,13		0,25	**	0,14		0,27	**	0,44	***	1									
RPE	0,32	**	0,24	**	0,12		0,01		0,05		0,07		0,35	***	0,61	***	1							
NKRE	0,27	**	0,22	**	-0,02		0,14		0,10		0,09		0,70	***	0,59	***	0,73	***	1					
KW	0,29	**	0,22	*	0,12		0,22	**	0,07		0,24	*	0,66	***	0,88	***	0,62	***	0,75	***	1			
GY	0,21	*	0,14		0,12		0,17		0,07		0,11		0,53	***	0,61	***	0,47	***	0,54	***	0,71	***	1	
RD	0,15		0,12		0,17	*	0,16		0,10		0,28	**	0,19	*	0,27	**	0,05		-0,01		0,26	**	0,47	***

TP – Tasseling period; ESP – Ear Silking period; PH – Plant height (cm); FEH – Ear height (cm); ST – Stalk thickness (mm); EL – Ear length (cm); ED – Ear diameter (mm); RPE – Rows ear^{-1}; NKRE, kernel rows ear^{-1}, RD – Rachis diameter (mm); KWE – Kernel weight in ear (g ear^{-1}); GY – grain yield (kg ha^{-1})

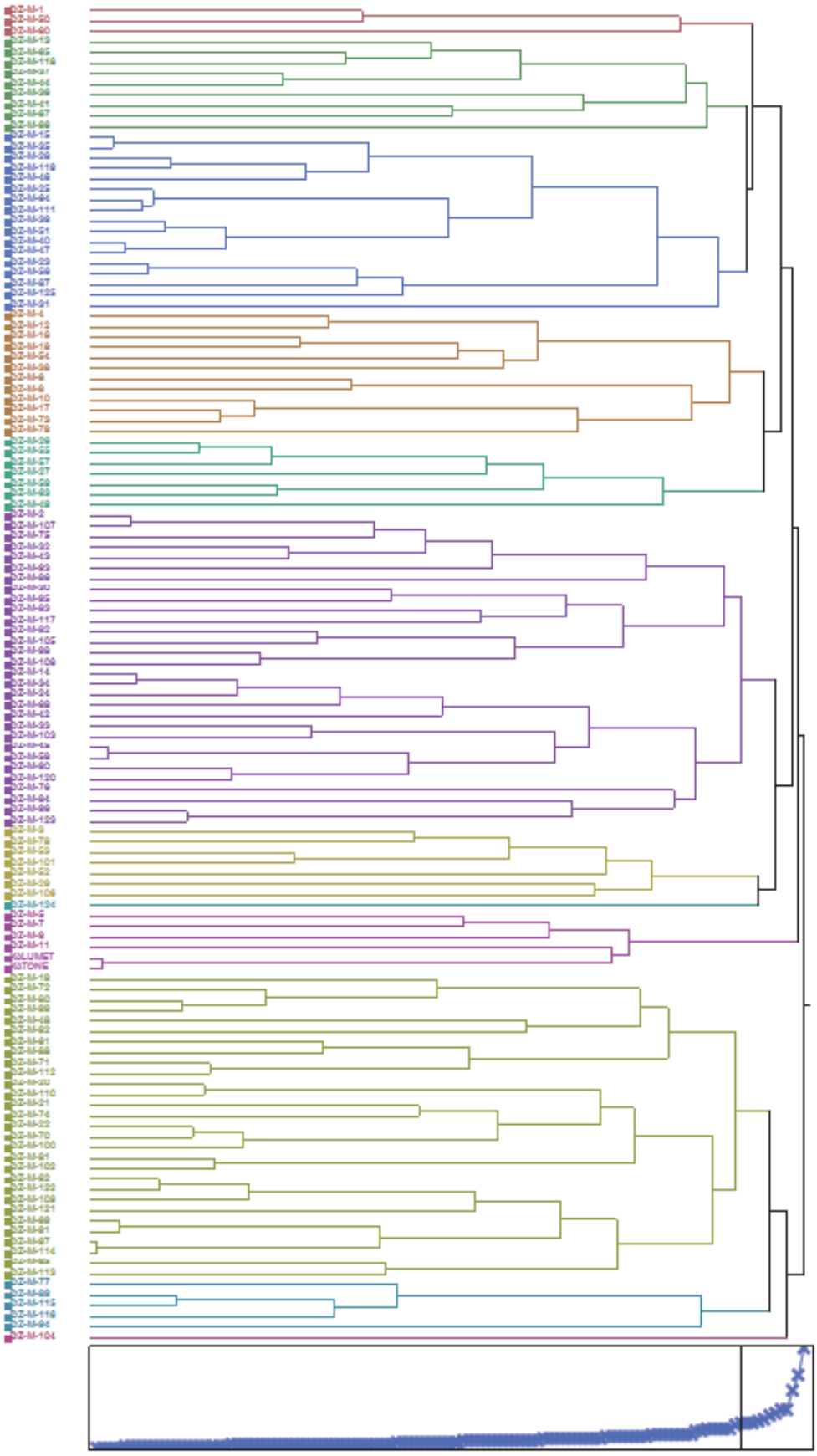

Figure 1 Dendrogram of the centroid clustering of 125 maize landraces and two commercial maize genotypes ('Kalumet' and 'Katone') based on twelve traits observed in agro-ecological region of Diyarbakır, Turkey

the regression analysis in respect of stem diameter, ear weight, and plant height remained in the final model of regression analysis and were considered as the effective components on the forage yield.

3.6 Cluster analysis

Understanding the extent and patterns of genetic diversity within germplasm accessions, particularly landraces of a particular region, is essential for successful future collection, improvement of conservation strategies of these genetic resources (Frankel et al., 1995). To determine the genetic distance between the populations and the variation within the population, the hierarchical analysis method was applied. According to morphological data, the hierarchical dendrogram differed grouped into 12 clusters (Figure 1), although some maize landraces collected in same area are included in different groups because of their different characteristics. Agronomic and ecological properties impact the genotypic constitution of landraces during domestication, and hence a relation exists between the agro-ecology of the exploration sites and the morpho-physiological make-up of the landraces (Kumar et al., 2015).

4 Conclusions

From the results and discussion of the present study, it can be concluded that all maize landraces collected Black Sea Region of Turkey had very large range for all traits (as compared with two commercial genotypes). Therefore, it was determined that they have potential to be used for developing the suitable maize varieties as well as to plan new genetic improvement program for different agro-ecological conditions.

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Original Paper

Bioaccumulation of macronutrients in herbaceous plants of the Sławno glaciolacustrine plain, northern Poland

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The studies aimed to compare bioaccumulation and translocation of macronutrients from roots to above-ground organs for six species of herbaceous plants (*Taraxacum officinale*, *Rumex acetosa* L., *Plantago major* L., *Plantago lanceolata*, *Potentilla anserina* L. and *Hypericum perforatum* L.) growing in the area of the Sławno Plain, northern Poland. Soil and plant samples were collected in June 2015 from 30 locations (five replications per species) and analysed using standard procedures, including content of nitrogen, phosphorus, potassium, calcium and magnesium. Mean contents of elements in the soil, roots and above-ground organs were calculated based on the results obtained. The content of organic carbon and pH were additionally determined in soil samples. The studied soils have been developed from silty-clayey glaciolacustrine deposits. They were characterised by acid and strongly acid reaction and contained from 9.5 to 28.7 g kg^{-1} of organic carbon. They were relatively abundant in nitrogen (1.44–1.87 g kg^{-1}) and potassium (4.30–5.34 g kg^{-1}), whereas poor in phosphorus (0.41–0.57 g kg^{-1}), calcium (1.63–2.84 g kg^{-1}) and magnesium (3.21–4.08 g kg^{-1}). The content of these elements in roots and above-ground parts of the studied plants was usually higher as compared to the soil. It was typical for herbs, reflecting their physiological demands. Only K occurred in higher amounts. The observed contents of nutrients suggest sufficient supply. The lowest bioaccumulation factors in roots were noticed for *Hypericum perforatum* L. (for N, P, Ca and Mg) or *Rumex acetosa* L. (for K) and the highest for *Plantago major* L. (for N, P, K and Ca) or *Rumex acetosa* L. (for Ca). In above-ground organs weakest bioaccumulation occurred in *Hypericum perforatum* L. (for K, Ca and Mg), *Rumex acetosa* L. (for P) or *Potentilla anserina* L. (for N) and the strongest in *Plantago major* L. (for N and Ca), *Taraxacum officinale* (for K and Mg) or *Plantago lanceolata* (for P). The values of translocation factors from roots to above-ground organs ranged from 1.3 to 3.1 for nitrogen, from 0.8 to 2.0 for phosphorus, from 1.3 to 3.3 for potassium, from 1.1 to 3.7 for calcium and from 1.1 to 3.1 for magnesium. Potassium and calcium were strongly translocated in *Taraxacum officinale*, whereas nitrogen, phosphorus and magnesium in *Hypericum perforatum* L.

Keywords: herbs, macronutrients, bioaccumulation, translocation, nutrient cycling

1 Introduction

Biogeochemical cycling of elements cover various interrelated links associated with marine and terrestrial ecosystems and countless physical and chemical processes leading to qualitative and quantitative transformation of these substances. It can be considered at different spatial and temporal scales and living organisms play an important role at any scale. Most of elements are nutrients and soils and rocks are major sources of these substances in terrestrial ecosystems. Abundance of elements in soils is strongly conditioned by origin and mineral composition of parent material. Its weathering constitutes primary source of bioavailable forms of these substances (Augusto et al., 2000). Litterfall, throughfall and stemflow are major secondary sources in forest ecosystems (Jonczak, 2013; Kozłowski, 2013), whereas mineral and organic fertilizers in agroecosystems (Rutkowska et al., 2014). Bioavailability of elements is controlled by soil ecochemical state, particularly pH (Kowalkowski, 2002; Devau et al., 2009), redox conditions (Tokarz and Urban, 2015), water regime (Mistra and Tyler, 2000), as well as a number of other environmental factors. The same factors determine toxicity of some elements (Cronan and Grigal, 1995). Demand for nutritional elements in natural and some modified ecosystems usually exceeds stocks of their bioavailable forms in rhizosphere, therefore some organisms have developed active (enzymatic) mechanisms of uptake

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(Antibus et al., 1992). Retranslocation is another adaptive mechanism to nutrients deficit in site (Dziadowiec et al., 2007). Abundance of elements and soil ecochemical state are reflected in chemistry of plant organs (Jonczak and Parzych, 2015; Šimanský et al., 2018). Close plant-soil interrelationships in this regard have been noted not only for nutritional elements, but also for heavy metals, as hazardous substances in environment (Tangahu et al., 2011). This relationship becomes the basis of bio-indicative methods in the assessment of environmental pollution with these substances (Čeburnis and Steinnes, 2000). Although the studies on elements uptake from soil, translocation and bioaccumulation in plants are conducted from many years under natural and controlled conditions, there are many gaps in knowledge in this area. Getting knowledge about some of them can be of great practical importance.

Our studies aimed to compare bioaccumulation and translocation of macronutrients from roots to above-ground organs of selected herbaceous plants growing in the area of the Sławno Plain, northern Poland. The studies included nitrogen, phosphorus, potassium, calcium and magnesium in six plant species – *Taraxacum officinale*, *Rumex acetosa* L., *Plantago major* L., *Plantago lanceolata*, *Potentilla anserina* L. and *Hypericum perforatum* L and associated soils.

2 Material and methods

Study area

The studies have been conducted in the area of the Sławno Plain near the villages of Mazów and Stary Kraków, northern Poland. This is a part of glaciolacustrine plain developed at the close of Pleistocene. Silty and clayey deposits constitute parent material of the soils, which according to the WRB classification system (IUSS 2014) should be classified as Stagnosols, Alisols or Luvisols. Mild climatic conditions are strongly influenced by the Baltic Sea. Average annual temperatures are about 7.5 °C, showing variability from 4.9 °C to 9.7 °C during the years 1871–2000. Mean annual sum of precipitation for this period ranged from 483 mm to 1,013 mm (Kirschenstein and Baranowski, 2009). The studied area is used in a variety of ways, including forests, arable fields, meadows and pastures. The history of agriculture dates back to the 14th century.

Soil and plant sampling and analysis

Soil and plant samples were collected in June 2015 from 30 locations within meadows, pastures and arable fields. The locations included five replications for each of six species of herbaceous plants – *Taraxacum officinale* (TO), *Rumex acetosa* L. (RA), *Plantago major* L. (PM), *Plantago lanceolata* (PL), *Potentilla anserina* L. (PA) and *Hypericum perforatum* L. (HP). Soil samples were collected from root zone. After removal of plant remains they were dried at 40 °C and sieved through a 2.0 mm mesh sieve to remove skeleton fraction. A part of sample was milled into powder for the purposes of elemental analysis. Soil analysis included determination of pH with potentiometric method (Elmetron CPC-401), total organic carbon (TOC) by Tyurin method (Dziadowiec and Gonet, 1999), total nitrogen (TN) by Kjeldahl method and the content of P, K, Ca and Mg after digestion of ashed samples in 20% HCl using microwave digestion system (Milestone ETHOS PLUS). The content of P in a solution was determined with molybdenum-blue method, whereas the remaining elements by flame atomic absorption spectrometry (Perkin Elmer 2100) at wavelengths 766.5 nm for K, 422.7 nm for Ca and 285.2 nm for Mg.

Plant samples have been washed with distilled water, dried at 65°C, milled into powder and analysed, including content of total nitrogen by Kjeldahl method and total content of P, K, Ca and Mg after samples digestion in a mixture of 65% HNO_3 and 30% H_2O_2. Concentrations of elements in the solutions were determined using the same methods and equipment like for soils.

Data processing

Average concentrations of elements in soils and plants and standard deviations were calculated for the individual plant species using Excel software. Bioaccumulation factors (BF) were calculated for the studied elements as a quotient of element concentration in plant (above-ground organs or roots) and in the soils (Zang et al., 2009). Translocation factors (TF) were calculated as a quotient of element concentration in plant roots and above-ground parts (Bose et al., 2008).

Results

3.1 Basic characteristics of the soils

The studied soils were characterised by acid and strongly acid reaction, with average pH at 4.9–6.0. The lowest value was noticed under HP, whereas the highest under TO and PA (Figure 1). Differences in pH measured at five stands of the individual herb species ranged from 0.6 to 1.6 of unit. The soils were relatively abundant in organic carbon, which content was from 9.5 to 28.7 g kg^{-1}. The lowest mean content of this element was observed at RA (15.6 g·kg^{-1}) and the highest at PA (20.0 g kg^{-1}) stands (Figure 1).

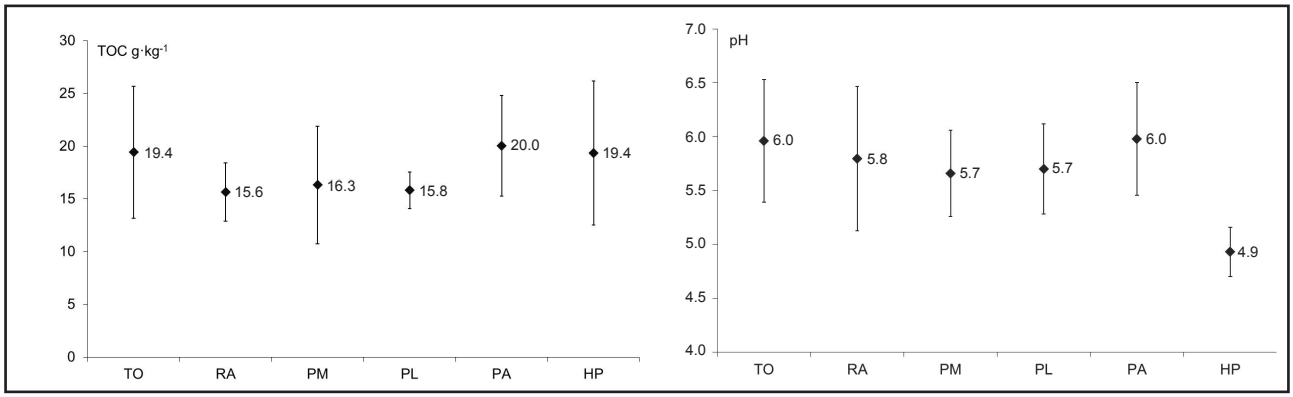

Figure 1 Mean contents of total organic carbon and pH in the soils under plant species (mean ±SD)

3.2 Retention and translocation of nitrogen

Although nitrogen is one of the most widespread elements in the environment, it is usually present in deficient amounts in soils. Therefore, it constitutes an important limiting factor for plant growth and succession (Oren et al., 2001), as well as biomass primary production (Elser et al., 2007). Nitrogen strongly influences also microorganisms and soil fauna (Aira et al., 2006). The content and forms of nitrogen in soils are regarded as useful indices of their quality and tools in the assessment of fertility and productivity (Schloter et al., 2003). Average contents of nitrogen in the studied soils ranged from 1.44 g kg^{-1} under PL to 1.87 g kg^{-1} under TO, showing relatively low variability between herb species (Figure 2). Much greater variability showed nitrogen in plants, both roots and above-ground organs. In roots its content ranged from 4.70 to 13.09 g kg^{-1}, whereas in above-ground biomass from 15.16 to 19.47 (Figure 2). In TO, RA, PA and HP the differences between roots and above-ground organs were statistically significant at p <0.05. The observed contents were among natural for most of plants (Ostrowska and Porębska, 2002). They also indicate sufficient supplying with this element. All studied plants accumulated nitrogen. Higher intensity of this process was observed in above-ground parts than in roots, which is typical for herbaceous plants (Yu

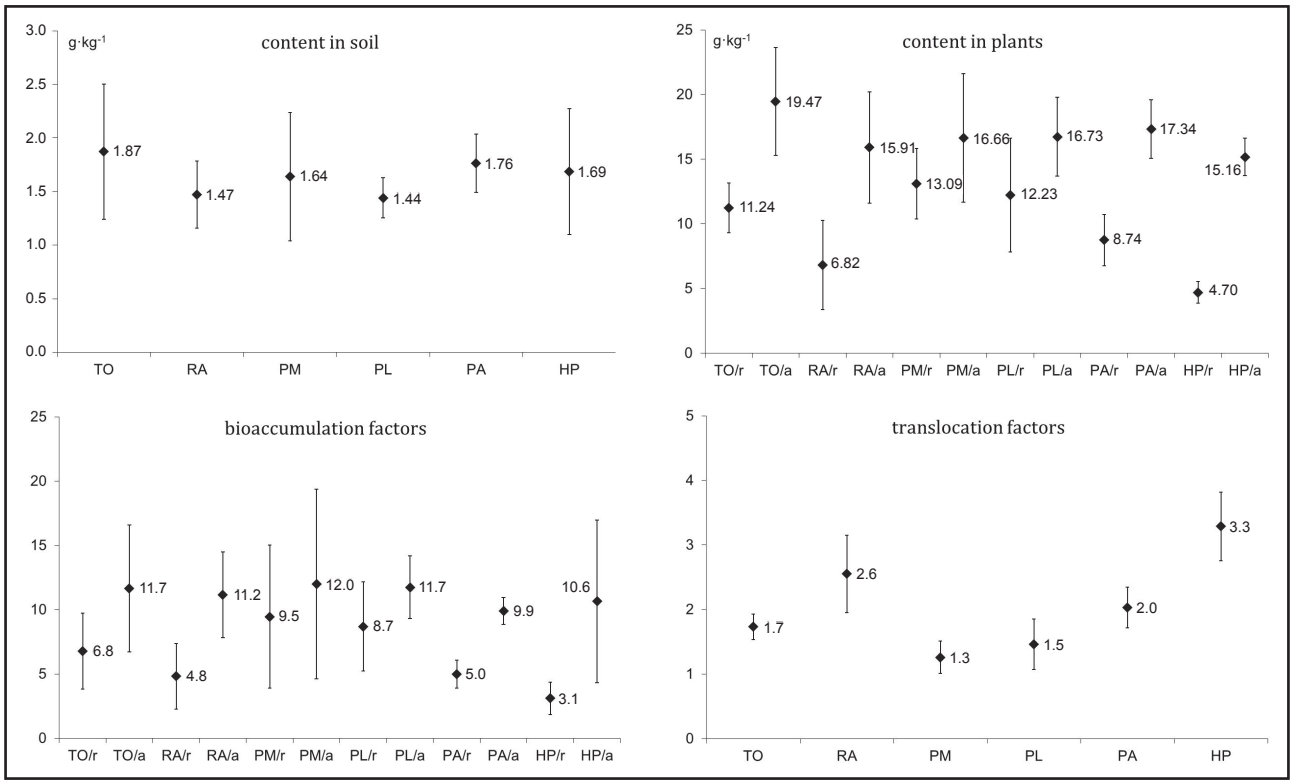

Figure 2 The content of total nitrogen in soils and plants, bioaccumulation factors in roots (/r) and above-ground parts (/a) and translocation factors from roots to above-ground organs (mean ±SD)

et al., 2014). High values of BF confirm high demand of the studied plants for nitrogen, which plays a key role in many physiological processes (Ostrowska and Porębska, 2002). The observed differences between plant species in BF were not significant statistically in most cases. Nitrogen was characterised by varied mobility from roots to above-ground organs in the studied plants, which is confirmed by significant differences (p <0.05) in translocation factors (Figure 2). It was the highest for HP, whereas the lowest for PM and PL. The observed differences can be due to varied acidification of the soils.

