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Agronomic evaluation of different lettuce (*Lactuca sativa* L.) varieties under unheated plastic tunnel

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Summary: Among the leafy vegetables, lettuce (*Lactuca sativa* L.) is the most widely grown species in the world. Wide ranges of physical properties of the products are used to assess the degree of maturity. In this study, different lettuce varieties were grown under an unheated plastic tunnel in springtime. We evaluated the agronomic properties of different lettuce varieties, evaluated the condition of the plants, and determined the bioactive substances. Larger head weight can be achieved by the 'King of May' butterhead (259.31 g) and 'Great Lakes 659'crisphead (220.40 g) genotypes. A very strong correlation (r = 0.995) was observed between the lettuce head index, and leaf index and both had a strong positive correlation (r = 0.828 and 0.760) with NDVI. The highest SPAD values were measured for cos lettuce 'Romaine lettuce' (44.01) and iceberg lettuce 'Great Lakes 659' (42.71). However, these genotypes showed the highest (9.52%; 8.74%) dry matter content, too. The red leaf variety 'Lollo Rosso' had the highest total polyphenol content (181.53 GAE/100 g FW). Among the evaluated properties, iceberg lettuce showed favorable morphology, plant condition, and good dry matter content. In addition, between the loose-leaf lettuces, the red leaf lollo type was outstanding with bioactive content.

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Key words: lettuce, NDVI, SPAD, morphology, total phenol content, dry matter content

Introduction

Among the leafy vegetables, lettuce (*Lactuca sativa* L.) is one of the most popular species in the world. According to the FAO (2018) data, the lettuce and chicory production was 27 million tons, and the harvested area was 1.3 million ha in the world. Lettuce is grown on all continents, but the greatest consumers and producers are in China (14 million tons) and the USA (4 million tons). In Europe, lettuce production was 2.9 million tons, and the harvested area was 123 thousand ha. Lettuce consumption contributes provitamin A, vitamin C, vitamin E, carotenoids, and fiber to consumers' diet (Agüero et al., 2008). So, the importance of consuming vegetables is due to the many nutrients they contain and the different bioactive substances. They include but are not limited to, phytochemicals (carotenoids, phenols, and flavonoids), minerals (calcium, magnesium, and potassium), vitamins (vitamin C, folic acid, and provitamin A), and dietary fiber (Oz & Kafkas, 2017; Liu, 2013).

In lettuce genus has more than 100 species and 6 types. Different types of lettuce are the following – butterhead, crisphead (iceberg), romaine (cos), stem (asparagus), leaf (cutting), and oilseed lettuce (Mousavi et al., 2013; Křístková et al., 2008).

Maturity of harvest is the stage of development in which a plant or plant part possesses the conditions for utilization by consumers for a proper purpose. Wide ranges of physical properties of products are used to assess the degree of maturity – changes in diameter, head length, head width, colour, firmness, compactness and surface. These parameters are used as general ripening indices (Barg et al., 2009). The compactness of the head, which can be compressed with moderate hand pressure is considered optimal maturity. Depending on the destination, different ripeness indicators can be used. These different purposes may be needed for the fresh-cut processing sector.

Head weight is an important factor for quality evaluation of the raw material for the fresh-cut produce industry. Furthermore, the ripe leaves and petiole length are great maturity indicators to ensure the quality of the processed product (Gil et al., 2012; Jenni & Yan, 2009).

In lettuce cultivation, it appears plant spacing is an important criterion for achieving maximum vegetative growth, thereby maximizing yield. In addition, optimum plant spacing helps to increase the number of healthy leaves and foliage (Hasan et al., 2017; Maboko & Du Plooy, 2009; Zemichael et al., 2017).

In recent years, the market for fresh-cut, minimally prepared or "ready to eat" leafy vegetables have been continuously increasing. Consumer acceptance is associated with leafy vegetables' visual characteristics such as shape, colour, size and freshness, and texture and flavour (Ferrante et al., 2004; Nicola & Fontana, 2014).