3.3 Retention and translocation of phosphorus

Apatites constitute major primary source of soil phosphorus. These are relatively susceptible to weathering minerals, therefore abundance in this element is decreasing during pedogenesis. This process is accompanied by qualitative changes of the element and its complexation with various soil components (Walker and Syers, 1976; Lair et al., 2009). The studied soils contained phosphorus in amounts from 0.41 g kg^{-1} under PL to 0.57 g kg^{-1} under RA (Figure 3). These are low contents, in particular considering the silty-clay character of the soils. Strongly acidic and acidic pH of the soils (Figure 1) can be an important factor limiting bioavailability of phosphorus. At pH <6.5 it is bounded by Fe and Al oxides, especially their amorphous fraction (Richardson, 1985; Achat et al., 2013). However, formation of phosphates can be inhibited by humic substances (Kodama and Schnitzer, 1980; Borggaard et al., 1990). Phosphorus is present in soils usually in deficient amounts, therefore it is strongly accumulated by plants. Its concentration in the studied herb species was several times greater as compared to the soils. In the roots it ranged from 1.50 g kg^{-1} in HP to 3.41 g kg^{-1} in MP, whereas in above-ground biomass from 2.41 g kg^{-1} in RA to 3.72 g kg^{-1} in PL on average (Figure 3). Mean phosphorus contents were higher in above-ground parts than in roots in most cases, however only under HP the differences were statistically significant. The noticed values suggests sufficient accumulation of this element (Ostrowska and Porębska, 2002). It is important, because phosphorus plays many physiological functions, i.a. accelerates development of generative organs (Gaj and Grzebisz, 2003). Bioaccumulation factors of phosphorus in the studied plants ranged from 3.2 to 7.4 in roots and from 4.6 to 9.2 in above-ground organs. Translocation factors ranged from 0.8 to 2.0 and were the highest for HP, whereas lowest for PM.

3.4 Retention and translocation of potassium

Soils are in general abundant in potassium, however its content gradually decreases during pedogenesis as an effect of weathering of primary and secondary minerals and leaching of the products of this process (Graham and

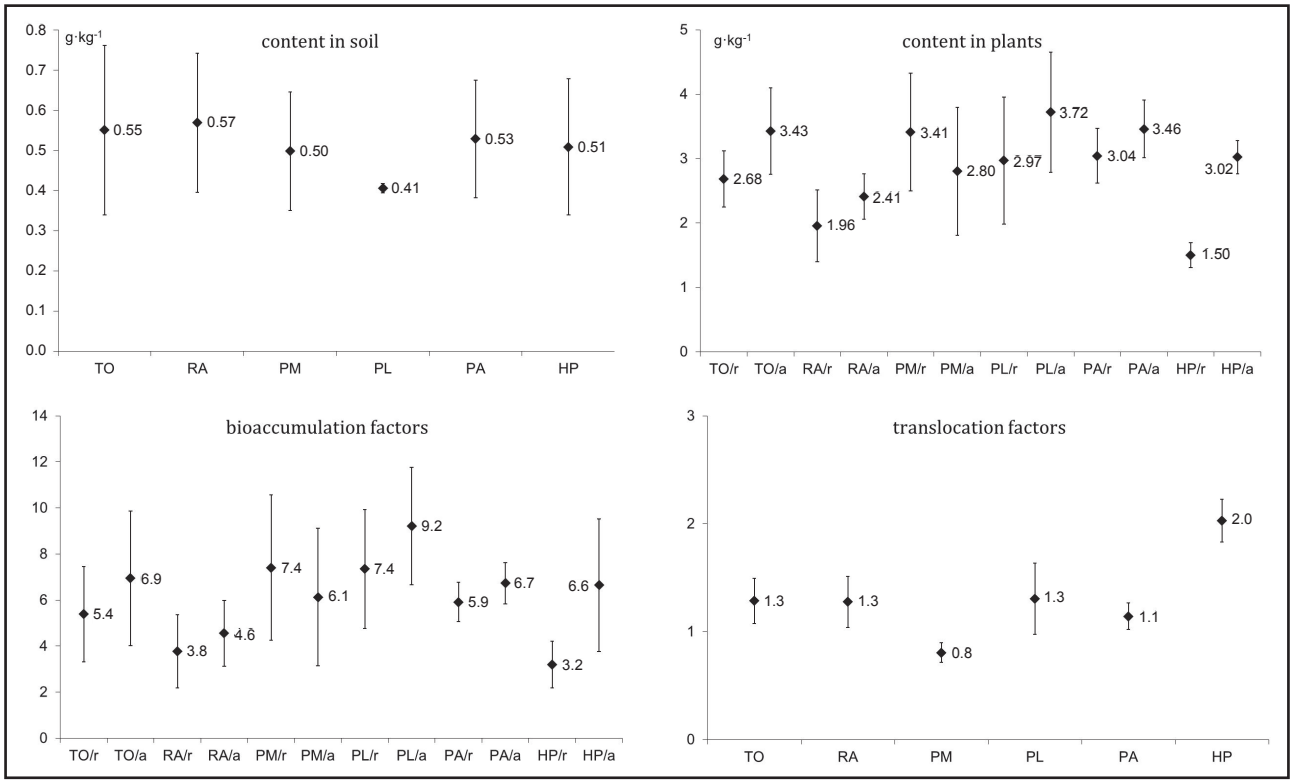

Figure 3 The content of phosphorus in soils and plants, bioaccumulation factors in roots (/r) and above-ground parts (/a) and translocation factors from roots to above-ground organs (mean ±SD)

Fox, 1971). K^+ ions are highly mobile in the environment, which was confirmed by lysimetric studies (Katutis et al., 2007), as well as in the studies on litter decomposition in forest ecosystems using litterbag method (Rutigliano et al., 1998; Jonczak et al., 2015). The studied area is located in young-glacial landscape developed just over 10,000 years ago. Young age of parent materials and their abundance in silt and clay textural fractions are main factors conditioning relatively high content of potassium in the studied soils. Its average contents were comparable at every stands and ranged from 4.30 g kg^{-1} under PL to 5.34 g kg^{-1} under PA (Figure 4). Potassium was strongly accumulated by the studied herb species, which is confirmed by much higher concentrations both in roots (from 5.97 g kg^{-1} in RA to 18.24 g kg^{-1} in MP) and above-ground biomass (from 9.83 g kg^{-1} in HP to 43.31 g kg^{-1} in TO) as compared to the soil, as well as by BF, which ranged from 1.4 to 4.6 in roots and from 2.6 to 10.6 in above-ground parts. The observed contents sometimes exceed values considered natural for plants (Ostrowska and Porębska, 2002). This phenomenon was observed also in our previous studies (Parzych and Jonczak, 2018) and by other authors (Czerwiński and Pracz, 1995; Krzywy, 2007). It is due to large mobility and bioavailability of the element. Strong accumulation of potassium can have some negative effects, including limiting uptake of other nutrients, especially Mg.

3.5 Retention and translocation of calcium

The content and profile distribution patterns of calcium in soils are conditioned by primary abundance of parent materials, water regime, leaching intensity, inflow of Ca^{2+} and carbonates from external sources (including groundwater and fertilizers) and many other natural and anthropogenic factors. The content of Ca is strongly varied among deposits/sediments and soils developed from them, as well as groundwater, where Ca^{2+} constitutes main cation. Vegetation, as a source of acidifying substances promotes decalcification of the soils in general. However, there are large differences between the individual species, in particular between deciduous vs coniferous trees (Quideau et al., 1996; Augusto et al., 2000). In the studied soils the content of calcium was low, which is typical for the soils developed from glaciolacustrine deposits of the Sławno Plain (Jonczak, 2015). It was on average from 1.63 g kg^{-1} under HP to 2.84 g kg^{-1} under PA (Figure 4). The content of Ca in plants was much higher, however strongly varied among the species. It was lower in roots (on average from 2.57 g kg^{-1} in HP to 13.44 g kg^{-1} in RA) than in above-ground parts (on average from 5.65 g kg^{-1} in HP to 33.79 g kg^{-1} in PM). BF ranged from 1.6 to 6.7 in roots and from 3.7 to 16.9 in above-ground organs. The observed contents of Ca in plants were at optimal level (Ostrowska and Porębska, 2002).

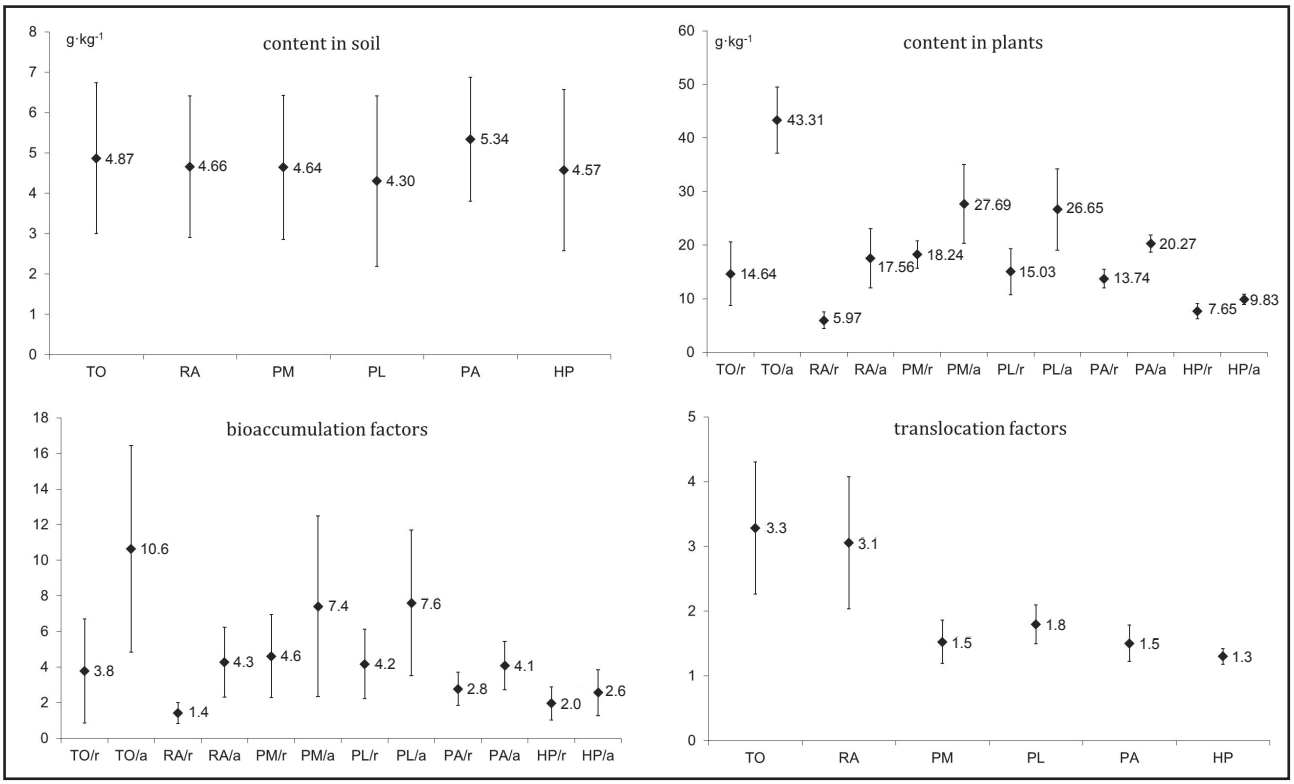

Figure 4 The content of potassium in soils and plants, bioaccumulation factors in roots (/r) and above-ground parts (/a) and translocation factors from roots to above-ground organs (mean ±SD)

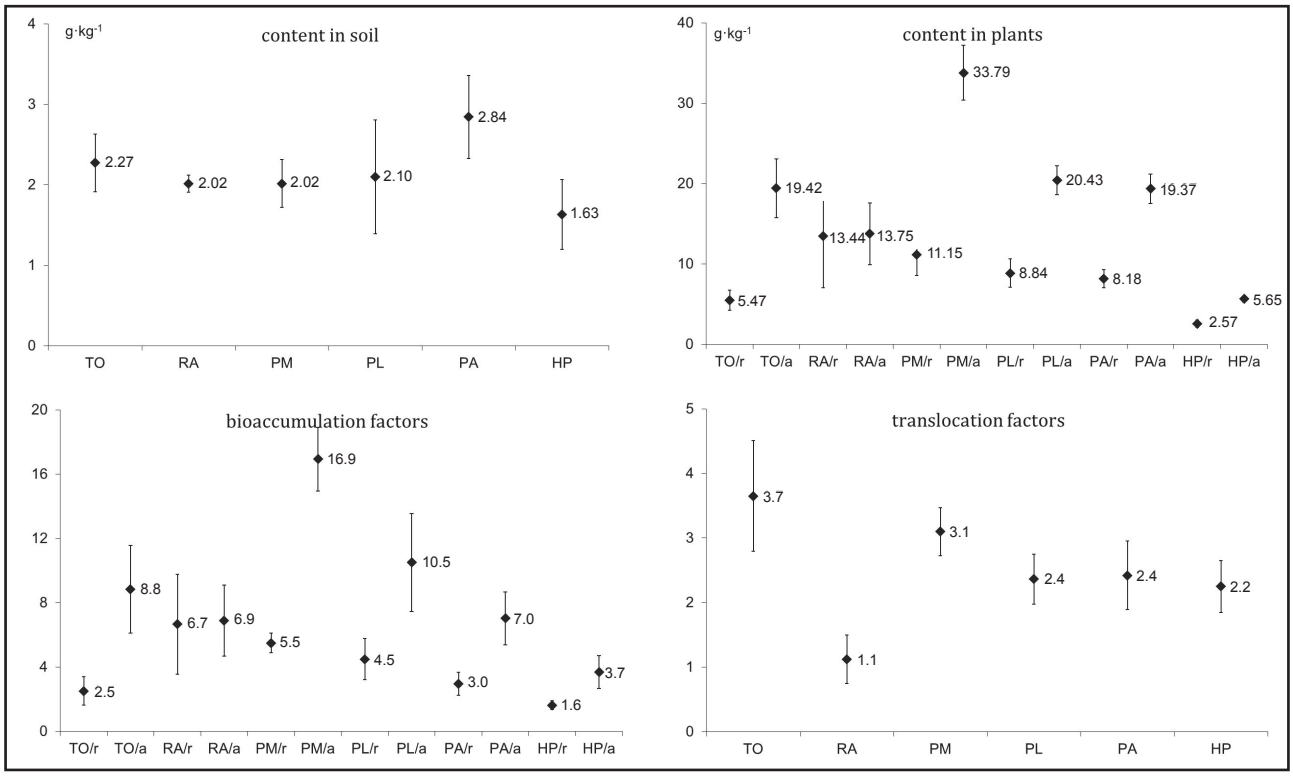

Figure 5 The content of calcium in soils and plants, bioaccumulation factors in roots (/r) and above-ground parts (/a) and translocation factors from roots to above-ground organs (mean ±SD)

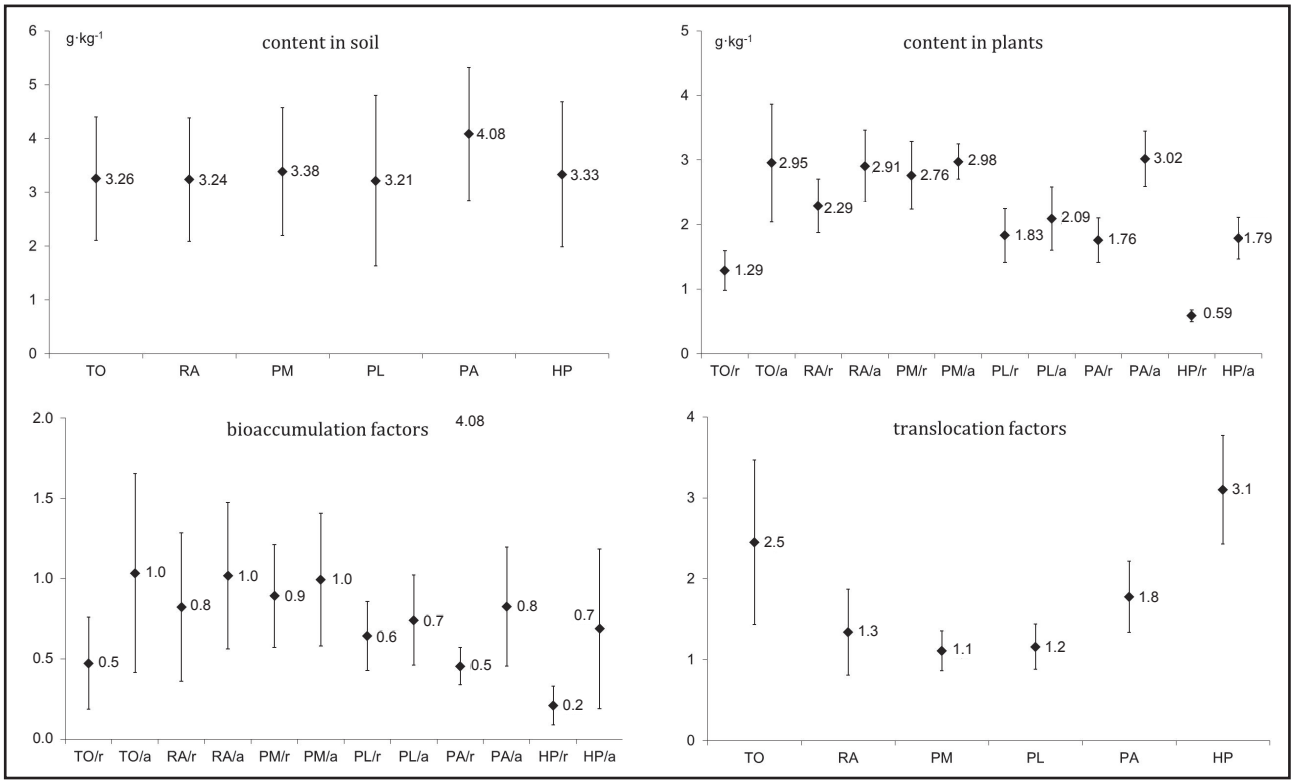

Figure 6 The content of magnesium in soils and plants, bioaccumulation factors in roots (/r) and above-ground parts (/a) and translocation factors from roots to above-ground organs (mean ±SD)

3.6 Retention and translocation of magnesium

Chlorite and mica are major sources of magnesium in soils developed from sedimentary rocks. Its content and profile distribution is conditioned by a complex of the same factors like for calcium, however contents are usually several times lower. It is reflected also in chemical composition of biomass of associated plants (Dziadowiec et al., 2007; Jonczak, 2013). In the studied soils content of Mg ranged on average from 3.21 g kg^{-1} under PL to 4.08 g kg^{-1} under PA, showing large variability within the stands of the individual herb species (Figure 6). In plants were noted comparable or lower amounts, slightly higher in above-ground organs as compared to the roots. Plants require at least 1–1.3 g kg^{-1} of Mg in their biomass for normal growth (Falkowski et al., 2000). The observed concentrations were much higher, despite potential negative effect of elevated contents of K. Magnesium showed different mobility depending on the species. It was the highest in HP and TO, whereas the lowest in PM (Figure 6).

4 Conclusions

The contents of N, P, Ca and Mg in roots and above-ground organs of the studied plants were among typical for herbs, reflecting their physiological demands. The content of K was higher. In general, the observed concentrations suggest sufficient bioavailability of elements in the studied soils and relatively high abilities to uptake by the studied plants. Based on mean bioaccumulation factors in roots and above-ground organs, the studied species form the following series:

N: roots – HP < RA < PA < TO < PL < PM
above-ground organs – PA < HP < RA < TO < PL < PM
P: roots – HP < RA < TO < PA < PL < PM
above-ground organs – RA < PM < HP < PA < TO < PL
K: roots – RA < HP < PA < TO < PL < PM
above-ground organs – HP < PA < RA < PM < PL < TO
Ca: roots – HP < TO < PA < PL < PM < RA
above-ground organs – HP < RA < PA < TO < PL < PM
Mg: roots – HP < PA < TO < PL < RA < PM
above-ground organs – HP < PL < PA < PM < RA < TO.

Presented the above list shows that the weakest bioaccumulation of most of macronutrients in roots was noticed for *Hypericum perforatum* L. and the strongest for *Plantago major* L., whereas for above-ground organs the weakest for *Hypericum perforatum* L. or *Rumex acetosa* L. and the strongest for *Plantago major* L., *Taraxacum officinale* or *Plantago lanceolata*. Translocation factors were typical for macronutrients and ranged from 1.3 to 3.1 for nitrogen, from 0.8 to 2.0 for phosphorus, from 1.3 to 3.3 for potassium, from 1.1 to 3.7 for calcium and from 1.1 to 3.1 for magnesium. The highest translocation was observed for *Taraxacum officinale* (K, Ca) and *Hypericum perforatum* L. (N, P, Mg).

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Original Paper

The morphological changes of uterus in postnatal development of heifers

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The aim of this work was to describe the microscopic and submicroscopic changes of the uterus of 20 Pinzgau heifers in their postnatal development (12th, 24th and 36th week of age) and in the experimental form of subclinical hypoglycaemia. Uterine specimens were obtained from the uterine horns by a vivisection for histological studies. Samples were fixed for light microscopy (LM) in formaldehyde and for scanning by electron microscope (SEM) and transmissive electron microscopy (TEM) in glutaraldehyde. Subsequently, the samples were processed in the usual manner as used in the LM and electron microscopic studies laboratories. The uterus increased to 24th weeks by 19.4%. The uterine weight decreased significantly (P <0.01) at the time of hypoglycemia (-14.6%). When the ovarian weight increased (+48.8%) to 36th week of age, the uterine weight increased by 39.9%. At the time of hypoglycaemia, the ovarian weight decreased by 4.4% and the uterine weight decreased by 17.1%. Endometrial development was mostly pronounced between 12th and 24th week of age (P <0.05). In particular, the superficial (+24.8%) and glandular epithelium (+25.9%) developed. Slower development continues up to 36th week, but in animals with hypoglycaemic development it stagnates (-4.1% and -18.8% respectively). The nucleo-cytoplasmic ratio was gradually reduced in luminal epithelial cells (N : C 1 : 3.2 to 1 : 2.9 respectively) and narrowed in glandular epithelial cells (N : C 1 : 2.3 to 1 : 1.7 respectively). A larger decrease was observed in the glandular epithelium. Mitochondria (M) increased the volume in both epithelium types (22.4% and 28.2%). In a hypoglycaemia is volume of M low (-18.4% and -16.2%). The rough endoplasmic reticulum (rER) increased in volume (+6.5% and +5.6% respectively) in both types of epithelial cells. Hypoglycaemia has been shown to decrease the volume of rER approximately equally (-10.9% and -10.2%). Macroscopic, microscopic and submicroscopic cell changes of endometrium are described in postnatal development and experimental subclinical hypoglycemia of heifers. There is a clear manifestation of the energy deficit in the retardation of growth and developmental changes.

Keywords: heifers, uterus, postnatal development, histology, hypoglycaemia

1 Introduction

The uterus of the mammals is the organ of pregnancy (Senger, 2011). It is a two-armed, muscular organ consisting of cervix, corpus and two cornua. The body of the uterus of a cow is short. Bielański (1972) reports a length of 9–12 cm, but Salisbury et al. (1985) only 2–5 cm. The body is formed from the caudal parts of the corners which form the longest part of the uterus, 25–30 cm (Bielański, 1972; Salisbury et al., 1985), but according to McDonald (1975) it is 35–40 cm. The wall is composed of the mucosa (endometrium), the muscular layer (myometrium) and the entire uterus is covered by serosus (perimetrium). The size of the uterus varies depending on the breed, age, birth rate, pregnancy, and, if appropriate, its health. The cervix is a sphincter, has a thick wall and a narrow canal. The cervix creates a barrier between the uterus and the external environment. The length of the neck is from 1.5 cm in heifers to 8 cm in cows. According to Bielański (1972), heifers have 6.6 cm long necks and cows 5–11 cm long and according to McDonald (2003) 8–10 cm in multi-breeding cows. The length and diameter of the cervix is greater for non-pregnant adult cows than for heifers. Most non-pregnant exotic cattle have a 7–10 cm long cervix (Ali et al., 2003), but Bello et al. (2012) found cervix of 8.0 cm with the same diameter. The mucous membrane of the cervix forms transverse folds. According to Salisbury et al. (1985) is the uterus 24–40 cm long. The postnatal development of the reproductive organs is continuously linked to the prenatal development and can continue even after puberty until sexual maturity. Intense development up to the 6th month of age also indicates an increase in uterus weight, which is related to the level of gonadal hormones. By the 6th–8th month of age it is relatively stable and, according to Foote (1972), consistent with physical development. The results of the Desjardins and

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Hafs studies (1969) confirm that the weight of the uterus is multiplied 22.5 times by the 10th month of age, but its enlargement continues beyond that time. In contrast, heifer multiplies its body weight by only 7.8 times. With increased endocrine ovarian activity associated with the onset of the estral cycle (40th week of age), a marked uterine development is seen (Foote, 1972; Williams and Amstalden, 2010). Desjardins and Hafs (1969) describe the slow, linear ovulation of the uterine cervix to the first ovulation. Similarly, the growth of the cervix and vagina is slow until the 4th month of age but followed by rapid growth after the first ovulation. It correlates with the growth of ovaries until the 5th month of age when their growth is stabilized. Honaramooz et al. (2004) describes intense postnatal development of the uterus of heifers, excluding the two-week period, up to the age of 6 months, followed by a tranquilization phase and re-intensive growth from the 8th to the 15th month of age. The reason for the two-phase development of the ovaries and the tubular reproductive tract is not entirely clear. This suggests that increased gonadotropin secretion (especially Luteinizing Hormone – LH) in the early postnatal period of heifers may reflect maturing changes in the regulation of gonadotrophin secretion prior to an estrogen suppression (Evans et al., 1994). Subsequent reduction of LH secretion by negative feedback and further enhancement of a follicular estrogen capacity can prevent and predict pubertal ovulation of heifers (Day et al., 1984, 1987). Bartol et al. (1995) studied the effect of progesterone (P) and estradiol (E) implants in neonatal heifers and changes in their adult endometrium. Regardless of the age at the time of application, P and E neonatal exposure caused a reduction in uterocervical weight by 35%, myometrial reduction by 23%, and endometrium by 27% compared to untreated animals. The endometrial gland density was reduced by 40%. This effect depends on the age in which the implant was administered. Endometrial gland density was reduced by 65% in case of treatment at birth, 22%, and 33% in case of treatment on day 21 or 45 after birth.

2 Material and methods

2.1 Animals

Twenty Pinzgau bred heifers of 4 weeks of age, weighing 52.4 kg, were selected for the experiment. Diet was permanently optimized for 15 animals. In 5 animals was at 32 weeks of age (body weight 148.9 kg) induced the subclinical hypoglycaemia (glucose in blood plasma achieved – 1.9–2.2 mmol, – using commercial spectrophotometric kits, plasma was analyzed for glucose – Sigma Tech, Bull.; Sigma Chemical, St. Louis). This condition lasted until the 60th week of age (241.3 ±3.1 kg). Average daily gain was 447.0 g. Heifers with the hypoglycaemia were at this time slaughtered too. 15 heifers were divided by 5 animals into 3 groups according to the age at which they were killed (12, 24 and 36 weeks). The animals were periodically weighed and their metabolic and health status checked.

2.2 Sampling and samples processing

All animals were slaughtered in line with the current state by usual method and the reproductive organs were removed immediately after draining of blood. The samples from uterus were taken from endometrium for light (LM), transmission (TEM) and scanning electron microscopy (SEM). Samples for LM were fixed in 10% formol, dehydrated by sequence of alcohols and deluged into paraffin. 8–10 μm thick slices were made from blocks, they were coloured by haemalauneeosine and by greens trichrome. For histochemical proof of glycogen and PAS – positive substances were used samples fixed in Gendres solution (Vacek, 1974), with PAS reaction (Schiff´s reaction periodic acid – Schiff). Sections were evaluated with LM (Olympus Provis AX) with a program bound for assessment of individual morphological structures – Statgraphics ver. 7, Image ProPlus (Spectra Services Inc, NY) and MS Excel 2000. Samples from the same parts and places of oviduct were taken for electronmicroscopic studies (TEM, SEM). They were fixed in 4% solution of glutaraldehyde paraformaldehyde (pH 7.4) with 0.08 M – cacodylate buffer (pH 6.9–7.1). We used 1% osmiumoxid with phosphate buffer (Milloning, 1962) for post fixation for TEM, samples were rinsed by Milloning´s phosphate buffer and sucrose. They were dehydrated by ascending sequence of ethanols, rinsed by propylene oxide and deluged in the compound Durcupan ACM (Fluka). Semi-thick (1 μm) and ultra-thin slices were made on ultramicrotome (LKB 8800 III). Semi-thick slices were coloured by Toluidine blue and assessed (Olympus Provis AX). Samples for SEM were rinsed and dehydrated in ascending sequence of acetones and desiccated with the help of CO_2 (CPD Polaron, England) after fixation (3 hours). On the fixtures, the dry samples were then metalized with 20 nm thick layer of gold by vacuum steaming. Ultra-thin slices were contrasted with lead citrate (Reynolds, 1963) and uranyl acetate. Electronograms were made with TEM (TESLA BS 500) and SEM (TESLA BS 301).

Morphometric methods were used for objectification of results (Weibel et al., 1966; Mráz and Polónyi,1988).

3 Results and discussion

According to Mukasa and Mugerwa (1989) is the uterus a muscular organ consisting of a body, about 4 to 5 cm long, and two uterine horns, each 15 to 25 cm in length and 1 to 3 cm in diameter. The length of the cervix

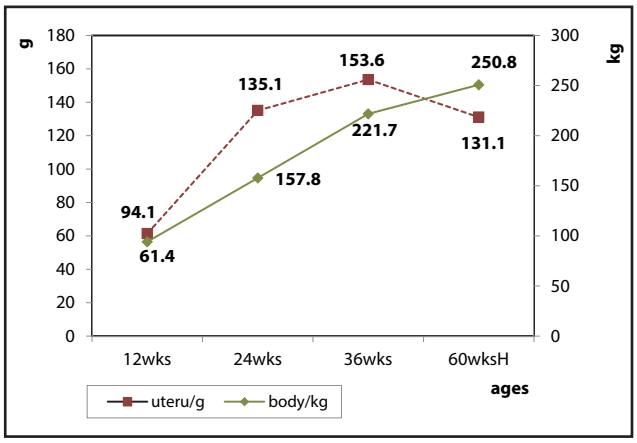

Figure 1 Comparison of the uterus weight (g) and the body weight (kg) of the heifers at different ages and with hypoglycaemia

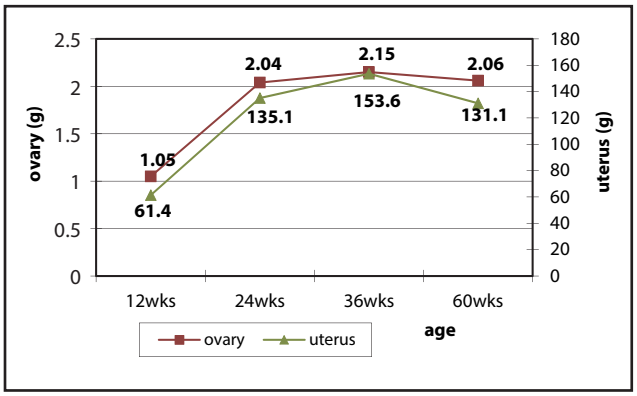

Figure 2 The weight of the ovary (g) and the weight of the uterus (g) in different ages and with hypoglycaemia of heifers

varies from 1.5 cm in heifers to 8 cm in multiparous cows of larger breeds. With the exception of the cervix lengths, it is not interpreted whether these attributes are from the cow uterus or from the heifer uterus. Age of the animals is absent too. Similarly, others (Salisbury et al., 1985; McDonald,1975; Ali et al., 2003; Bello et al., 2012) do not report the ages of the animals. According to our results it is probably about cyclic heifers or cows, because these attributes are near to 36 wks and 60 wks old animals (Table1). The smallest changes of a development were on the body of the uterus. In other parts were obvious changes (P <0.05, resp. P <0.01). After rapid growth of the uterus till 24th week of life, stagnation occurs in the next period. The uterus increased to 24th week by 19.4% but till 36th week by only 3.5%. There was small correlation (r 0.135) between the length and weight of the uteri and the body weight. The weight of the uterus has dropped significantly (P <0.01) at the time of hypoglycaemia (-14.6%, Figure 1). Honaramooz et al. (2004) describes the intensive postnatal development of the uterus of heifers, with the exception of the two-week period, up to 6th month of age, followed by a resting phase and a recurrent growth from 32nd to 60th month of age. The reason for the biphasic nature of ovarian and tubular reproductive tract development remains unclear. It has been suggested that increased gonadotropin secretion (particularly LH) in the early post-natal heifer, may reflect maturational changes in the regulation of gonadotropin secretion prior to suppression by estrogen (Evans et al., 1994). The weight of the uterus was much more affected by changes in ovaries (r 0.995) of the first 36 weeks, as well as at the time of hypoglycaemia (Figure 2). An increase in weight of ovaries +48.8% to 36th week of age, the weight uterus increased +39.9%. At the time of hypoglycaemia, the weight of the ovaries decreased -4.4% and the uterine weight decreased -17.1%. This confirms the significant influence of ovaries on the changes occurring in the uterus during its development and the lesser dependence on changes in body weight. Administration of estrogens to neonatal gilts from birth affects uterine growth and endometrial development acutely at both structural and biochemical levels (Yan et al., 2008). Treatment with estradiol valerate 2 weeks after birth increased uterus wet weight and advanced endometrial development to post-natal day 14 as reflected by increased glandularity and premature development of endometrial folds (Tarleton et al., 1999).

This fact also supports the trend in terms of growing follicles (±8 mm), which has a tendency to increase number of follicles and to 36th week of age of heifers. This is also the same, declining trend at the time of hypoglycaemia in both (Figure 3). Foote (1972) and

Table 1 The length and weight of the uterus and the body weight of heifers (No5/group)

Ages/wks	Cervix (mm)	Body (mm)	Horn (mm)	Uterus (mm)	Uterus weight (g)	Body weight (kg)
	x ±s	x ±s	x ±s	x ±s	x ±s	x ±s
12 wks	29.3^{a} ±0.05	29.4 ±0.41	69.7^{a} ±0.47	128.1^{b} ±1.12	61.4^{b} ±22.0	94.1^{b} ±7.22
24 wks	35.0 ±0.12	30.3 ±0.33	93.9 ±0.29	159.0 ±1.51	135.1 ±31.0	157.8^{b} ±11.3
36 wks	35.8 ±0.15	30.1 ±0.10	96.4 ±0.35	164.7 ±1.99	153.6^{a} ±10.0	221.7 ±10.1
60 wksH	36.6 ±0.326	30.7 ± 0.46	97.1 ± 0.96	164.4 ±2.86	131.1 ±18.0	241.3 ±3.1

^{a} P <0.05; ^{b} P <0.01; H – hypoglycaemia

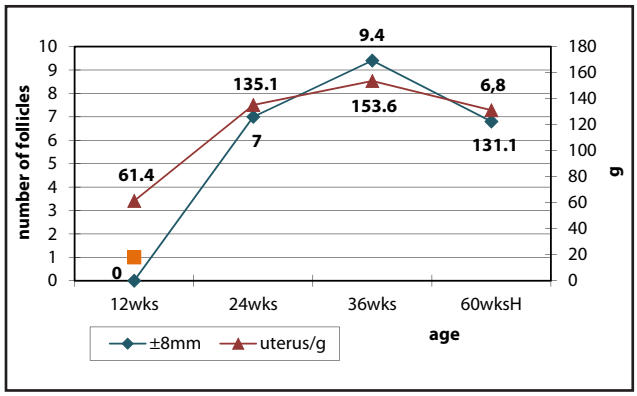

Figure 3 Number of follicles (± 8mm) and weight of the uterus (g) in different age of heifers

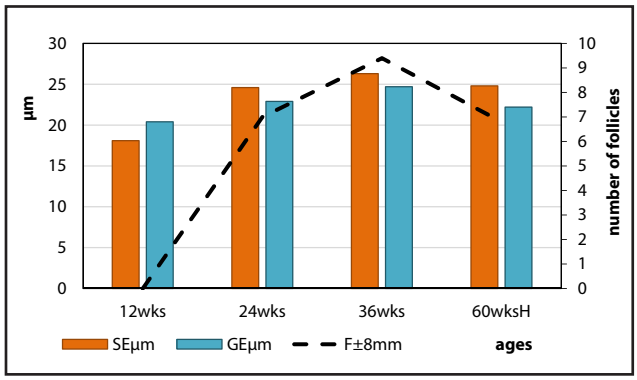

Figure 4 The dependence of the heigth of endometrial epithelium on the number of ovarian follicles
SE – superficial epithelium, GE – gladular epithelium, wks – weeks, H – hypoglycaemia

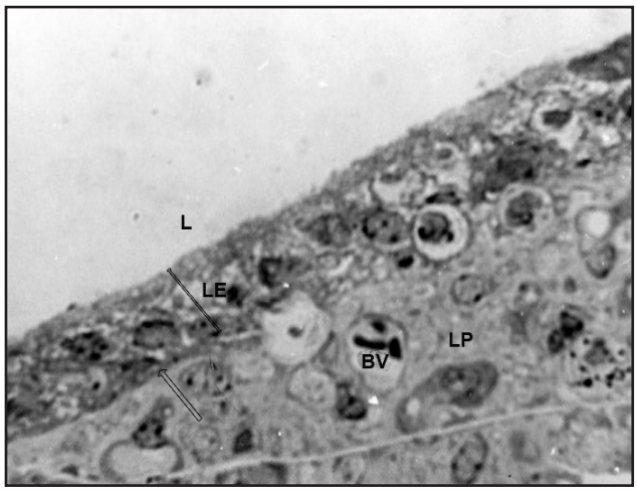

Figure 5 Part of the 12-weeks old heifer endometrium
LE – luminous epithelium, L – lumen, LP – lamina propria, BV – blood vessel, arrow basal membrane;, Toluidine blue, ×1,260

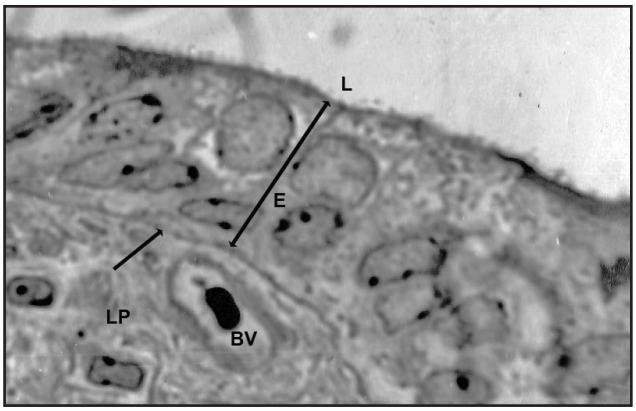

Figure 6 Part of the 24-weeks old heifer endometrium
E – luminous epithelium, L – lumen, LP – lamina propria, BV – blood vessel, arrow basal membrane; Toluidine blue, ×1,260

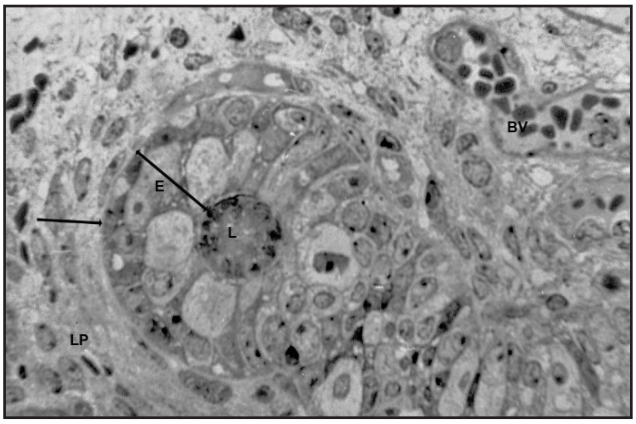

Figure 7 Uterine gland of heifer at 12 weeks of age
E – epithelial gland, L – lumen, BV – blood vessel, LP – lamina propria, arrow basal membrane; Toluidine blue, ×1,260

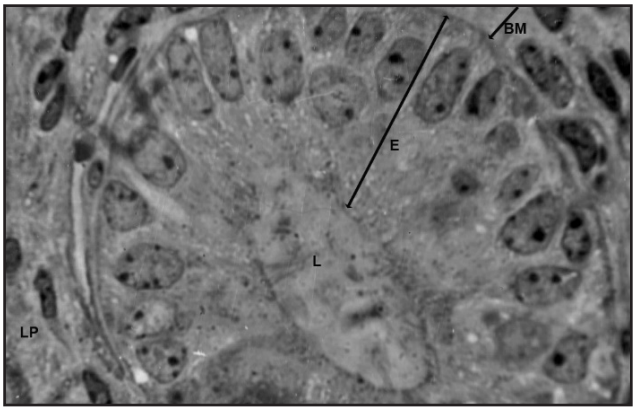

Figure 8 Uterine gland of heifer at 24 weeks of age
E – epithelial gland, L – lumen, LP – lamina propria, arrow with BM basal membrane; Toluidine blue, ×1,260