The Green Seeker Model 505 measuring device specifies the NDVI value from which we can conclude the crop cover of the field and the vegetation distribution. The NDVI is computed as (NIR - Red)/(NIR + Red) where NIR and Red are the sum of near-infrared and red light, reflected by the surface. The green leaves have great visible light absorption together with great near-infrared reflectance, resulting in positive NDVI

values (Pettorelli et al., 2011). The vegetation index can help assess vegetation cover density, identify plants, and monitor plant growth (Zhang et al., 2019). In addition, the early detection of pest infestation by non-destructive methods is an important prerequisite for accurate and targeted control of pests and diseases during crop production (Sandmann et al., 2018).

The Minolta SPAD-502 instrument is used for quick and non-destructive determination of relative leaf chlorophyll concentration. The nitrogen content of plants can be inferred from the chlorophyll content of the leaves since the amount of chlorophyll is closely, positively and linearly related to the nitrogen content of the leaves (Zebarth et al., 2002). Furthermore, based on the SPAD values, we can conclude the chlorophyll content of the plant, which is correlated to the health of the plant and the yield (Duzs et al., 2019). Some reports suggest that SPAD is a dependable measuring device for estimating nitrogen concentration in lettuce plants. Thus, early evaluation of chlorophyll and nitrogen status can be used to more effective and scheduled fertilization during the growing season (Mendoza-Tafolla et al., 2019). Several factors influence the accumulation of nitrate in plants such as the soil pH, the weather conditions (precipitation, light), the harvest time, the type of varieties and the use of nitrogen fertilizers (Žnidarčič & Kmecl, 2018). Based on the chlorophyll content, we can conclude the health status of the plant as it is one of the most important indicators of photosynthetic capacity, nutritional stress and growth status of plants (Kizil et al., 2012; Barry et al., 2009)

Our research aimed to evaluate the agronomic properties of different lettuce varieties, to evaluate the condition of the plants based on the NDVI and SPAD values, and to determine the bioactive substances.

Materials and methods

The experiment was conducted at the Botanical and Exhibition Garden of the Farm and Regional Research Institute in the University of Debrecen in an unheated plastic tunnel (in springtime), on limestone chernozem soil. The soil test results of the experimental area are given in ***Table 1***, which was performed at the Agricultural Laboratory Centre of the University of Debrecen.

In the experiment, we evaluated the following types and varieties of lettuce – non-heading type ('Lollo Rosso', 'Lollo Bionda'), heading type ('Great Lakes 659', 'King of May') and cos type ('Romaine lettuce').

Regarding the conditions of the experiment, sowing was performed in 84-cell trays on 9 March 2019. Transplants of 5–6 leaves were planted out on 9 April 2019 with row and plant distances 25 cm under unheated plastic tunnel. Ferticare fertilizer (24:8:16 + 3,8MgO + microelement) was applied in 0.75 % concentration 3 times during the vegetation period.

The following morphological parameters were measured at the harvest:

- Head weight (g)
- Stem length (cm)
- Head firmness (1-5 scale) 1 – very soft → 5 – extra hard
- Closing of base (1-5 scale) 1 – open → 5 – completely closed
- Head shape index (height / diameter)
- Leaf shape index (length / width)

During instrumental measurements, the following were measured:

Measurement of photosynthesis activity:

- SPAD value (Minolta SPAD-502) – easy non-destructive measurement of the chlorophyll content of plant leaves without damaging the leaves.
- NDVI value (Green Seeker Model 505) – can be used to follow crop growth and development.

Laboratory measurements the following were measured:

- Total dry matter content (%) – Dry matter was determined after drying in an oven at 105°C.
- Total polyphenol content (TPC) was analyzed by the Folin–Ciocalteu method. The results were given in Gallic acid equivalent value (Meda et al., 2005).

Table 1: The soil analysis of experimental area.