Williams and Amstalden (2010) confirms that marked increase in uterine development is seen in increasing ovarian endocrine activity associated with the onset of estral cycle (40th week of age). These data suggest that uteri of heifers are influenced by significant levels of gonadal hormones during the seventh month of age before the onset of first estrus. These reflections are in agreement with similar investigation for rats (Desjardins

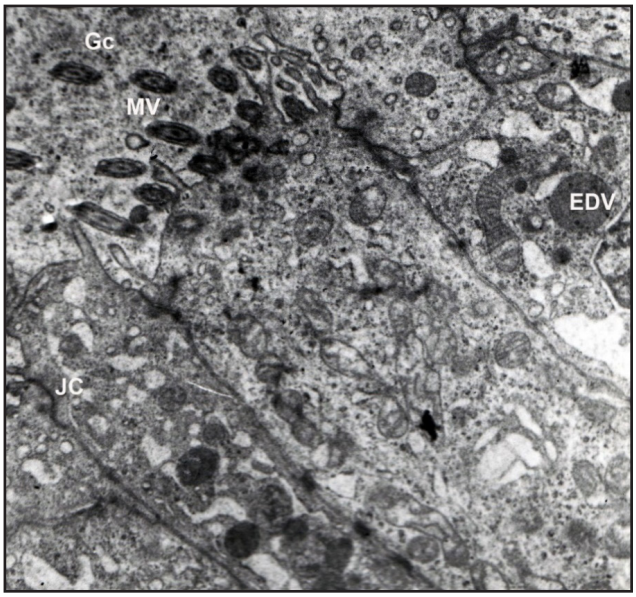

Figure 9 The epithelial cells of lumen (24 wks of age), they have the microvilli at the apical end
MV – microvili, Gc – glycocalyx, EDV – electron densite vesicles, JC – juctional complex; TEM, ×14,600

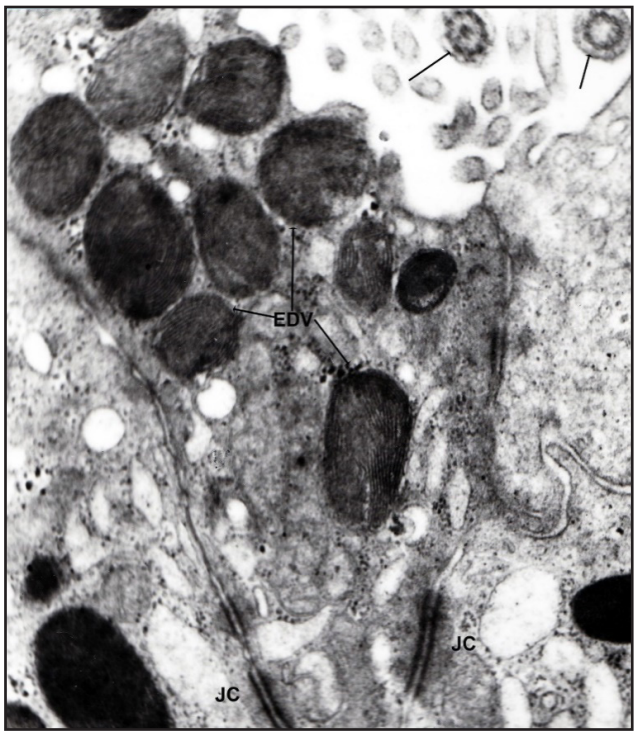

Figure 10 The cells of the luminal epitelium (36 wks old), they have on the apical ends well structural villi (arrows) and cells contain electrodensite vesicles (EDV)
JC – juctional complex, TEM, ×14,000

et al., 1968). Development of the uterus involves a series of morphogenetic and cytodifferentiative events that establish the framework for tissue function in adulthood. In the pig, uterine glands are absent at birth (postnatal day 0) and the uterus is estrogen receptor-α negative (Tarleton et al., 1998; Yan et al., 2006). However, during the first 2 weeks of life, uterine glands differentiation is evident in both nascent glandular epithelium and endometrial stroma (Tarleton et al., 1999). Post-natal development of the uteri before puberty can also be seen in changes in endometrial structures. Endometrial development was most pronounced between 3rd and 6th month of age (P <0.05). In particular, the superficial (+24.8%) and glandular epithelium (+25.9%) developed in contrast to interstitial tissue (Table 2). Slower development continues till 9th month, but in animals with hypoglycaemia the development stagnates and in the 15th month of age (Figure13, 14), the superficial and glandular epithelium has a volume of approximately six month heifers (-4.1%, resp. -18.8%). Chelikani et al. (2003) reported that the development of follicles depends only on age and not on the energy value of the feed. Changes in epithelial volume were caused by a change in epithelial cell height, probably due to an increase in the number of larger follicles (±8.0 mm) as documented in Figure 4 and 6. The luminal uterine epithelium of 12 weeks old heifer is unlike the epithelium at the age of 24 weeks, it is low and irregularly layered (Figure 5). A similar picture to see even in the glandular epithelium, which is highly heterogeneous (Figure 7, 8). The cells of the epithelium of the glands are not very dense, "quasi empty ". The cells of the superficial epithelium at the age of 24 weeks already have the apical end of microvilli, which were not available before.

Table 2 The relative volume of the endometrial components (% from mucous)

Age/wks	Superficial epitelium	Glandular epitelium	Glandular lumen	Interstitium
	x ±s	x ±s	x ±s	x ±s
12 wks	1.73 ±0.62	4.01 ±0.67	0.97 ±0.42	93.30 ±2.37
24 wks	2.30[a] ±0.66	5.41[a] ±0.41	1.19 ±0.51	91.10 ±3.43
36 wks	2.41 ±0.59	5.97 ±0.74	1.17 ±0.46	90.45 ±4.21
60 wksH	2.31 ±0.54	4.85 ±0.61	1.20 ±0.44	91.34 ±3.52

[a] 12 : 24; P <0.05

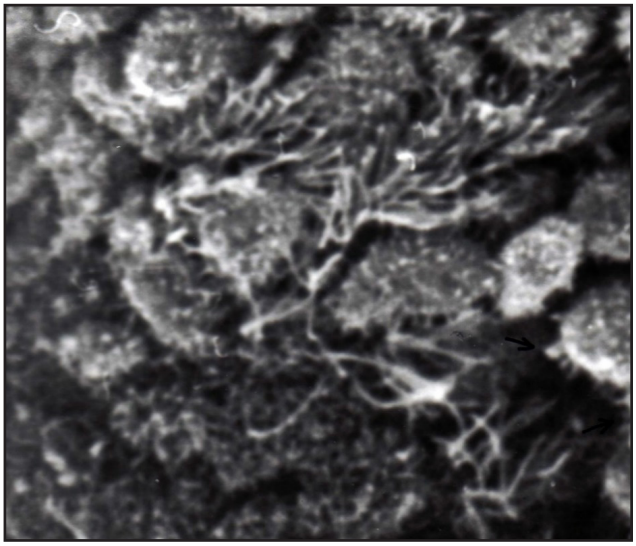

Figure 11 Ciliations of the endometrial cells of 36-wks old heifers, SEM, covered by gold, ×16,200

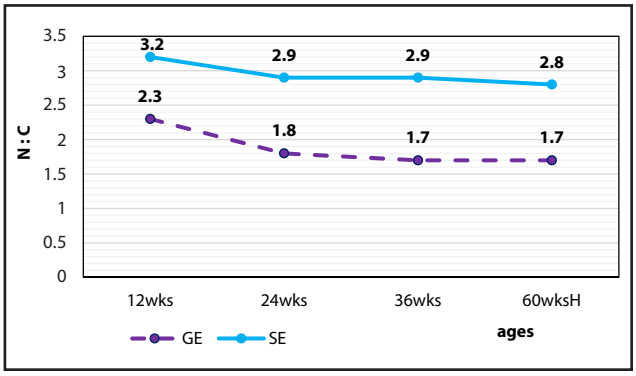

Figure 12 The nucleo-cytoplasmatic ratio of the endometrial epithelium
N = 1; GE – glandular epitelium; SU – superficial epitelium; N – nucleus; C – cytoplasm

Endometrial cell ciliations (Figure 10, 11) are usually associated with the proestral and estral stages of the animal cycle (Wick and Kress, 2002, Akinloye and Oke, 2014). Postnatal development and energy deficit also marked the internal structure of the cells. Although the closest nucleo cytoplasmic (N : C) ratio in immature

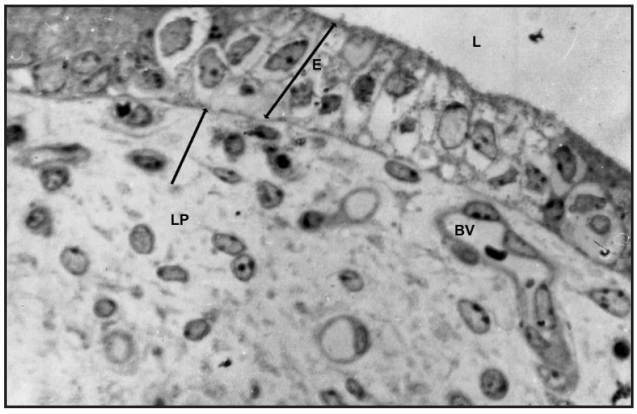

Figure 13 Uteri luminal epithelium of heifers with subclinic hypoglycaemia is heterogeneous, without ciliation
L – lumen, E – epithelium, LP – lamina propria, BV – blood vessel) Toluidine blue, ×1,260

and young cells is described and later relatively stable, in luminal epithelial cells and glandular epithelial cells, this ratio gradually narrowed down (Figure 9). A larger decrease was observed in the glandular epithelium (Figure 12). The lowest values can be seen in animals with hypoglycaemia.

Postnatal development of the uterus was also reflected in ultra-structural changes in epithelial cells of the luminal and glandular epithelium. Mitochondria (M) increased the volume in both epithelium types (22.4% or 28.2% respectively), (Table 3). However, over a 24-week period of hypoglycaemia, the volume M fell approximately equally (18.4% and 16.2% respectively). A much more stable value preserved the rough endoplasmic reticulum (rER). Both types of epithelial cells increased the volume in the cytoplasm (+6.5% and +5.6% respectively). The negative effect of hypoglycaemia has been shown to decrease the volume of rER approximately as much in the luminal as in the glandular epithelium (-10.9% and -10.2% respectively). The occurrence of lysosomes (L) has been unstable and it is difficult to evaluate changes in volume.

Table 3 Volume of the organelles of the endometrial epithelium (% of the cytoplasm)

Age/wks	Superficial epitelium			Glandular epitelium		
	x ±s			*x* ±s		
	M	rER	L	M	rER	L
12 wks	9.7 ±4.2	8.6 ±3.7	3.03 ±0.1	8.4 ±2.2	8.3 ±3.9	6.1 ±0.2
24 wks	10.3 ±4.7	8.4 ±2.6	8.4 ±0.07	9.6 ±2.9	8.8 ±2.8	4.09 ±0.4
36 wks	12.5 ±6.4	9.2 ±3.4	7.04 ±0.04	11.7 ±4.4	8.8 ±3.1	6.3 ±0.2
60 wksH	10.2 ±5.3	8.2 ±4.1	9.7 ±0.2	9.8 ±4.6	7.9 ±3.7	6.6 ±0.9

M – mitochondria; rER – rough endoplasmatic reticulum; L – lysosome

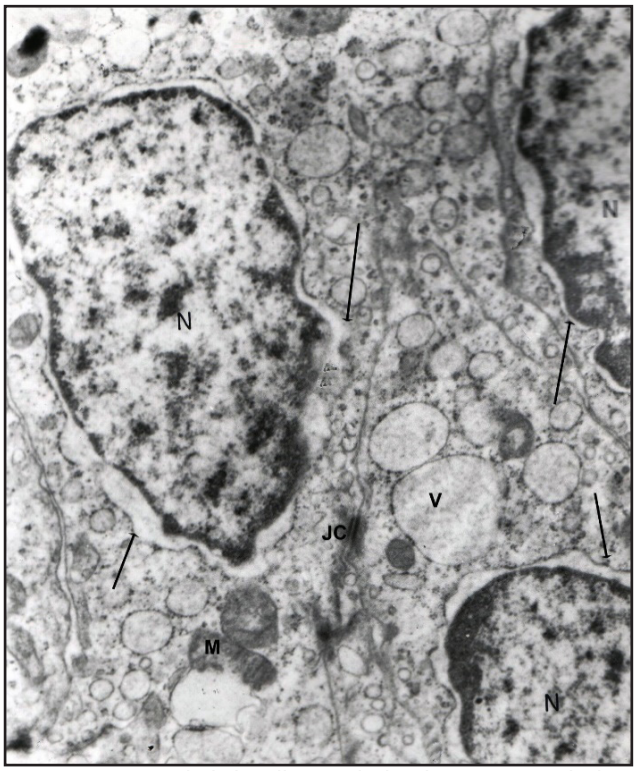

Figure 14 Epithelial cells are little denseness and they contain many vesicles, we see starting of karyolysis
N – nucleus, V – vesicles, M – mitochondria, JC – juctional complex, arrows collapse of karyolema), TEM, ×29,500

4 Conclusions

This work describes structural quantitative and qualitative changes of heifer uterus during their post-natal development until the pubertal period (from 12th wk to 36th wk). Changes in the heifer uterus structure with inducted subclinical experimental hypoglycaemia from 36th wk of age up to 60th wk were also assessed. Progressive development changes in mucous structures, especially epithelium mucous component were confirmed. We discovered different development and quantitative changes in superficial and glandular cells which are under steroid pre-pubertal control. Based on the changes in hypoglycaemic animals we can assume the importance of sufficient energy subsidising during development, because these animals based on comparison with other observed animals and despite their age of 60 wks (reproduction age) they had the development of uterus structures on the level of animals in pre-pubertal age.

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Original Paper

Energy content of hybrid *Rumex patientia* L. × *Rumex tianschanicus* A.Los (Rumex OK 2) samples from spring months and June

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Aim of this study was to determine the gross energy concentration of fresh, wilted and ensiled Rumex OK 2 (*Rumex patientia* L. × *Rumex tianschanicus* A.Los.) from spring months and June. Samples were collected in spring months and June of the year 2018. The plant of Rumex OK 2 consist in March mainly from rosette of leaves and the stalk is not higher than leaves, from April is stalk higher than rosette of leaves. The height of Rumex OK 2 during analysed months was following, March 60.96 ±5.22 cm; April 114.70 ±35.15 cm, May 168.31 ±39.74 cm and June 197.41 ±48.44 cm. Rumex OK 2 silage was made from wilted matter, with or without of addition of dried molasses. Gross energy was determined as the heat released after combustion of a sample (Leco AC 500) in MJ per kilogram of dry matter (DM) of the sample. The dry matter and gross energy concentration of fresh Rumex OK 2 increased during study, dry matter from 7.42% in March to 56.97% in June and gross energy from 18.00 MJ kg^{-1} of DM in March to 18.88 MJ kg^{-1} of DM in June. Statistically significant (P <0.05) higher concentration of dry matter, as well as gross energy was detected in wilted Rumex OK 2 samples and silages from May compared to April. Addition of dried molasses to wilted Rumex OK 2 did not affected concentration of gross energy in silages (P >0.05). From all analysed Rumex OK 2 samples the highest concentration of gross energy had silage from May with addition of dried molasses, 19.04 MJ kg^{-1} of DM. The utilisation of Rumex OK 2 from spring months can by neither for bioenergy production as a source of renewable energy, or after evaluation of nutritive value as a source of energy and nutrients in animal nutrition in form of pasture and silage. Rumex OK 2 from summer months seems to be utilized only as a source of heat via direct combustion.

Keywords: Rumex OK 2, silage, gross energy, dry matter

1 Introduction

Production of biomass is desired for bio-energy production. Planting of Rumex OK 2 has been considered as critical point of providing sufficient amount of biomass, mainly in areas with soil that has lower potential for agricultural crops. On the other hand there is ongoing demand for replacement of fossil fuel. Biomass is considered as a renewable source of energy (Petříkova, 2006). Advantage is, that production of green energy from Rumex OK 2 do not compete with food producing agricultural crops. During summer months is available also the straw from Rumex OK 2. Petříková (2006) published energy concentration of straw from Rumex OK 2 with value 19.17 MJ kg^{-1} of dry matter, which equals to heating capacity of 17.89 MJ kg^{-1} of dry matter. Straw from Rumex OK 2 is more valuable fuel than straw from cereals and show higher power. The next advantage is that Rumex OK 2 can be combusted also by dry matter concentration 70%, whereas straw from cereals by dry matter concentration at least 80% (Petříková, 2006). Other possible was for utilization of Rumex OK 2 in bioenergy industry is via fresh biomass or in form of silage. Fresh biomass or silage from Rumex OK 2 can be used also as a feed for animal, mainly ruminants. As it was published previously, Rumex OK 2 produced during autumn months interesting amount of energy in form of biomass (Rolinec et al., 2018). Research of energy concentration in Rumex OK 2 plants was realized also in spring months and June of the year 2018, and results are presented in this article.

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2 Material and methods

Methodoltogy of this study is similar to study of energy concentration of Rumex Ok 2 during autumn months of the year 2017 published in article (Rolinec et al., 2018). Rumex OK 2 (*Rumex patientia* L. x *Rumex tianschanicus* A. Los.) was used for this study. Plants of Rumex OK 2 were grown in experimental fields under Institute of Biodiversity Conservation and Biosafety (SUA in Nitra). Samples of fresh matter were collected in the year 2018, during months March, April, May and June (in March at the end of month, and in other months around 15th day of the month). During sampling, the height of leaves and stalk with flowers and/or seeds was measured in centimetre from ground (together were measured 26 samples in March, 76 samples in April, 67 samples in May and 63 samples in June. Fresh samples were wilted for three days. Wilting was realized in indoor conditions, by the open windows and without heating. After wilting, Rumex OK 2 plants were cut to the theoretical length of cut 1.5 cm and ensiled. First variant (Rumex OK 2 silage) was ensiled without additives. Second variant (Rumex OK 2 silage + molasses) was ensiled with a 1.0% addition of dried molasses to wilted Rumex OK 2 matter. All samples prepared for ensiling were stored in plastic bags without air (hermetic sealed). During fermentation process, which last for five weeks, plastic bags with silage samples were stored in room without light and at 20 °C. Fresh, wilted and silage samples were prepared for dry matter and energy concentration determination. Dry matter was determined by drying at 103 ±2 °C to constant weight. Gross energy concentration was determined by Calorimeter LECO AC 500 (Leco Corporation, USA). Each sample was analysed in triplicate. Gained results were statistically processed with IBM SPSS v. 20.0. Differences of means between months within type of sample were tested by Tukey HSD test. Differences of means between silages samples (with or without an addition of dried molasses) within month were tested by independent samples *T*-test. A P <0.05 was considered as significant.

3 Results and discussion

Compared to autumn months, the average surface air temperature during spring months increases, which is good for plants with early spring growth as for example Rumex OK 2. Crop growth rate is besides temperature, soil moisture content and nutrition affected by many factors (Hric et al., 2013; Hric et al., 2018). At the end of March consist Rumex OK 2 from leaves and stalk, which one is not higher than leaves. The height of Rumex OK 2 in the end of March was 60.96 ± 5.22 centimetres that is higher than average of all autumn samples (Rolinec et al., 2018). In following months the stalk with flowers or seeds of Rumex OK 2 is higher than rosette of leaves. During maturity Rumex OK 2 has 4 to 6 stalks with average height 235 cm (Usťak, 2007; Bazhay-Zhezherun and Rakhmetov, 2014). The average height of Rumex OK 2 was in April 114.70 ±35.15 centimetres, in May 168.31 ±39.74 centimetres and in June 197.41 ±48.44 centimetres, which is similar to publication of Usťak (2004). Concentration of dry matter and gross energy of Rumex OK 2 in spring months and June is shown in Table 1. Concentration of dry matter in fresh Rumex OK 2 increases and the peak reached in last analysed month, June. Similar development of dry matter revealed all crops and plants growing outdoors (Gálik et al., 2016). In March was dry matter concentration (Table 1) similar to that in November (Rolinec et al., 2018). With increase of dry matter increase the gross energy concentration too. Highest gross energy concentration of fresh matter was detected in June, when Rumex OK 2 reached maturity and stalk contains a mature seeds that increases energy concentration of whole plant. Hejduk and Doležal (2004 and 2008) wilted *Rumex obtusifolius* for 24 hour and reached dry matter 16.84%. Despite low concentration of dry matter of Rumex OK 2 fresh matter in March and April is wilting, as well as ensiling problematic. As published Bíro et al. (2014) and Skládanka et al. (2014) low concentration of dry matter of wilted matter causes outflow of silage effluent during fermentation and problems during fermentation process. Statistically

Table 1 Energy value of different Rumex OK 2 samples from March, April, May and June (MJ kg^{-1} of DM)

Month of the year 2018	Fresh Rumex OK 2		Wilted Rumex OK 2		Rumex OK 2 silage		Rumex OK 2 silage + molasses		SEM
	DM	GE	DM	GE	DM	GE	DM	GE	GE
March	7.42^{a}	18.00	–	–	–	–	–	–	0.007
April	9.99^{a}	18.49	12.51^{a}	18.01^{a}	12.06a	18.09^{a+}	13.20^{a}	18.20^{a+}	0.014
May	16.91^{a}	18.28	18.99^{b}	18.72^{b}	18.33^{b}	19.03$^{b□}$	18.85^{b}	19.04$^{b□}$	0.330
June	56.97^{b}	18.88	–	–	–.	–	–	–	0.132

DM – dry matter concentration of sample in %; GE – gross energy concentration of sample in MJ kg^{-1} of dry matter; SEM – value of standard error of the mean for gross energy in that month; *abc* – means within a column bearing different superscript differ significantly at P <0.05; $^{+□}$ difference of mean values of GE between Rumex OK 2 silage and Rumex OK 2 silage + molasses were within month nonsignificant (P >0.05)

significant (P <0.05) higher concentration of dry matter, as well as gross energy was detected in wilted Rumex OK 2 samples and silages from May compared to April (Table 1). Addition of 1% of dried molasses did not affect the concentration of gross energy in analysed silage samples (P >0.05). All four analysed Rumex OK 2 silage samples from April and May (Table 1) contains more gross energy than Rumex OK 2 silage samples from autumn months (Rolinec et al., 2018). Higher energy concentration of Rumex OK 2 samples in April and May was due to presence of stalk with flowers and seeds, whereas autumn Rumex OK 2 samples consisted only from rosette of leaves (Rolinec et al., 2018). Fresh Rumex OK 2 from summer months is used for direct combustion and production of heat. The lowest concentration of dry matter in Rumex OK 2 for combustion is 70% (Petříková, 2006). The average dry matter concentration of fresh Rumex OK 2 in June was 56.97%, however some plants had dry matter 70%. Harvesting of Rumex OK 2 at the end of June could increase dry matter concentration and thereby also the production of heat. On the other hand it depends on weather condition and rainfall totals during harvesting. Energy concentration of Rumex OK 2 fresh matter in June was 18.88 MJ kg^{-1} of dry matter (Table 1), which is less than published (Petříková, 2006) 19.17 MJ kg^{-1} of dry matter.

4 Conclusions

Rumex OK 2 is in agricultural condition of V4 countries unknown plant. Results of this article bring closer look on development of dry matter and energy concentration of fresh Rumex OK 2 in early spring and next in each month to June. Different types of samples were analysed, fresh, wilted matter and silage. The utilisation of Rumex OK 2 from spring months can by neither for bioenergy production as a source of renewable energy, or after evaluation of nutritive value as a source of energy and nutrients in animal nutrition in form of pasture and silage. Rumex OK 2 from summer months seems to be utilized only as a source of heat via direct combustion.

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Original Paper

Can soil properties of Fluvisols be influenced by river flow gradient?

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The occurrence of Fluvisols is associated with the rivers, which means that their properties can be greatly influenced by the fluvial activity of the rivers. Therefore, the aim of this work were to (1.) find out whether the flow gradient along the river influenced the soil properties of Fluvisols (2.) evaluate the soil properties of Fluvisols. Soil samples were taken from Nitra River Catchment between villages Výčapy-Opatovce and Jelšovce near Nitra city. There were excavated five soil pits and soils were classified according to the World Reference Base for Soil Resources as follows: Profile 1 as Eutric Fluvisol (Loamic, Humic) (soil use: restored forest), Profile 2 as Eutric Fluvisol (Loamic, Humic) (soil use: arable soil), Profile 3 as Eutric Fluvisol (Loamic, Humic) (soil use: fallow soil), Profile 4 as Eutric Gleyic Fluvisol (Loamic, Humic) (soil use as: forest), Profile 5 as Eutric Fluvisol (Loamic, Humic) (soil use: raid forest). The investigated Fluvisols had different chemical and physical properties, but not as a consequence of the flow gradient along the river. Differences in chemistry and physical properties of Fluvisols developed along the Nitra River have been significantly affected mainly by its use, soil management practices and depth of the soil profile.

Keywords: physical and hydrophysical properties, soil structure, soil sorptive parameters, Fluvisols

1 Introduction

Fluvisols occupy less than 350 million ha worldwide (WRB, 2015). In Slovak Republic the area of Fluvisols is 309.7 thousand hectares, representing 12.6% of the agricultural land fund (Bielek, 2017). The original natural undergrowth for Fluvisols were forests and floodplain meadows. On deep alluvial and texturally heavy Fluvisols with groundwater 1.5 to 2.0 m beneath the surface are good conditions for planting of cereals, technical crops and root crops. Sandy Fluvisols are good soils for growing of vegetables and for forage crops (Zaujec et al., 2009). Fluvisols are genetically young soils developed in predominantly recent, fluvial deposits. There are located along the river plains and valleys, lake depressions and tidal marshes on all continents and in all climate zones; no groundwater and no high salt contents in the topsoil; many Fluvisols under natural conditions are flooded periodically. Soil horizons are weak differentiated, but a distinct topsoil horizon may be present (Zaujec et al., 2009; WRB, 2015). The A-horizon is often sorptive saturated, mostly alluvial texture, with a low humus content of inferior quality and a weak acidic soil pH. A-horizon of Fluvisols does not contain carbonates even when the soil is developed on carbonate alluviums. The production potential of Fluvisols is relatively wide, ranging from 33 to 90 points in a 100-point scale. This means that fertility is significantly limited by soil properties (Zaujec et al., 2009). The properties of Fluvisols are significantly influenced by soil management practices (Kotorová, 2007; Kotorová and Šoltýsová, 2011; Kotorová, 2013; Polláková and Šimanský, 2015). Fluvisols are among the azonal soils. As their occurrence is associated with the rivers, it is evident that their properties can be greatly influenced by the fluvial activity of the rivers (Zaujec et al., 2009, WRB, 2015, Bielek, 2017).

Based on the above context, the aim of this study was to find out whether the flow gradient along the river influenced the soil properties of Fluvisols.

2 Material and methods

The soil surveys were carried out to determine the soil properties of several soils along the Nitra River. Soil sampling sites are shown in the Figure 1. These localities are located between villages Výčapy-Opatovce and Jelšovce near Nitra city. Soil pits were dug on both sides of the Nitra River (the youngest part of investigated area). The parent material consists of fine particles loess and loess silt

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Figure 1 Soil sampling sites

from the Pleistocene epoch. Sometimes there are areas with sand and gravels from Neogene epochs and areas along the river, where loess and loess silt are formed from Holocene drains. The site has a temperate climate, with a mean annual air temperature of 9.7 °C. The mean annual precipitation at this site is 595 mm (333 mm between April and September). More information about the Nitra River Catchment is published in Tarník and Igaz (2015).

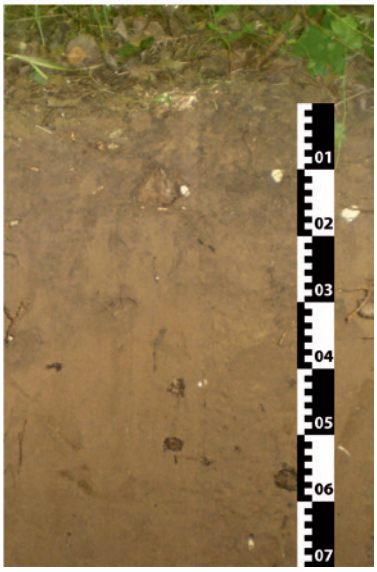
In each locality, before soil sampling a pit was excavated and the soils were classified according to the World Reference Base for Soil Resources (WRB, 2015) based on the whole-profile soil morphology. In the soil pits, the soil samples were collected (in triplicate) after 10 cm layers to a depth of 50 cm to cylinders with an inner diameter of 5 cm and height of 5 cm. Determination of physical (bulk and particle densities, pore size distributions), hydrophysical properties (soil moisture, capillary absorption, 30 minute moisture, maximum capillary water capacity, and retention water capacity) was then conducted using standard methods (Hrivňáková et al., 2011). The soil samples for determination of chemical properties, soil organic carbon and soil structure parameters (vulnerability coefficient, index of aggregate stability) were taken from described soil horizons. In laboratory, the large clods of soil were gently broken up along the natural fracture lines, and soil samples for the determination of individual size fractions of aggregates (undisturbed soil samples) were obtained. In undisturbed soil samples, individual size fractions of aggregates were determined by dry sieving. These size fractions of air-dried aggregates (>7, 7–5, 5–3.15, 3.15–1, 1–0.5, 0.5–0.25 and <0.25 mm) were used for the determination of water-stable aggregates (WSA) distribution by Baksheev method (Vadjunina and Korchagina, 1986). Following size fractions of WSA >5, 5–3, 3–2, 2–1, 1–0.5, 0.5–0.25 (macro-aggregates) and <0.25 mm (micro-aggregates) were determined. Part of the soil samples collected from the soil horizons were grinded before analysis. The soil samples were determined for: soil pH – potentiometrically in the supernatant suspension of a 1 : 2.5 soil/distilled water and 1 : 2.5 soil/1M KCl (Hrivňáková et al., 2011). Soil colloidal complex was characterized by the hydrolytic acidity (H), sum of basic exchangeable cations (SBC) and cation exchange capacity (CEC) and base saturation (Bs) which were determined by Kappen method (Hrivňáková et al., 2011). Carbonates were determined by volumetric method using a Jankov calcimeter. Soil organic carbon content (SOC) was measured using the wet combustion method (Gonet et al., 2002). The content of total iron, content of free iron oxides, the content of amorphous iron oxides by means of the microwave plasma atomic emission spectrometry (Agilent 4100MP-AES) after samples digestion in a mixture of 60% $HClO_4$ and 40%HK, after samples extraction by means of Jackson´s method (Mehra and Jackson, 1960) and after samples extraction by means of the Schwertmann method (Van Reeuwijk, 1995), respectively, were measured. In the grinded soil samples, particle-size distribution was determined by pipette method (Hrivňáková et al., 2011). Mineral composition of soil samples was determined using X-ray diffraction (XRD) method. Samples were dried at 20 °C and ground. Powders were analysed by means of Bruker AXS D5005 diffractometer equipped with the KRISTALLOFLEX® 760 X-ray generator, the vertical goniometer, 1 mm divergence slit, 2 mm anti scatter slit, 0.6 mm detector slit, and a graphite diffracted-beam monochromator. CoKα radiation was used with the applied voltage of 40 kV and 30 mA current. Random mounts of the ground materials were scanned at a counting time of 2 s per 0.01° step from 3 to 70 °2θ. XRD analyses were performed in the Department of Soil Environment Sciences, Warsaw University of Life Sciences – SGGW, Poland.

The statistical analysis was performed using the computer program Statgraphics Centurion XV.I (Statpoint Technologies, Inc., USA). The data were analyzed using one-way ANOVA, and the means (average values of soil properties) were compared with LSD test at P <0.05. The relations between chemical and physical properties in soil profiles of Fluvisols were determined through correlation matrix.

3 Results and discussion

3.1 Description of the soil profiles

Profile 1



Localization: On the left side of actual river bed Nitra (48° 23‘ 48.83“ N 18° 5‘ 5.99“ E).

Description: Soil pit located at the area approx. 150 m from protection wall of Nitra River. Before 2010, poplar forest (approx. 45 years’ old woods) was planted. In 2015 (time of sampling) here was neglected area no cultivation, no planting – appearance of raid plants/trees.

Morphology of soil profile: Eutric Fluvisol (Loamic, Humic)

0–5 cm (Aka) slightly humid, colour: moist wet 10YR 2/3, moist-dry 10YR 6/2, loose, silty-clay-loam, crumb soil structure, aggregates of spherical shape, intensive root growth, CO_3^- 1–3%.

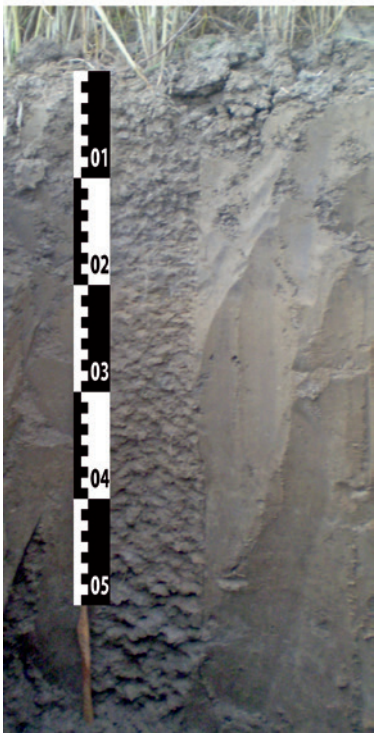
5–20 cm (Akp) slightly humid, colour: moist wet 10YR 4/3, moist-dry 10YR 7/2, oppressed, silty-loam, polyhedron, angular aggregates, intensive root growth, CO_3^- 1–3%.

20–61 cm (Ck) slightly humid, colour: moist wet 10YR 4/4, moist-dry 10YR 7/3, oppressed, silty-loam, granular soil aggregates, rust stains after Fe^{3+}, CO_3^- 1–3%.

61–72 cm (Ckgr1) moderately humid, colour: moist wet 10YR 4/4, moist-dry 10YR 7/3, oppressed, silty-loam, rust stains after Fe^{3+} on granular aggregates, CO_3^- 1–3%.

>72 cm (Ckgr2) wet, colour: moist wet 10YR 4/4, moist-dry 10YR 7/3, oppressed, silty-loam, rust stains after Fe^{3+} on granular aggregates, CO_3^- 3–5%.

Profile 2



Localization: On the right side of actual river bed Nitra (48° 23‘ 47.60“ N 18° 4‘ 52.86“ E).

Description: Soil pit located on right side of the artificially excavated channel connecting the two dead branches of Nitra River (Ľudovítová III. and II.). In the past this area was flooded every year. Last 10 year the area is without flood and this one is used for agriculture (planting of crops). There was planted winter wheat during sampling.

Morphology of soil profile: Eutric Fluvisol (Loamic, Humic)

0–21 cm (Akp1) slightly humid, colour: moist wet 10YR 3/3, moist-dry 10YR 6/2, oppressed, loam, crumb soil structure, aggregates of spherical shape, intensive root growth, CO_3^- 0.3–1%.

21–29 cm (Akp2) slightly humid, colour: moist wet 10YR 3/4, moist-dry 10YR 6/3, compacted, silty-loam, polyhedron, angular aggregates, weak root growth, CO_3^- 0.3–1%.

29–55 cm (Ck) slightly humid, colour: moist wet 10YR 4/3, moist-dry 10YR 6/3, oppressed, loam, granular soil aggregates, weak root growth, CO_3^- 1–3%.

55–71 cm (Ckg) moderately humid, colour: moist wet 10YR 4/3, moist-dry 10YR 6/3, loose, loam, weak root growth, granular soil aggregates, weak root growth, rust stains after Fe^{3+} on granular aggregates, CO_3^- 1–3%.

>71 cm (Ckgr) moderately humid, colour: moist wet 10YR 4/2, moist-dry 10YR 6/2, loose, loam, granular soil aggregates, rust stains after Fe^{3+} with grey coats on granular aggregates, CO_3^- 1–3%.

Profile 3

Localization: On the right side of actual river bed Nitra (48° 23' 35.09" N 18° 4' 49.00" E).

Description: The soil pit was located 20 m from the artificially excavated channel connecting the two dead branches of Nitra River (Ľudovítová II. and I.). In the past, this area has been dealt with by common agricultural practice. In 1965 the Nitra River stream was modified and this part of the area was damaged by working mechanisms. In 1986 the area (field) was again intensively used for agricultural activities and monoculture maize cultivation until 2010. From 2010 neglected area. The area is covered with raid vegetation (sallow, poplar). The dominant plant species is casuarina.

Morphology of soil profile: Eutric Fluvisol (Loamic, Humic)

0–12 cm (Akp1) moderately humid, colour: moist wet 10YR 3/3, moist-dry 10YR 6/4, loose, loamy, crumb soil structure, intensive root growth, CO_3^- 1–3%.

12–24 cm (Akp2) moderately humid, colour: moist wet 10YR 3/3, moist-dry 10YR 6/4, oppressed, loamy, chestnuts soil structure, aggregates of spherical shape, intensive root growth, CO_3^- 1–3%.

24–37 cm (Ck) moderately humid, colour: moist wet 10YR 3/3, moist-dry 10YR 6/4, oppressed, loamy, polyhedron, angular aggregates, rust stains after Fe^{3+}, corridors after earthworms, CO_3^- 1–3%.

37–58 cm (Ckg) moderately humid, colour: moist wet 10YR 4/3, moist-dry 10YR 7/4, oppressed, silty-loam, polyhedron, angular aggregates, rust stains after Fe^{3+} with grey coats on aggregates, up to 1% of CO_3^- >58 cm (Ckgr) wet, colour: moist wet 10YR 4/3, moist-dry 10YR 7/4, oppressed, silty-clay-loam, prismatic, aggregate of columnar shape, rust stains after Fe^{3+}, corridors after earthworms, CO_3^- 1–3%, underground water at depth 70 cm.

Profile 4

Localization: On the right side of actual river bed Nitra (48° 23‘ 33.55“ N 18° 4‘ 46.53“ E).

Description: The soil pit was located 20 m from the artificially excavated channel connecting the two dead branches of Nitra River (Ľudovítová II. and I.). Profile 4 was located approx. 80 m by the profile 3. In the past, this area has been dealt with by common agricultural practice. In 1965 the Nitra River stream was modified and this part of the area was damaged by working mechanisms. After the mechanical regulation of the banks of the Nitra River, the area was enhanced by raid of plants/trees. The age of the oldest woods (willow and poplar) is estimated at about 55 years. The surface of the soil was not covered by any herbal communities. On the surface of the soil was a litter fall (leaves of trees in different degrees of decomposition).

Morphology of soil profile: Eutric Gleyic Fluvisol (Loamic, Humic)

0–8 cm (Aa) slightly humid, colour: moist wet 10YR 2/2, moist-dry 10YR 5/2, loose, loamy, crumb soil strucutre, aggregates of spherical shape, intensive root growth, CO_3^- <0.3%.

8–19 cm (Akp) moderately humid, colour: moist wet 10YR 2/3, moist-dry 10YR 6/2, oppressed, loamy, plate-shaped aggregates, coarse rock occurrence up to 20% in grain-size up to 1 cm, CO_3^- 1–3%.

19–27 cm (Ck) moderately humid, colour: moist wet 10YR 4/3, moist-dry 10YR 7/3, oppressed, silty-loam, plate-shaped aggregates, rust stains after Fe^{3+}, corridors after earthworms, CO_3^- 1–3%.

27–60 cm (Ckg) moderately humid, colour: moist wet 10YR 4/4, moist-dry 10YR 7/3, oppressed, loamy, rust stains after Fe^{3+} with grey coats on granular aggregates, CO_3^- 1–3%.

>60 cm (Ckgr) wet, colour: moist wet 10YR 4/4, moist-dry 10YR 7/3, oppressed, loamy, rust stains after Fe^{3+} with grey coats on granular aggregates, CO_3^- 1–3% underground water at depth 60 cm.

Profile 5

Localization: On the left side of actual river bed Nitra (48° 23‘ 14.98“ N 18° 4‘ 35.71“ E).

Description: Soil pit located the left side of actual river bed Nitra behind a hydropower plant. In past this area was used as arable and horticulture land originally. Upon completion of the hydroelectric power plant, this site was neglected. During sampling time, the area was covered with raid vegetation (poplar, elder, acacia). There were growing neglected apple trees as well.

Morphology of soil profile: Eutric Fluvisol (Loamic, Humic)

0–17 cm (Akp) slightly humid, colour: moist wet 10YR 3/3, moist-dry 10YR 5/2, loose, silty-loam, crumb soil structure, aggregates of spherical shape, intensive root growth, CO_3^- 1–3%.

17–24 cm (Ak/C) slightly humid, colour: moist wet 10YR 3/3, moist-dry 10YR 5/2, loose, silty-loam, crumb soil structure, aggregates of spherical shape, intensive root growth, CO_3^- 1–3%.

24–63 cm (Ck1) slightly humid, colour: moist wet 10YR 4/4, moist-dry 10YR 7/3, oppressed, silty-loam, granular aggregates, CO_3^- 3–5%.

>63 cm (Ck2) moderately humid, colour: moist wet 10YR 4/4, moist-dry 10YR 7/3, oppressed, loam, granular soil aggregates, CO_3^- 3–5%.