Measured parameters (unit of measurement)	Amount
pH (KCl)	7.35
Plasticity index according to Arany (K_A)	42
Total water-soluble salt (%)	0.10
$CaCO_3$ (%)	4.20
Humus (%)	2.88
AL-soluble P_2O_5 (mg kg^{-1})	313.7
AL-soluble K_2O (mg kg^{-1})	459
KCL-soluble NO_3^- (mg kg^{-1})	130
AL-soluble Na (mg kg^{-1})	69.7
KCL-soluble Mg (mg kg^{-1})	584
KCL-soluble S (mg kg^{-1})	98.7
EDTA-soluble Mn (mg kg^{-1})	135
EDTA-soluble Zn (mg kg^{-1})	35.9
EDTA-soluble Cu (mg kg^{-1})	9.514

Statistical analysis

The data were submitted for analysis of variance (ANOVA) and differences between means were determined by post hoc Duncan and Tukey's test at the 5% (0.05) level of significance (n=5). Pearson's correlation coefficient between some data pairs was also calculated (n=25). Correlation was significant at $p \leq 0.05$ and $p \leq 0.01$ level (2-tailed). The analyses were carried out using IBM SPSS software (version 25).

Results and discussion

Relationship between fresh weight and stem length of the head by different lettuce varieties

The length of the stem can determine the number of leaves, in this way the weight of the head. It is important because lettuces are also determined by their head weight. It can be stated that a similar tendency can be observed between the development of stem size and head weight for different lettuce types (***Figure 1***). Among the cultivars, the smallest (1.95 ± 0.27 cm) stem size was measured by the non-heading lettuce 'Lollo Rosso'. This genotype also had the smallest head weight (129.00 ± 14.05 g). Drăghici et al. (2016) have detected a similar tendency for lollo type lettuce varieties

('Lollo Bionda': 201.33 g/plant; 'Lollo Rosso': 187.25 g/plant) by applying chemical fertilizer (NPK ratio of 4-1-2) for the growing. The 'King of May' butterhead type lettuce has improved the largest stem size (4.22 ± 0.48 cm) and head weight (259.31 ± 16.83 g).

Evaluations of head firmness

The head firmness was classified into five groups from 1 to 5. In group 1 there is lettuce with very loose heads, while in the group 5 had belonged the most compacted heads. Considering the market acceptance, firmness and compactness are important requirements. But it is known that the varieties with compact heads are more sensitive to physiological diseases, leading to quality decline. ***Figure 2*** shows that the highest head firmness (4.01 ± 0.32) was detected by butterhead lettuce 'King of May' (***Figure 3***), while the loosest head structure (1.00 ± 0.00) was improved by 'Lollo Rosso' (***Figure 4***).

Visual assessment of the closing of base of lettuce heads

This parameter was classified into five classes – from open (1) to completely closed (5). Varieties with a well-closed base are more resistant to *Botrytis* sp. and sclerotic diseases. The appearance of this fungus (*Sclerotinia sclerotiorum*) is very frequent by field-grown lettuce production causing great problems worldwide (Clarkson et al., 2014). It can be seen in ***Figure 5*** that the butterhead type lettuce has a significantly more closed base (4.08 ± 0.41) compared to the other lettuce types, which is a favorable property. For the lollo type lettuce ('Lollo Rosso') we have detected a very open (1.00 ± 0.01) base part, to respond more sensitively to diseases.

Evolution of head shape and leaf shape index by different lettuce types and varieties

Different lettuce varieties have different head shape indexes (ratio between plant height and diameter), which play a significant role in the appearance of diseases and determining the growing area. Moreover, the upright leaf varieties are favorable, because the lower leaves nearly have not to contact with the soil, in this way, the occurrence of fungal infection is rather less. For the planting design, it is major to know the head size of the different lettuce types because the larger headed varieties need greater row- and plant distance (25x30 or 30x30 cm), while the smaller ones are enough 20x20 cm. The higher plant density can decrease the head weight and earliness, while the too large distance reduces the profitability of production per unit area.