3.2 Particle size distribution and mineral composition

Based on visual evaluation of soil profiles there were not observed any clay coatings on aggregate surfaces which means that re-distribution of individual grain-size fractions is the consequence of the deposition of layers of different grain by fluvial activity of river. Laboratory analysis also showed that the particle-size distribution of the studied soil profiles was considerably different, so classification of the whole profile in terms of grain-size is not possible. In all Fluvisols an individual soil horizons were classified (Table 1). In A-horizons of Fluvisols, the soil texture varied among loamy (Profile 2, 3 and 4), silt loam (Profile 5 and 1) and silty-clay-loam (Profile 1), with the clay content ranging from 14.2% to 25.1%. The content of clay decreased with the depth almost in all soil profiles of Fluvisols (except Profile 3). In profile 1, clay content from Ckg and Ckgr horizons to the depth layers increased what was evident because of cohesive soil structure. In these soils, soil aggregates are not obvious but the soil is solid and plastic what is typical feature of hydromorphic soils (Zaujec et al., 2009). Overall, the fraction of silt was a predominant grain-sizes in all soil profiles of Fluvisols. There was not determined any decrease or increase along the river flow gradient for portion of particle-size distribution.

There was determined a mineral composition of Fluvisols at depth of 5–25 cm (Fig. 2). Quartz was a predominant phase in the studied soils. They also contained feldspars (albite and orthoclase) and micas (most likely muscovite). Moreover, chlorite and some other clay minerals which presence was corroborated by the occurrence of broad peak around 1.4 nm (e.g. samples 4 and 5) were found. Some samples contained trace contents of amphibole. Furthermore, carbonates (calcite and dolomite) were identified in all studied soils apart from sample 5. That sample did not contain calcite, but contains trace amounts of dolomite. In general, carbonates are not abundant constituents of the sample 5 and they can be overlooked on XRD patterns. The chemical analysis confirmed carbonates in profile 5 as well. As mentioned above the soil samples of Fluvisols contained from clay minerals mainly chlorite which is a phyllosilicates with

Table 1 Particle-size distribution of Fluvisols

Soil pit	Horizons	Depth (cm)	Sand	Silt	Clay	Texture Δ
			(%)			
Profile 1 Restored forest	Aka	0–5	19.92	54.97	25.11	silty-clayey-loamy
	Akp	5–20	16.91	60.35	22.74	silty-loam
	Ck	20–61	9.68	68.34	21.98	silty-loam
	Ckgr	61–72	32.12	53.47	14.41	silty-loam
Profile 2 Arable soil	Akp1	0–21	42.97	38.84	18.19	loamy
	Akp2	21–29	30.16	52.13	17.71	silty-loam
	Ck	29–55	43.91	41.28	14.81	loamy
	Ckg	55–71	46.67	40.49	12.84	loamy
	Ckgr	>71	41.06	48.10	10.84	loamy
Profile 3 Fallow soil	Akp1	0–12	48.05	37.26	14.69	loamy
	Akp2	12–24	40.08	39.35	20.57	loamy
	Ck	24–37	39.09	44.42	16.49	loamy
	Ckg	37–58	18.94	57.54	23.52	silty-loam
	Ckgr	>70	15.54	57.73	26.73	silty-clayey-loamy
Profile 4 Forest	Aa	0–8	43.37	37.53	19.10	loamy
	Akp	8–19	48.76	34.82	16.42	loamy
	Ck	19–27	28.47	53.05	18.48	silty-loam
	Ckg	27–60	47.98	39.11	12.91	loamy
Profile 5 Raid forest	Akp	0–17	25.16	56.55	18.29	silty-loam
	Ak/C	17–24	22.23	63.57	14.20	silty-loam
	Ck1	24–63	25.78	55.44	18.78	silty-loam
	Ck2	>63	40.12	49.31	10.57	loamy

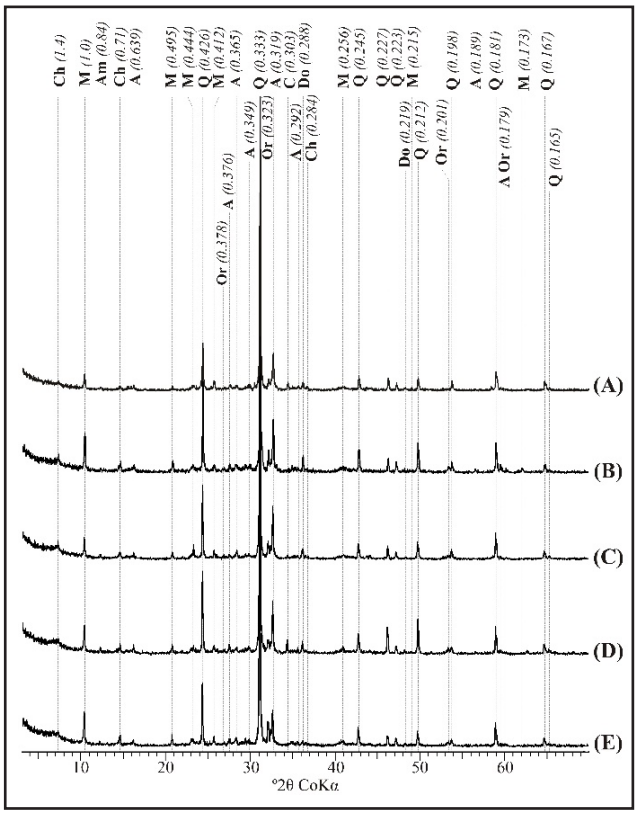


Figure 2 XRD patterns for the studied soil samples in layer 5–25 cm of studied Fluvisols
(A) sample 1, (B) sample 2, (C) sample 3, (D) sample 4, (E) sample 5. The d values (in brackets) in nm. Symbols of phases: A – albite, Am – amphibole, C – calcite, Ch – chlorite, Do – dolomite, M – muscovite, Or – orthoclase, Q – quartz

a 2 : 1 layer complex which instead of the interlayer cation contain another octahedral layer (Wiewióra and Weiss, 1990). With the increase of temperature and humidity, the 2 : 1 type clay mineral content gradually decreased and was transformed into 1 : 1 type clay mineral, and the active clay mineral was transformed into inactive clay mineral (Wu et al., 2016). In our case, nothing similar has been observed, which may be the consequence of both the fluvial activity of river, but also of the mild space on which the research was carried out. Within a small space, no significant temperature and humidity change is observed, which would have a significant effect on the intensive conversion of clay.

3.3 Physical and hydrophysical properties

The mineralogical composition is also related to the values of the particle density (ρs). The values of ρs were fairly equalized in all profiles of Fluvisols (Table 2). Smaller differences could be explained by river activity, which at different time periods created layers with different material and composition. For example, in Figure 3 it is easy to see the individual layers that were created by the fluvial activity of Nitra River (Profile 5). Lower values of the soil density were found in A-horizons in all profiles and this is due to the higher content of SOC (Table 4), since the values of this parameter depend mainly on the mineral and organic content of the soil (Zaujec et al., 2009). Based on mineral composition (Fig. 2) Fluvisols contained quartz, feldspars such as: albite and orthoclase and from micas mainly muscovite. As presented Scheffer and Schachtschabel (1970) ρs values of quartz, albite, orthoclase and muscovite are 2.65, 3.02–3.45, 2.55–2.63 and 2.77–2.88 t m^{-3}, respectively and results of soil organic carbon are mentioned in Table 4.

In soil profiles of Fluvisols, values of bulk density (ρd) ranged from 0.86 to 1.67 t m^{-3}. Lower values of ρd were identified in the upper layers and increased with the depth. Generally, the lowest values of ρd were determined in arable soil. In Fluvisols, an average values of ρd were higher by 14, 20, 28 and 37% under restored forest, fallow and raid forest, respectively compared to the arable soil. There wasn't found any decrease or increase of ρd along the river flow gradient. Significant factor which influence ρd is a particle-size distribution, soil structure and soil water content (Fulajtár, 2006; Mati et al., 2011). Generally, soils containing high percentage of clay are prone to compaction and higher values of bulk density (Polláková, 2012; Safadoust et al., 2014). The effects of human activity such as: soil tillage on changes of bulk density but other physical and hydrophysical properties use are evident also in Fluvisols (Kotorová, 2007; Kotorová and Šoltýsová, 2011; Kotorová, 2013). The highest values of total porosity (P) were determined in arable soil, while the lowest in raid forest. In A-horizons of Fluvisols under fallow, forest, restored forest, arable soil and raid forest the total porosity was slightly compacted, loose, slightly compacted, slightly compacted and compacted, respectively. In Ckg and Ckgr-horizons of all Fluvisols except profile 4 (under arable soil) the values of

Figure 3 Soil layer in C horizon of Fluvisol (soil profile FM 5)

Table 2 Physical properties of Fluvisols

Soil pit	Depth (cm)	BD	PD	Θ_{CA}	Θ_{30}	Θ_{MCWC}	Θ_{WRC}	P	Pn	Pc	Ps
		(g cm^{-3})		(%)							
Profile 1 Restored forest	0–10	0.90	2.49	38.4	37.2	36.2	35.1	64.0	26.9	35.1	2.0
	10–20	1.24	2.55	39.6	38.4	37.4	36.1	51.4	13.0	36.1	2.3
	20–30	1.37	2.60	38.9	38.2	37.5	36.4	47.2	9.1	36.4	1.8
	30–40	1.47	2.56	39.5	38.8	38.1	37.3	42.6	3.8	37.3	1.5
	40–50	1.27	2.61	39.2	37.2	35.6	33.7	51.5	14.3	33.7	3.5
Profile 2 Arable soil	0–10	0.97	2.56	42.2	40.4	38.2	35.1	62.3	21.9	35.1	11.6
	10–20	1.10	2.59	39.7	37.9	34.9	30.8	57.8	19.9	30.8	7.2
	20–30	1.04	2.61	41.7	38.5	34.4	28.8	60.4	21.9	28.8	9.8
	30–40	1.15	2.61	41.9	39.5	36.4	30.7	55.9	16.4	30.7	8.9
	40–50	1.25	2.62	42.7	41.2	39.2	33.8	52.4	11.2	33.8	7.4
Profile 3 Fallow soil	0–10	1.17	2.56	34.4	31.4	30.1	27.6	54.4	23.0	27.6	3.8
	10–20	1.41	2.60	36.8	34.7	33.6	31.5	45.9	11.2	31.5	3.2
	20–30	1.44	2.63	35.5	33.6	32.7	30.6	45.3	11.7	30.6	3.0
	30–40	1.49	2.38	36.7	35.6	34.7	33.2	37.2	1.6	33.2	2.3
	40–50	1.52	2.60	39.9	39.3	39.0	38.1	41.7	2.4	38.1	1.2
Profile 4 Forest	0–10	0.86	2.52	52.2	49.6	47.7	44.6	65.8	16.2	44.6	5.0
	10–20	1.37	2.61	38.1	35.4	33.9	31.8	47.3	12.0	31.8	3.6
	20–30	1.53	2.65	35.3	33.4	32.1	30.0	42.5	9.1	30.0	3.5
	30–40	1.52	2.64	37.0	35.1	34.1	32.3	42.6	7.4	32.3	2.9
	40–50	1.34	2.66	39.1	36.5	31.5	32.8	49.9	13.4	32.8	3.7
Profile 5 Raid forest	0–10	1.28	2.58	37.9	35.4	32.9	30.7	50.3	14.9	30.7	4.7
	10–20	1.39	2.61	38.3	36.3	34.4	32.5	46.9	10.6	32.5	3.9
	20–30	1.67	2.64	22.7	32.7	32.2	31.6	36.8	4.0	31.6	1.1
	30–40	1.62	2.64	33.9	33.3	32.8	32.2	38.7	5.4	32.2	1.1
	40–50	1.59	2.65	34.3	33.6	32.9	31.9	40.0	6.5	31.9	1.6

BD – bulk density, PD – particle density, Θ_{CA} – capillary absorption, Θ_{30} – 30 minute moisture, Θ_{MCWC} – maximum capillary water capacity, Θ_{WRC} – retention water capacity, P – total porosity, Pn – non-capillary porosity, Pc – capillary porosity, Ps – semi-capillary porosity

P signalled soil compaction mentioned layers. In profile 4 the values of P signalled slightly compaction according to criteria published by Kutílek (1966). Both the critical values of ρd and P according to Fulajtár (2006) for loamy soils (in most case our results) under fallow, forest, restored forest and raid forest were determined at the depths >30 cm, >20 cm, 30–40 cm and >20 cm, respectively. Volume of capillary, non-capillary and semi-capillary pores from total porosity represented on average 55–74.8, 19.5–31.7, and 5.84–7.94%, respectively. There wasn't found any decrease or increase along the river flow gradient for the individual categories of the soil pores. A highest values of capillary absorption (Θ_{CA}) were determined under arable soil, however, the water retention capacity (Θ_{WRC}) of this soil was the lowest. The highest ability to retain water in the soil for plants (maximum capillary water capacity – Θ_{MCWC}) was found under restored forest. Soil use of Fluvisols did not have statistically significant influence on maximum capillary water capacity and water retention capacity. Soil profiles were balanced also from the point of view of these water parameters (Θ_{WRC} and Θ_{MCWC}). As is mentioned in Kotorová (2007) in Gleyic Fluvisols these water parameters are rather affected by the water supply in the soil, heterogeneity of soil profile and content of clay particles than the soil cultivation. Opposite Polláková and Šimanský (2015) in Calcaric and Hortic Calcaric Fluvisols found the changes in soil hydrophysical properties due to different soil use and cultivation.

Significant feature of soil structure is their shape and size of soil aggregates (Shukla, 2014). For example, the

Figure 4 Shape and size of soil aggregates A) crumb structure, B) ploty structure, and C) polyedric (angular) structure

A-horizons of Chernozems have crumb structure and for Bt and Bn-horizons is typical prismatic and columnar structure, respectively. Eluvial horizons of Luvisols or Podzols have platy or laminated structure (Fulajtár, 2006). In our case, shape and size of soil aggregates were different. For example, under forest soil in A-horizon the shape of soil aggregates was spherical (Fig. 4a), while platy in Ck-horizon (Fig. 4b). Under the fallow in Ck-horizon the aggregates were angular (Fig. 4c) typical of the polyedric soil structure.

In laboratory, the contents of individual size fractions of water-stable aggregates (WSA) were determined (Table 3). Content of water-stable micro-aggregates (WSA_{mi}) ranged from 2.43 to 64.1% and covered the largest proportion whereas the size fraction water-stable macro-

Table 3 Soil structure parameters of Fluvisols

Soil pit	Horizons	Depth (cm)	Size fractions of water-stable macroaggregates in mm					WSA_{mi}	WSA_{ma}	MWDd	MWDw	Kv	Sw
			>5	5–3	3–1	1–0.5	0.5–0.25	(%)					
Profile 1 Restored forest	Aka	0–5	52.5	8.93	7.96	3.23	3.81	23.6	76.4	3.48	3.10	1.13	0.94
	Akp	5–20	24.6	11.1	4.88	4.52	6.56	48.4	51.6	3.61	1.70	2.13	0.61
	Ck	20–61	1.54	1.47	1.40	3.79	9.83	81.9	18.0	3.23	0.21	15.4	0.19
	Ckgr	61–72	0.28	1.16	0.98	1.04	8.35	88.2	11.0	2.37	0.10	25.1	0.13
Profile 2 Arable soil	Akp1	0–21	0.33	0.56	0.92	1.07	5.12	92.0	7.99	2.11	0.08	28.3	0.08
	Akp2	21–29	4.21	0.74	0.99	1.60	7.98	84.5	15.5	3.60	0.28	13.4	0.19
	Ck	29–55	0	0	0.29	0.62	3.64	95.5	4.55	3.20	0.02	156.1	0.01
	Ckg	55–71	0	0	0.46	0.93	11.2	87.4	12.6	2.61	0.05	62.8	0.16
	Ckgr	>71	2.08	6.53	6.53	7.65	11.2	70.7	29.3	2.52	0.27	10.8	0.48
Profile 3 Fallow soil	Akp1	0–12	1.01	1.15	4.29	6.36	13.1	74.1	25.9	3.81	0.17	22.7	0.38
	Akp2	12–24	0.62	0.87	3.21	4.93	11.2	79.2	20.8	3.50	0.18	20.9	0.29
	Ck	24–37	3.88	2.62	1.51	3.72	14.7	73.6	26.4	3.97	0.38	10.7	0.31
	Ckg	37–58	15.4	3.09	1.23	3.06	12.2	65.1	34.9	4.42	0.94	4.76	0.40
	Ckgr	>70	10.6	11.1	11.6	11.5	15.4	39.7	60.3	2.53	1.19	2.13	1.00
Profile 4 Forest	Aa	0–8	3.25	6.63	12.7	11.6	14.3	51.6	48.4	2.63	0.71	3.71	0.86
	Akp	8–19	2.27	2.15	3.75	4.53	9.18	78.1	21.9	2.96	0.32	9.27	0.27
	Ck	19–27	1.41	0.94	1.10	1.07	4.51	90.9	9.02	2.08	0.14	15.4	0.09
	Ckg	27–60	0.59	2.48	2.84	4.73	11.1	78.3	21.7	2.27	0.22	10.7	0.26
Profile 5 Raid forest	Akp	0–17	0.41	2.13	3.99	5.37	12.4	75.7	24.3	2.39	0.25	9.76	0.29
	Ak/C	17–24	0.60	1.07	2.14	2.61	7.24	86.4	13.7	2.52	0.14	18.5	0.15
	Ck1	24–63	0.70	0.68	1.84	1.44	1.75	93.6	6.40	0.68	0.11	7.11	0.05

WSA_{mi} – water-stable micro-aggregates, WSA_{ma} – water-stable macro-aggregates, MWDd – mean weight diameter of aggregates for dry sieving, MWDw – mean weight diameter of water stable aggregates, Kv – vulnerability coefficient, Sw – index of aggregate stability

aggregates (WSA_{ma}) 5–3 mm occupied the least. There wasn't determined any decrease or increase along the river flow gradient for portion of water-stable aggregates. The aggregate size distribution was significantly affected by soil use and one-way ANOVA analysis also showed the significant differences between soil horizons for contents of WSA. Land use change has an important influence on soil properties. For example, with changes in land use, soil micro-aggregates may form macro-aggregates through the action of temporary and transient binding agents (Elliott, 1986). Forestry influences soil organic matter (SOM), which in turn influences aggregation in comparison with conventional managed systems (Atsivor et al., 2001). Forest soil dynamics improves soil aggregation while transferring organic carbon into deeper soil horizons (Podrázský et al., 2009). Arable soil had the largest content of WSA_{mi} while that soil under restored forest had the lowest. On the other hand, the highest average content of WSA_{ma} was found under restored forest and then under forest > fallow > raid forest > arable soil. In A-horizons of Fluvisols the significant higher contents of WSA_{ma} and lower contents of WSA_{mi} compared to Ck or Ckgr horizons were observed. Contents of WSA_{mi} negative correlated with MWDw (r = -0.901; P <0.001) and aggregate stability (r = -0.968; P <0.001) but on the other hand positively correlated with vulnerability of soil structure (r = 0.488; P <0.05). Values of MWDw, Sw and Kv were not affected along the river flow gradient. ANOVA showed significant effects of land use and soil depth on soil structure parameters. The highest aggregate stability resulted the lowest vulnerability under forest soil. Opposite, in arable soil the lowest aggregate stability with the highest Kv values were determined. Based on MWDw, Kv and Sw values, better soil structure was observed in A than Ck, Ckg or Ckgr-horizons of Fluvisols. Our results confirmed that the soil structure is affected except soil intrinsic properties as well as by soil use and soil management (Bronick and Lal, 2005).

3.4 Content of soil organic carbon, sorptive parameters, soil pH and content of iron and its oxides

The content of Fe in the soil is conditioned mainly by primary abundance of parent materials. Progressively released as a result of weathering, metals from complexes with the remaining components of soils, particularly with the clay minerals and the organic matter, and are included into biological turnover (Jonczak et al., 2015). Contents of Fe and its oxides could be changed due to different soil management practices (Šimanský and Jonczak, 2017). The results of the Table 4 shows that the land use as well as soil depth displayed had significant influence on the total iron content (Fet) and its amorphous (Feo) and crystalline (Fec) oxides. The values of Fet was lower by 23%, 12%, 24% and 30% under forest, restored forest, arable soil and raid forest respectively than fallow soil. The lowest content of Feo was determined under raid forest while the highest under restored forest. The highest average content of Fec was found under restored forest > under forest = raid forest > arable soil > fallow soil. In all cases higher but no significant differences in Fe and its oxides were determined in Ck, Ckg or Ckgr-horizons compared to A-horizons.