This relationship between the head- and leaf shape index is rather clear (***Figure 6***), there is a strong correlation (r = 0.955) for each lettuce type and variety. Higher head shape (***Figure 7***) and leaf shape (***Figure 8***) were detected by 'Roman lettuce' (cos lettuce). Furthermore, the upright leaves are not in contact with the soil surface, in this way, the probability of soil borne infection is reduced. Among the lollo type varieties 'Lollo Bionda' showed (0.60 ± 0.03; 0.83 ± 0.01) the less head shape (***Figure 9***) and leaf shape (***Figure 10***). This genotype produced a round leaf shape, which is closer to the soil, therefore the lower leaves have a higher risk of diseases.

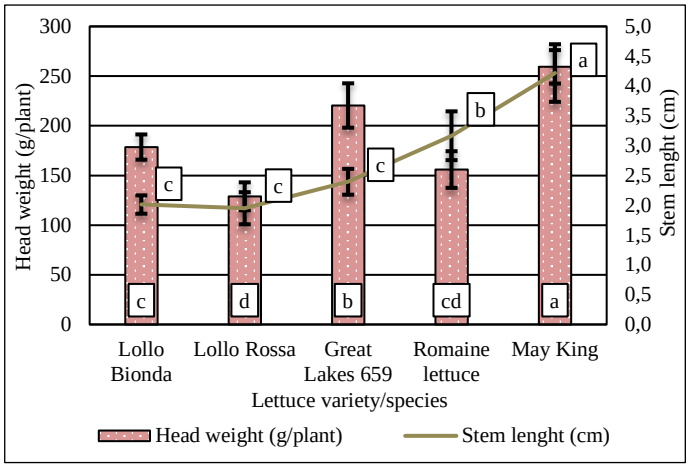

Figure 1: Relationship between head weight (g) and stem length. Means followed by the same letters do not different according to the Tukey test (P ≤ 0.05).

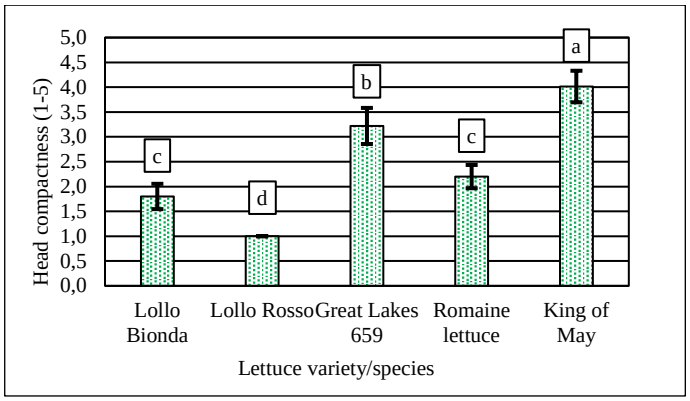

***Figure 2*:** Lettuce head structure (1-5) by different types lettuce varieties. Means followed by the same letters do not differ according to the Tukey test (P ≤ 0.05).

Figure 3-4: 'King of May'– compact head and 'Lollo Rosso' – non-heading variety.

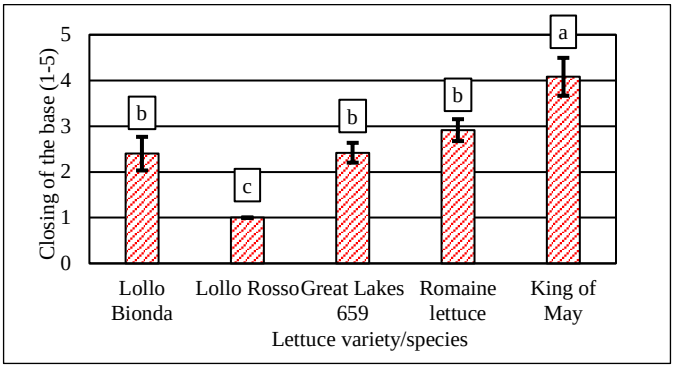

Figure 5: Closing of the base (1-5) by different types and varieties of lettuce. Different letters between cultivars denote significant differences (Tukey's test, p < 0.05).