The distribution of soil organic carbon, sorptive parameters and soil pH was not effected along the river flow gradient. There are significant differences for SBC, CEC and SOC in relation to the soil use as well as significant differences in soil pH, carbonates content, hydrolytic acidity, base saturation and SOC in relation to the soil depth. The average values of CEC was lower by 51%, 51%, 41% and 35% under forest, raid forest, arable soil and fallow soil respectively than restored forest. The sorptive complex was fully saturated in soil profiles of all investigated Fluvisols. It is known that the more intensively the soil is used the more intensively its properties are changed. An unsuitable change in land use due to human activities and agricultural management practices can affect the soil properties (Papini et al., 2011). Even differences in soil management practices can negatively as well as positively influence soil properties. In addition, tillage disrupts soil aggregates, compacts soil and disturbs plant and animal communities that contribute to aggregation and lowers SOM, CEC, nutrients, microbial activity and faunal activities that contribute to aggregation (Plante and McGill, 2002). The conversion of natural forests and grasslands to agricultural land also may cause important changes in soil physical and chemical properties, especially to reduce SOM (Haghighi et al., 2010). The SOC content was almost two and a half times and one and a half times lower under forest as well as restored forest than in arable soil. When compared to arable soil, the SOC content was higher by 69% and 73% under fallow soil and raid forest, respectively. Also, the mean SOC values were more than twice as high in A than Ck, Ckg and Ckgr-horizons.

3.5 Correlations between the chemical and physical properties in the soil profiles of Fluvisols

In Fluvisols, the soil pH in H_2O negatively affected water retention capacity and volume of capillary pores. Soil pH in KCl positively affected both particle and bulk densities, while it negative effected moisture states in Fluvisols. These effects depend on the carbonate and the soil organic contents, since positive correlations were observed between $CaCO_3$ and particle and bulk densities. On the other hand, negative correlation were determined between SOC and particle and bulk densities

Table 4 Soil pH, sorptive parameters, content of soil organic carbon content of iron and its oxides in soil profiles of Fluvisols

Soil pit	Horizons	Depth (cm)	pH_{H_2O}	pH_{KCl}	$CaCO_3$	Ha	SBC	CEC	Bs	SOC	Fet	Fed	Feo	Fed/Fet	Feo/Fed
					(%)	(mmol kg^{-1})			(%)						
Profile 1 Restored forest	Aka	0–5	7.42	7.18	1.2	5.15	82.9	88	94.1	3.67	2.89	0.67	0.19	0.23	0.29
	Akp	5–20	7.52	7.36	1.2	5.49	42.5	48	88.6	1.50	2.97	0.73	0.23	0.24	0.31
	Ck	20–61	7.81	7.44	1.6	2.49	101.5	104	97.6	0.77	3.06	0.81	0.23	0.26	0.29
	Ckgr	61–72	7.83	7.59	3.0	2.66	77.3	80	96.7	0.46	2.32	0.57	0.22	0.25	0.39
Profile 2 Arable soil	Akp1	0–21	7.57	7.24	1.8	3.82	36.2	40	90.4	1.26	2.49	0.59	0.19	0.24	0.33
	Akp2	21–29	7.73	7.36	1.2	2.49	85.5	88	97.7	0.82	2.62	0.59	0.18	0.23	0.31
	Ck	29–55	7.94	7.57	1.8	2.49	45.5	48	94.8	0.50	2.52	0.59	0.19	0.24	0.32
	Ckg	55–71	7.87	7.50	1.4	2.49	25.5	28	91.1	0.41	2.53	0.52	0.17	0.21	0.32
	Ckgr	>71	7.87	7.54	1.8	2.99	29.0	32	90.7	0.32	2.09	0.51	0.17	0.24	0.33
Profile 3 Fallow soil	Akp1	0–12	7.75	7.58	1.8	4.66	35.4	40	88.4	1.36	2.40	0.48	0.16	0.20	0.33
	Akp2	12–24	7.77	7.54	2.3	2.49	13.5	16	84.4	1.09	3.06	0.56	0.17	0.18	0.31
	Ck	24–37	7.82	7.59	2.0	4.16	43.8	48	91.3	0.93	3.15	0.50	0.16	0.16	0.32
	Ckg	37–58	7.73	7.42	2.0	4.16	59.8	64	93.5	0.93	3.55	0.72	0.23	0.20	0.31
	Ckgr	>70	7.65	7.26	1.8	3.66	88.3	92	96.0	0.93	3.87	0.92	0.23	0.24	0.25
Profile 4 Forest	Aa	0–8	7.22	7.19	1.2	6.48	9.52	16	59.5	5.63	2.58	0.48	0.22	0.19	0.45
	Akp	8–19	7.43	7.34	1.7	3.99	12.0	16	75.1	1.61	2.31	0.47	0.21	0.20	0.45
	Ck	19–27	7.67	7.50	1.4	2.66	69.3	72	96.3	0.89	2.58	0.65	0.22	0.25	0.34
	Ckg	27–60	7.78	7.74	1.8	1.66	50.3	52	96.8	0.41	2.44	0.58	0.20	0.24	0.35
Profile 5 Raid forest	Akp	0–17	7.25	7.16	1.0	6.98	25.0	32	78.2	2.74	2.10	0.63	0.17	0.30	0.26
	Ak/C	17–24	7.62	7.32	1.1	3.66	52.3	56	93.5	1.12	2.42	0.65	0.17	0.27	0.26
	Ck1	24–63	7.87	7.59	2.8	3.32	24.7	28	88.1	0.42	2.34	0.61	0.14	0.26	0.23

Ha – hydrolytic acidity, SBC – sum of basic cations, CEC – cation exchange capacity, Bs – base saturation, SOC – soil organic carbon, Fet – total iron content, Fed – free iron oxides, amorphous iron oxides, Fec – crystalline iron oxides

Table 5 Correlation coefficient between the soil pH, sorptive parameters, content of the soil organic carbon, texture and parameters of soil structure in the soil profiles of Fluvisols

	BD	PD	Θ	Θ_{KN}	Θ_{30}	Θ_{MCWC}	Θ_{WRC}	P	Pn	Pc	Ps
pH_{H_2O}	ns.	ns.	ns.	ns.	ns.	ns.	-0.495*	ns.	ns.	-0.495*	ns.
pHKCl	0.465*	0.503*	ns.	-0.429*	-0.472*	-0.540	-0.500*	ns.	ns.	-0.500*	ns.
$CaCO_3$	0.507*	ns.	ns.	-0.489*	ns.	ns.	ns.	-0.486*	ns.	ns.	ns.
H	ns.	-0,508*	ns.	ns.	ns.	ns.	ns.	ns.	ns.	ns.	ns.
SBC	ns.	ns.	ns.	ns.	ns.	ns.	ns.	ns.	ns.	ns.	ns.
CEC	ns.	ns.	ns.	ns.	ns.	ns.	ns.	ns.	ns.	ns.	ns.
Bs	ns.	ns.	ns.	ns.	-0.433*	-0.465*	ns.	ns.	ns.	ns.	ns.
SOC	-0.580**	-0.470*	ns.	0.495*	0.537**	0.580**	0.603**	0.536**	ns.	0.603*	ns.
Sand	ns.	ns.	ns.	ns.	ns.	ns.	ns.	ns.	ns.	ns.	0.530*
Silt	ns.	ns.	ns.	ns.	ns.	ns.	ns.	ns.	ns.	ns.	-0.460*
Clay	ns.	-0.552**	0.463*	ns.	ns.	ns.	0.429*	ns.	ns.	0.429*	-0.460*

H – hydrolytic acidity, SBC – sum of basic cations, CEC – cation exchange capacity, Bs – base saturation, SOC – soil organic carbon, BD – bulk density, PD – particle density, Θ_{CA} – capillary absorption, Θ_{30} – 30 minute moisture, Θ_{MCWC} – maximum capillary water capacity, Θ_{WRC} – water retention capacity, P – total porosity, Pn – non-capillary porosity, Pc – capillary porosity, Ps – semi-capillary porosity

of Fluvisols. SOC positively correlated with the moisture characteristics (capillary absorption, maximum capillary water capacity, water retention capacity) and total and capillary porosities. A decrease in SOC leads to increased bulk density and decreased porosity, thus reducing soil infiltration (Li et al., 2007). Water retention in Fluvisols depends on content of clay (Kotorová, 2007) which is in agreement with our results in Table 5. Higher clay content resulted in higher actual water content, water retention capacity and volume of capillary pores.

Soil pH effected aggregation through clay dispergation. The negative surface charge on clay particles increases with pH increase particle repulsion. Clay particles often flocculate at high pH values (Haynes and Naidu, 1998) and large aggregates form in the soils with high pH and high carbonate concentration (Boix-Fayos et al., 2001). The significant negative correlations were found between soil pH and WSA_{ma} >0.5 mm in our studied profiles of Fluvisols. In contrast, higher pH resulted in higher content of WSA. This means that with increase of soil pH is also increased the content of WSA_{mi} but on the other side content of WSA_{ma} is decreased. This could be connected with the content of carbonates in case of our Fluvisol. Carbonates were determined in the whole soil profiles of Fluvisols (except Profile 5) as it is shown in Figure 2. Carbonates positively correlated with WSA_{mi} but on the other hand negative correlation was observed between the carbonates and WSA_{ma} (Table 6). The effect of carbonates on the soil structure could be modified by soil organic carbon. Increase of SOC thereby accelerating formation of secondary carbonates. If soil contains low SOC the macroaggregate stability is enhanced by carbonates (Boix-Fayos et al., 2001), which is confirmed by negative correlation between SOC and WSA_{mi} and by positive correlation between SOC and WSA_{ma} (mainly WSA_{ma} >0.5 mm). The most important factor responsible for stabilization of WSA_{ma} in profile of Fluvisols was the SOC due to significant correlation between SOC and Sw. SOC is a key factor effected aggregate stability (Šimanský and Jonczak, 2016). A significant positive correlation was determined between SOC and MWDw (Table 6). Higher values of hydrolytic acidity resulted in higher content of WSA_{mi} and lower contents of WSA_{ma} 0.5–3 mm. Values of sum of base cations and CEC had significant effects on aggregation in profiles of Fluvisols despite the fact that CEC is one of the most important factor responsible for aggregate stability (Dimoyiannis et al., 1998). The results of Šimanský et al. (2014) also showed the fact that more intensive aggregation process in loamy soils is connected with the high content of basic exchangeable cations, and the high value of CEC and soil organic carbon content in WSA. Aggregation is stimulated by the interaction of polycationic bridging in which the repulsive forces between negatively charged clay and/or SOC are reduced. Aggregates containing polyvalent cations (Ca^{2+}, Al^{3+} and Fe^{3+}) are resistant to slaking (Tisdall, 1996). In profiles of Fluvisols, an increase of base saturation resulted in an increase of WSA_{mi} and opposite in a decrease of WSA_{ma}. Aggregation is controlled by different mechanisms in different soil types. The rate and stability of aggregation generally increases with SOC and clay surface area and CEC. In soils low in SOC or clay concentration, aggregation

Table 6 Correlation coefficient between the soil pH, sorptive parameters, content of soil organic carbon, texture and parameters of soil structure in soil profiles of Fluvisols

	Size fractions of water-stable macroaggregates					WSA_{mi}	WSA_{ma}	MWDd	MWDw	Kv	Sw
	>5	5–3	3–1	1–0.5	0.5–0.25						
pH_{H_2O}	-0.445*	-0.712***	-0.691***	-0.623**	ns.	0.721***	-0.721***	ns.	-0.569**	0.500*	-0.740***
pHKCl	-0.486*	-0.510*	-0.410	-0.366	ns.	0.620**	-0.620**	ns.	-0.551**	ns.	-0.577**
$CaCO_3$	ns.	ns.	ns.	ns.	ns.	0.451*	-0.451*	ns.	ns.	ns.	ns.
H	ns.	0.708***	0.560**	0.539**	ns.	-0.638**	0.638**	ns.	0.510*	ns.	0.633**
SBC	ns.	ns.	ns.	ns.	ns.	ns.	ns.	ns.	ns.	ns.	ns.
CEC	ns.	ns.	ns.	ns.	ns.	ns.	ns.	ns.	ns.	ns.	ns.
Bs	ns.	-0.554**	-0.728***	-0.764***	-0.484*	0.462*	-0.462*	ns.	ns.	ns.	-0.615**
SOC	0.521*	0.759***	0.728***	0.628**	ns.	-0.776***	0.776***	0.063	0.635**	ns.	0.806***
sand	ns.	ns.	ns.	ns.	ns.	ns.	ns.	ns.	ns.	ns.	ns.
silt	ns.	ns.	ns.	ns.	ns.	ns.	ns.	ns.	ns.	ns.	ns.
clay	0.602**	0.443*	ns.	ns.	ns.	-0.610**	0.610**	0.758***	0.607**	-0.440*	0.489*

H – hydrolytic acidity, SBC – sum of basic cations, CEC – cation exchange capacity, Bs – base saturation, SOC – soil organic carbon, WSA_{mi} – water-stable micro-aggregates, WSA_{ma} – water-stable macro-aggregates, MWDd – mean weight diameter of aggregates for dry sieving, MWDw – mean weight diameter of water stable aggregates, Kv – vulnerability coefficient, Sw – index of aggregate stability

may be dominated by cations, while the role of cations in aggregation may be minimal in soils with high SOC or clay concentration (Boix-Fayos et al., 2001). Clay content positive correlated with content of WSA_{ma} >3 mm but on the other side it negative correlated with WSA_{mi} in profiles of Fluvisols. A significant correlation were determined between clay and MWD, K and Sw. Higher clay content resulted in lower vulnerability of soil structure in profiles of Fluvisols. Silt and sand particles did not have significant effects on the soil structure parameters. Generally, soil texture has a significant influence on aggregation. In coarse-textured soils, the SOC has a greater influence on structure; while with increasing clay content the type of clay is more important than the amount in determining aggregation (Kay, 1998).

4 Conclusions

The investigated Fluvisols had different chemical and physical properties, but not as a consequence of the flow gradient along the river. Apparently, to have the flow gradient along the river observed, more data are needed to be available from the greater river flow. Differences in chemistry and physical properties of Fluvisols developed along the Nitra River have been significantly affected mainly by the use, soil management practices and depth of the soil profile. The relationships between the soil properties were also different in Fluvisols. The bulk density and hydrophysical characteristics of Fluvisols were effected in decreasing order of significance by SOC > pHKCl > clay content > $CaCO_3$. SBC and CEC did not have any significant effects on bulk density and hydrophysical properties of Fluvisols. Soil structure of Fluvisols was effected in decreasing order of significance by clay content = soil pH_{H_2O} > SOC > Bs = Ha = pHKCl > $CaCO_3$. SBC, CEC and silt content did not have any significant effects on the soil structure of Fluvisols.

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Original Paper

Zea mays L. hybrids kernels evaluated by image analysis tools

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The aim of this study was to distinguishing between kernels of maize hybrids by the use of image analysis tools. We analyzed 10 registered *Zea mays* L. hybrids (5 – dent, 2 – semi-flint to flint, and 3 – semi-flint to dent type). Different parameters on ventral, dorsal, corolla side, and lateral side cross section of kernel were measured. Sample per each hybrid comprised 50 maize kernels. Acquired bio-images were processed by software Zeiss AxioVision Rel. 4.8. We analyzed the segmented regions of interest on the kernels. The data for area (mm^2), height and width (mm) were gathered from these regions. The hybrid ZE EDOX significantly differed (p <0.05) from all other hybrids almost in all traits. It is the hybrid with the smallest area of the whole kernel, floury endosperm proportion, and depressed part on corolla. The new trait the area of the depressed part on the kernel corolla was measured. The hybrids with smaller proportion of floury endosperm had smaller area of depressed part, and vice versa. The image analysis methods can usefully contribute to selection of proper hybrids for different types of use.

Keywords: maize, *Zea mays* L., kernel, image analysis

1 Introduction

The maize (*Zea mays* L.) is one of the most important dietary staple food globally. Many different hybrids are producing by breeding and they are for different use. Appropriate type of use strongly depends on kernel characters, like size, shape, and color. The amount of different substances correlates with these bio-morphological kernel traits (Guelpa et al., 2015). The chemical content depends on convariety (group). They differ in content of starch, proteins, lipids, sugars, and other substances (Janda and Michalec, 1982).

The important way of maize kernel use is maize meal producing by dry-milling after de-germing (the germ and pericarp are removed). The quality of maize meal also depends on the amount of two types endosperm. The vitreous one is harder, of higher density, translucent and placed on outside part of kernel. The floury endosperm on the contrary is softer, of less density, and placed in the center of kernel (Watson, 1987). There were many ways of analyzing maize kernel hardness which mainly depends on the content of floury and vitreous endosperm (Fox and Manley, 2009). The macro imaging of morphological and anatomical parts of maize kernel can be also fast method to determine it.

Image analysis methods are successfully used in agriculture research. The number of papers about application of these methods is increasing (Glasbey and Horgan, 2001; Dell´Aquila, 2006; Rodríguez-Pulido et al., 2012; Wiwart et al., 2012; Blaschke et al., 2014). The advantage is that the numerous amount of numerical data can be extracted from acquired images. The color, size, shape characteristics of plant products can be quickly processed and then statistically elaborated.

The objective of this study was to use the methods of image analysis for distinguishing between kernels of maize hybrids. Different parameters on maize kernels were measured. This method can contribute to quality characterization of maize kernels utilized for different purposes.

2 Materials and methods

The experimental set consists of 10 registered maize hybrids (*Zea mays* L.) which were cultivated under the equal climatic and vegetation conditions of field experiment, with the uniform agrotechnics. The characterization of hybrids is documented in table 1. The hybrids were provided by company ZELSEED spol. s r.o Horná Potôň.

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Table 1 List of evaluated maize hybrids

Hybrid	Registration	FAO	Type of maize kernel	Color of kernel corolla	Use
ZE ADULAR	2009	350	dent	yellow	kernel
ZE EDOX	2008	280	flint to semi-flint	yellow	kernel and ensilage
ZE ELMO	2013	380	dent	yellowish-white	kernel
ZE HILDA	2015	350	semi-flint to dent	yellow	kernel and ensilage
ZE KARUZEL	2014	420	semi-flint to dent	yellow	kernel and ensilage
ZE OTIS	2010	300	dent	yellow	kernel
ZE ZEAMAX	2015	420	semi-flint to dent	yellow	kernel and ensilage
ZE ZELMA	2014	410	flint to semi-flint	yellow	kernel
ZE ZELSTAR	2015	330	dent	yellow	kernel and ensilage
ZE 4501	2005	450	dent	yellow	kernel and ensilage

The images processing and analyzing is always done in steps. The workflows how to automatically analyze bio-images have to be prepared (Uchida, 2013). In our experiment the image analysis was realized in four steps (Fig. 1). The first step was images acquiring. The kernels were imaged by fully automated macroscope Zeiss SteReo Discovery V20 with digital Camera AxioCam MRC5. For imaging were randomly selected 50 kernels for each hybrid. The sample images were prepared from whole kernels and cross-sections (Fig. 2). There were acquired 250 images for each hybrid. The kernels before cutting were placed to distilled water for 24 hours.

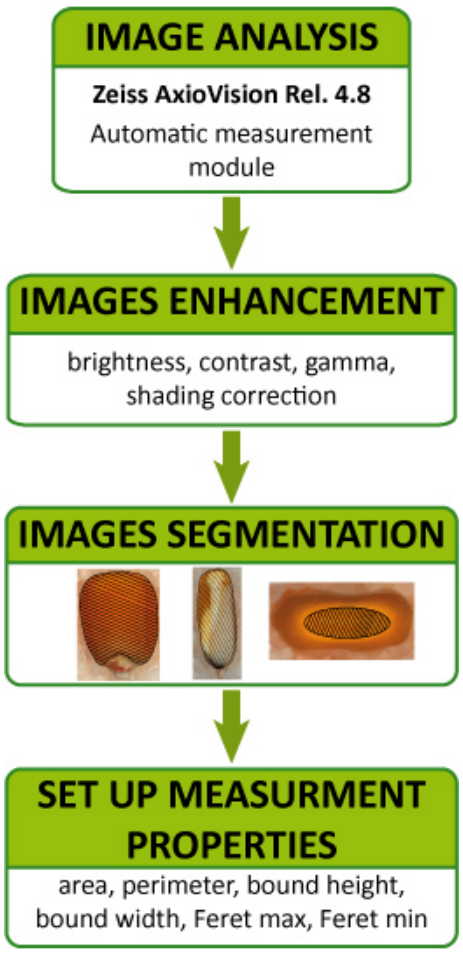

Figure 1 The workflow for image analysis of maize kernels

The images were then processed by software for image analysis Zeiss AxioVision Rel. 4.8 with module for automatic measurement. We used measurement program wizard. The images enhancement was the second step of analysis (Fig. 1). It was important to pre-process the images by several tools. We used brightness, contrast, gamma, shading correction.

The other essential step was the images segmentation. We segmented the objects of interest in the image. In some cases the images had to be processed to binary mode and then the concerning section was segmented.

The last step of image analysis was quantification of recognized regions of interest. The parameters of measurement were set up (Fig. 1). We measured the different parts of maize kernels in images. It depended on the kernel orientation. Figures (3 – 6) are documenting the measured regions.

The automatic measurement module was used for quantification of images with kernels oriented on the ventral side and lateral section (Fig. 3, 6). The concerning parts were automatically selected in images which were in binary mode.

All other images were not measured by automatic measurement module. In these cases, we used measuring tools like distance or outline.