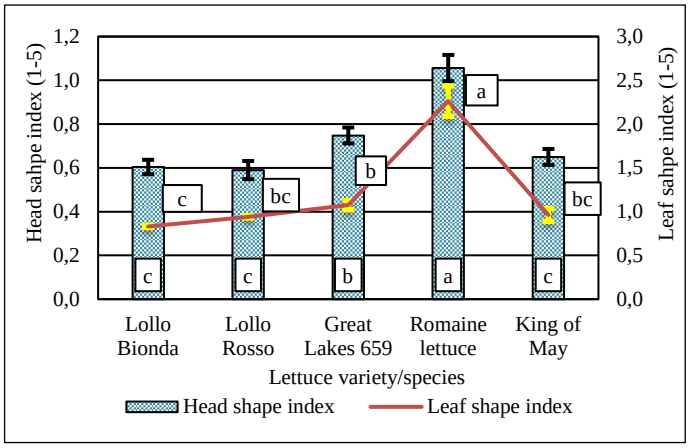

Figure 6: Evolution of the shape index of lettuce leaf and heads by different types. Means followed by the same letters do not differ according to the Tukey test ($P \leq 0.05$).

Figure 7-8: 'Romaine lettuce' – head shape and 'Romaine lettuce' – leaf shape.

Figure 9-10: 'Lollo Bionda' – head shape and 'Lollo Bionda' – leaf shape.

Relationship between lettuce heads shape index and NDVI value

With the NDVI value, we can conclude the vegetation cover of the growing area because it correlates well with leaf area index and biomass. This measurement is suitable for crop-related field phenotype determination (Bauer et al., 2019). ***Figure 11*** shows that there is a strong correlation (r = 0.828) between the head shape index of each lettuce type and the NDVI value. Among the evaluated lettuce types, the highest head shape index (1.06 ± 0.06) was measured for the cos lettuce ('Romaine lettuce') and this type also had the highest NDVI value (0.72 ± 0.03). The lowest values for this parameter were shown in loose-leaf lettuces, 'Lollo Bionda' and 'Lollo Rosso'.

SPAD value measured for different lettuce types

The chlorophyll content of the leaves directly determines the efficiency of plant photosynthesis. Based on the colour intensity (green) of the leaves of the plants, the nitrogen supply of the plant can be concluded. Furthermore, chlorophyll content is an indicator of plant health, and it can be used to optimize the timing of nutrient supply and the amount of supplemental fertilizer application to achieve higher quality and yield. Several studies mention that in many species, there is a high correlation between SPAD value and extractable chlorophyll content (León et al., 2007). The highest SPAD values were measured for iceberg lettuce ('Great Lakes 659') and cos-lettuce ('Romaine lettuce'), and these genotypes also had the most intense green colour (***Figure 12***). There was no statistically significant difference between the two types. The lowest SPAD value was measured by the 'Lollo Bionda'. Son & Oh (2013) have measured a higher SPAD value by the red leaf type 'Sunmang' (more than 20 SPAD value) compared to the green leaf type 'Grand Rapid TBR' (less than 20) under various combinations of red and blue light-emitting diodes (LEDs).

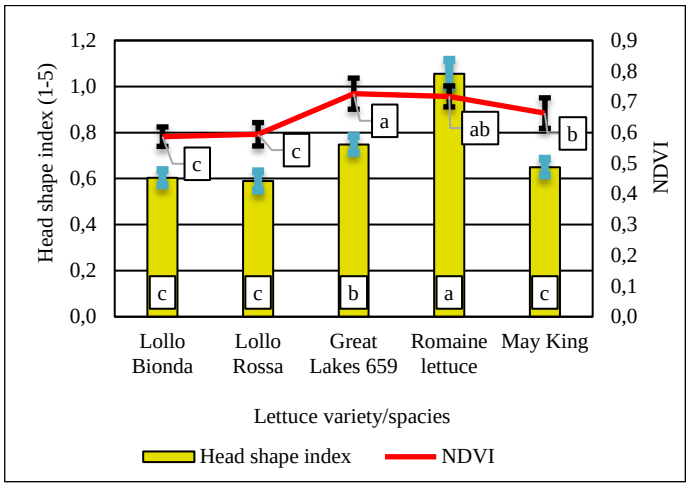

Figure 11: Evaluation of connection of shape index and NDVI value by different lettuce type and variety. Means followed by the same letters do not differ according to the Tukey test ($P \leq 0.05$).

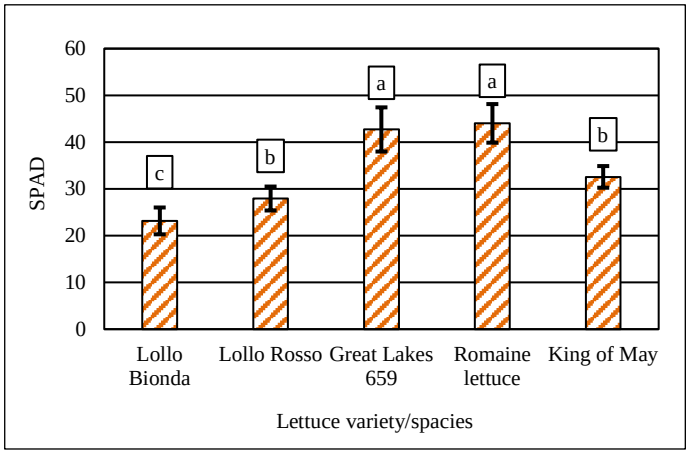

Figure 12: SPAD value of different lettuce types and varieties. Means followed by the same letters do not differ according to the Tukey test ($P \leq 0.05$).

Dry matter content (%)

Total dry matter content is an important component of vegetables. Among the varieties, we have found highly significant differences in this parameter (***Figure 13***). The 'Romaine lettuce' (cos lettuce) showed the highest (9.52 ± 0.12%) and the leaf lettuce 'Lollo Bionda' showed the lowest total dry matter content (6.02 ± 0.30%) on the chernozem soil. In their experiment, Barickman et al. (2018) evaluated two types of Roman lettuce ('Salvius' and 'Thurinus'). For the dry mass content (DM) they measured lower value for these varieties – 'Salvius' (5.26 %) and 'Thurinus' (6.21 %).

Total polyphenol content (TPC)

Polyphenols are secondary plant metabolites that have a beneficial effect on human health. The TPC value of the five selected lettuce varieties (***Table 2***) has changed between 37.80-181.53 mg gallic acid equivalent (GAE) per 100g fresh weight. Among the different types of lettuce, the red leaf variety ('Lollo Rosso') showed the highest TPC value (181.53 ± 3.29 mg GAE/100 g FW). The lowest value (37.80 ± 2.07 mg GAE/100 g FW) was measured by cos lettuce ('Romaine lettuce'). Gan & Azrina (2016) evaluated the TPC content of different leaf lettuce species (iceberg, butterhead, romaine, green coral and red coral) by DPPH assay. Similarly, they also measured the highest value in red leaf lettuce, although they found a lower value (76.05 mg GAE/100 g FW) than our results. Liu et al. (2007) evaluated the total phenolic content and antioxidant capacity of lettuce by Folin–Ciocalteu method and DPPH assay. The results proved the highest TPC content (52.4 mg GAE/g dry mass) and DPPH scavenging ability (estimated value of 76.4%) of red leaf lettuce compared to romaine (70.0%) and butterhead (51.7%) type.

Correlation analysis

There is a strong, high correlation between the parameters, which are highlighted (***Table 3***). Lettuce head index and leaf index had very strong correlations (r = 0.955) and strong positive correlations (r = 0.828 and 0.760) with NDVI. The dry matter content is also highly correlated with the head index, leaf index, NDVI and SPAD value. Pettorelli et al. (2011) also mentioned that NDVI measurements strongly correlated with total dry matter accumulation of herbaceous over an observation period. Our experiment proved a high correlation (r = 0.840) between NDVI and SPAD. Between the stem length and the head weight, there is a medium correlation (r = 0.664) and a significant relationship.