For statistical analysis was used software SAS 9.3 Enterprise guide 5.1. Obtained data were subjected to basic statistics and parametric test (ANOVA). We used Ryan-Einot-Gabriel-Welsch Multiple Range Test for contrasts testing. The images used for article publication

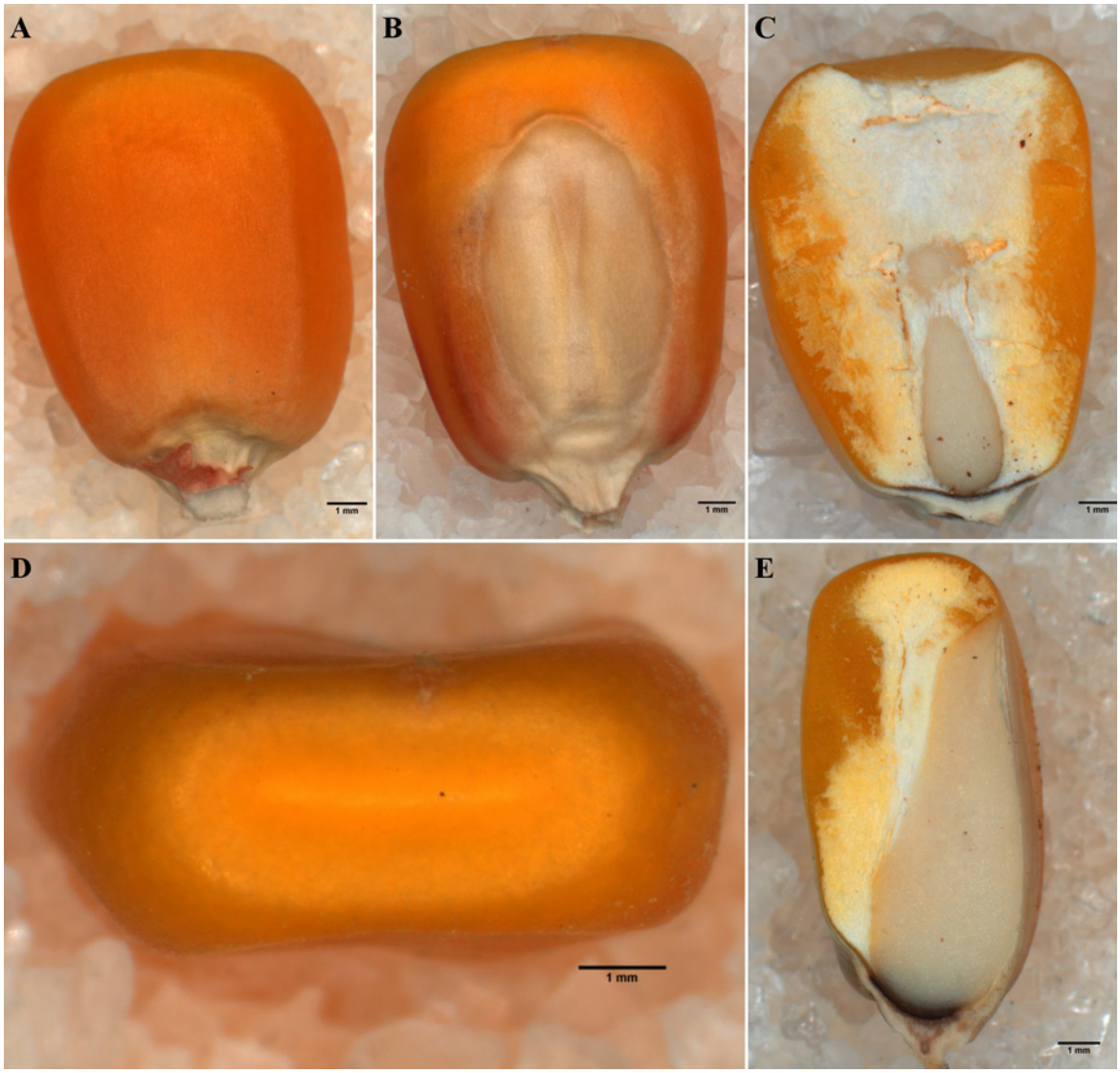

Figure 2 The images acquired from maize kernels: A – the ventral side of kernel; B – the dorsal side of kernel; C – the front side section; D – the corolla of kernel; E – the lateral side section

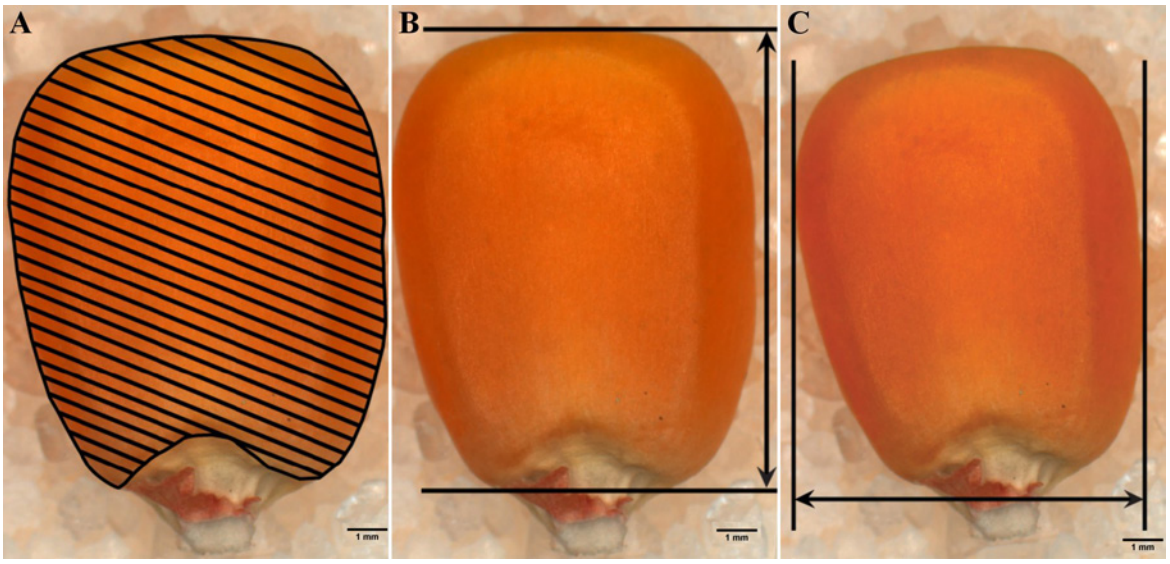

Figure 3 Parameters measured on the ventral side of kernel: A – area (mm^2), B – height (mm), C – width (mm)

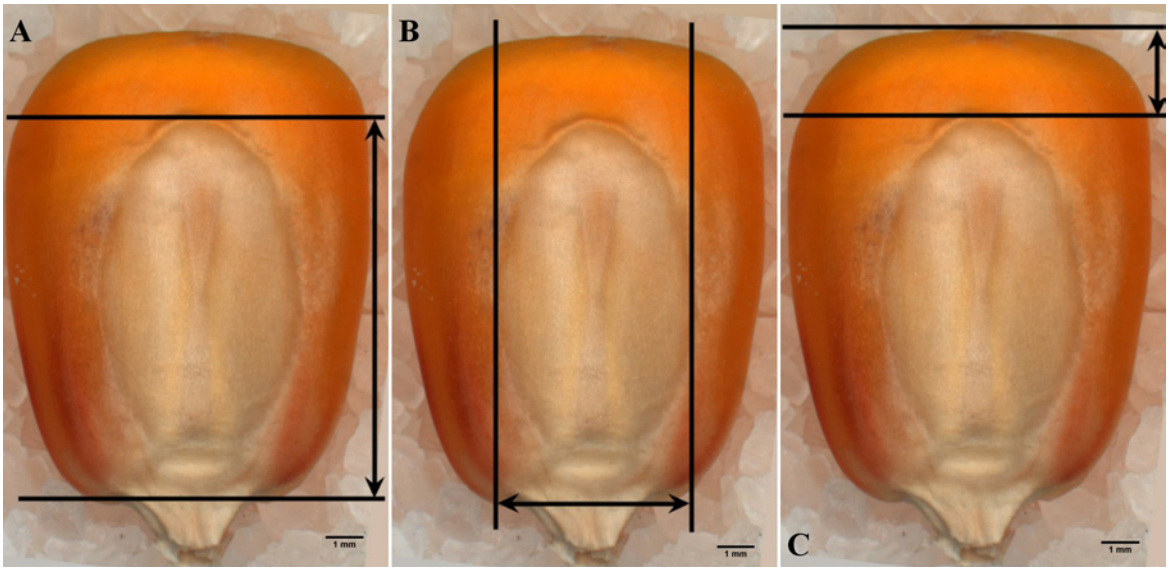

Figure 4 Parameters measured on the dorsal side of kernel by tool distance: A – length of embryo (mm), B – width of embryo (mm), C – the subtraction of embryo length from kernel length (mm)

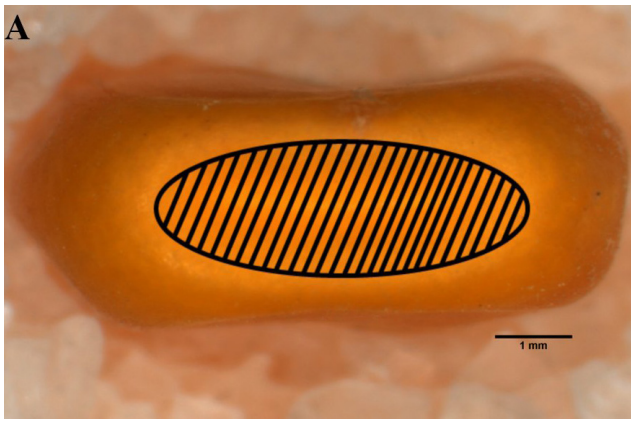

Figure 5 The area measured on the depressed part of corolla (mm^2)

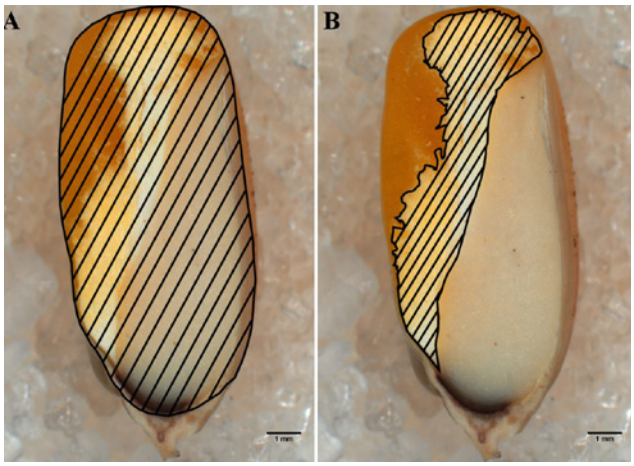

Figure 6 Parameters measured on the kernel lateral section: A – area of cross-section (mm^2), B – area of floury endosperm (mm^2)

were elaborated by open source software ImageJ and FigureJ (Schindelin et al., 2015; Mutterer and Zinck, 2013).

3 Results and discussion

In the article are presented the results obtained by measuring the traits on ventral and dorsal side of kernel, kernel corolla, and area of floury endosperm on kernel lateral section.

From the ventral side of kernel, we displayed the results of the following traits – area (mm^2), kernel width – measured by tool Bound height and kernel length – measured by tool Bound width (mm). Coefficient of variation was less than 10% for each trait (n = 50). It means that the maize kernels of each hybrid were even in evaluated traits.

The figure 7 illustrates the results gathered by measuring the area of the whole kernel (mm^2). The data show that the average values of area ranged from 69.67 mm^2 (ZE EDOX) to 89.06 mm^2 (ZE ELMO). The hybrid ZE ELMO and ZE KARUZEL differed significantly (P <0.05) from all others hybrids.

The second chart (Fig. 8) demonstrates the width of maize kernel which was measured by tool Bound height (mm). The data show that the widest average values reached the hybrids – ZE ELMO (9.14 mm), ZE KARUZEL (9.15 mm), ZE ZELMA (8.93 mm), and ZE EDOX (8.87 mm). The hybrid ZE 4501 reached the smallest value (8.12 mm).

The chart displayed in the figure 9 shows the results obtained by measuring the length of maize kernel. It is evident that the hybrid ZE EDOX (10.15 mm) significantly differs from other hybrids. Hybrid ZE ELMO reached the highest average value (11.97 mm) and also is significantly different from all other samples.

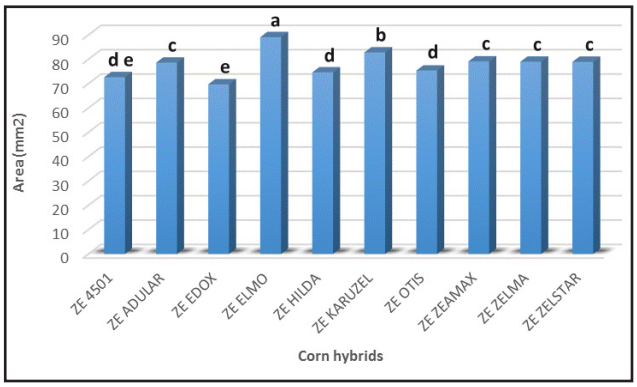

Figure 7 The area of kernel from ventral side (mm²)
Legend: $n = 50$; different letters (a, b, c, d, e, f) point out significant difference ($p < 0.05$)

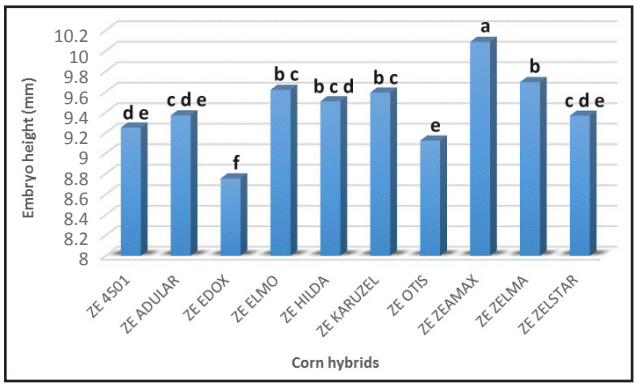

Figure 10 The length of embryo on dorsal side of kernel (mm)
The legend is the same as in Figure 7

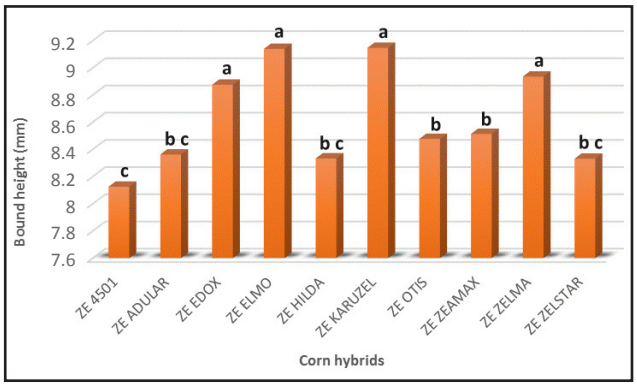

Figure 8 The width of kernel measured by Bound height (mm)
The legend is the same as in Figure 7

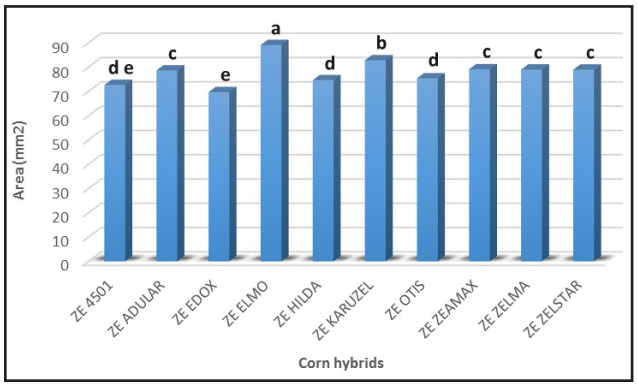

Figure 11 The area of depressed part on corolla (mm²)
The legend is the same as in Figure 7

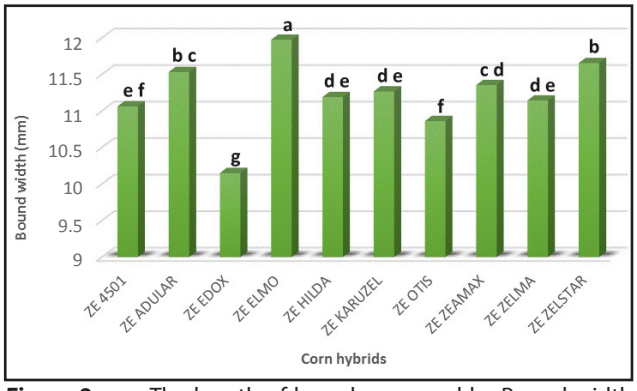

Figure 9 The length of kernel measured by Bound width (mm)
The legend is the same as in Figure 7

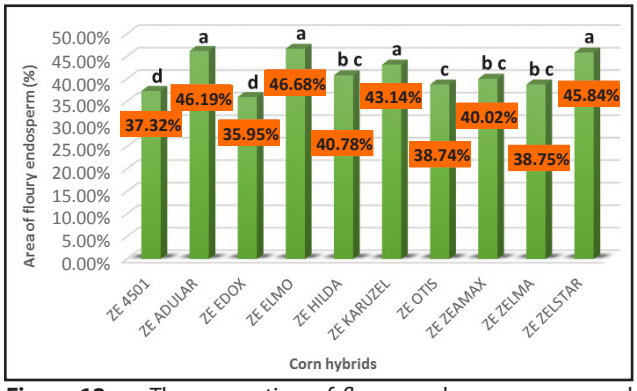

Figure 12 The proportion of floury endosperm measured on kernel lateral section (%)
The legend is the same as in Figure 7

On the dorsal side of kernel, we measured the embryo length (mm) and the results are documented in the Fig. 10. The smallest average value obtained hybrid ZE EDOX (8.75 mm) which is again significantly different from other hybrids. The highest average value had ZE ZEAMAX (10.09 mm), also differs from all other hybrids.

Another interesting morphological trait, which was measured, was the size of depressed part on kernel corolla. We assumed that the larger size of this area will cause the higher proportion of floury endosperm. This assumption was built on information that depressed part of corolla is the result of cracks in floury endosperm (Robutti et al., 1974; Erasmus, 2003, Guelpa et al., 2015), so the larger depressed part means the more cracks and

higher proportion of floury endosperm. The cracks can be the result of the endosperm dehydration (De Carvalho et al., 1999; Guelpa et al., 2015). So we expected that there will be significant differences between different types of maize hybrids.

The smallest depressed area of corolla was measured on the hybrid ZE EDOX (8.11 mm^2) significantly different from all other hybrids. The largest area was reached by hybrid ZE KARUZEL (23.98 mm^2) also significantly different from other hybrids (Fig. 11).

According to literature sources we know that the floury endosperm is placed into the center of the kernel (De Carvalho et al., 1999; Guelpa et al., 2015). Due to this we measured the area of the floury endosperm on the kernel lateral section. The results displayed in the Fig. 12 show that the smallest area was reached by hybrid ZE EDOX (18.6 mm^2 – 35.95%), which was together with ZE 4501 (18.04 mm^2 – 37.32%) significantly different from other hybrids.

4 Conclusions

From the mentioned results we can conclude the following findings:

- According to obtained results we can confirm that the chosen parameters evaluated on kernels by the use of image analyses software, can be good tools for identifying the maize hybrids. The hybrid ZE ELMO differed from all other hybrids almost in all cases. The hybrids ZE ADULAR, ZE ZELSTAR were significantly different only in one trait.
- The coefficient of variation was less than 10% in the cases of traits measured on ventral side of kernel, and on embryo. But the traits measured on depressed part of corolla and on floury endosperm reached higher than 20% the values of coefficient of variation. In this case the tools for image segmentation should be improved.
- The hybrid ZE ELMO (dent type, kernel use hybrid) had the largest kernels (trait area), the second largest the area of depressed part on corolla, and also the highest proportion of floury endosperm. On the other hand, the hybrid ZE EDOX (flint to semi-flint, kernel and ensilage use hybrid) had the smallest kernels (trait area), the smallest area of depressed part on corolla, and also the smallest proportion of floury endosperm. This can prove our assumption that the higher proportion of floury endosperm in the kernels can cause larger area of depressed part on corolla, and as a consequence to this more cracks in the floury endosperm.
- In this experiment the new trait – area of depressed part on corolla was used. We suppose that this trait has connection with the proportion of floury endosperm in the maize kernel. The larger area of this part of corolla can cause the larger proportion of floury endosperm. This statement was confirmed by hybrids ZE ELMO, ZE KARUZEL, ZE ADULAR, and also by hybrids ZE EDOX, ZE 4501 (small area of depressed part, small proportion of floury endosperm). This trait can be used non-destructive way.
- The image analysis method for evaluation of size and different parts of maize kernel can be used for determination of kernel types, and proportion of vitreous and floury endosperm. This is proved by statistically significant differences between examined hybrids.

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