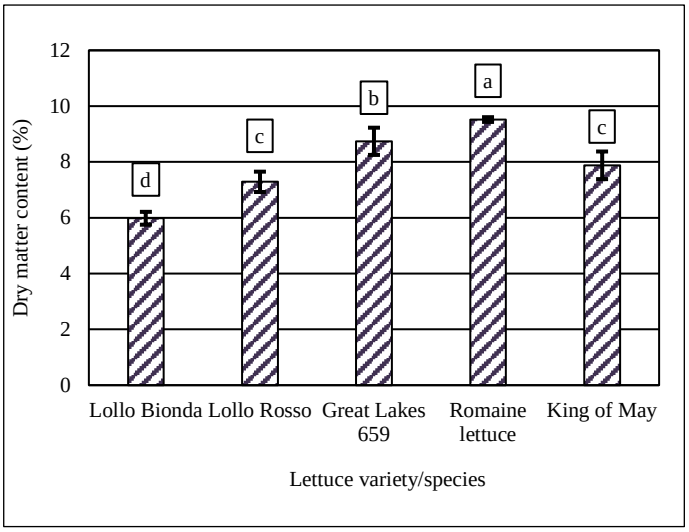

Figure 13: Dry matter content (%) of different lettuce types and varieties. Means followed by the same letters do not differ according to the Tukey test (P ≤ 0.05).

Table 2: Total polyphenol content (TPC) of different lettuce varieties.

Lettuce variety/species	***Total polyphenol content (TPC)***
'Lollo Bionda'	45.40b ± 2.80
'Lollo Rosso'	181.53a ± 3.29
'Great Lakes 659'	40.80b,c ± 2.58
'Romaine lettuce'	37.80c ± 2.07
'King of May'	46.13b ± 2.48

Table 3: Pearson's correlation coefficients among physiological and biochemical parameters by lettuce
Pearson correlation coefficient of a part of parameters. Correlation was significant at *p ≤ 0.05 and **p ≤ 0.01 (2-tailed).

Pearson Correlations

	Head weight (g/plant)	Stem length (cm)	Dry matter content (%)	Lettuce leaf index	Lettuce head index	NDVI	SPAD	Total polyphenol content (TPC)
Head weight (g/plant)	1	0.664**	0.119	-0.294	-0.135	0.061	0.076	-0.584**
Stem length (cm)		1	0.389	0.239	0.266	0.312	0.317	-0.432*
Dry matter content (%)			1	0.738**	0.796**	0.896**	0.785**	-0.273
Lettuce leaf index				1	0.955**	0.760**	0.612**	-0.300
Lettuce head index					1	0.828**	0.682**	-0.442*
NDVI						1	0.840**	-0.385
SPAD							1	-0.339
Total polyphenol content (TPC)								1

Conclusions

The head weight of lettuce is an important factor because the lower level can reduce the efficacy of the process for the fresh cut industry. A similar tendency can be observed between the development of head size and head weight by different lettuce varieties. Larger head weight can be achieved with butterhead and crisphead genotypes. However, the lollo type lettuce with a smaller head weight is asked, too, for raw material of various salad mixes. The firmness of lettuce heads is an important factor, too, because varieties with higher compactness may be more sensitive to fungal diseases and physiological disorders. For designing the growing area, it is important to know the head size of the lettuce to achieve optimal planting space and profitable yield. It is possible to apply a smaller growing area by upright leaf lettuces (cos lettuce) varieties.

According to our results, we can conclude, that the vegetation cover of the growing area (evaluated with NDVI value) strongly correlates with the head shape index. With intense green colour (iceberg and cos lettuce), lettuce has a higher SPAD value. The chlorophyll content is an indicator of plant health and can optimize fertilizer application to achieve higher quality and yields. NDVI and SPAD values showed a strong correlation with dry matter content (%), too. Different values were found for the total polyphenol content (TPC), but the highest value was detected by red leaf lettuce 'Lollo Rosso' and by butterhead lettuce 'King of May'.

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