Volume 7, Number 1 (2020)

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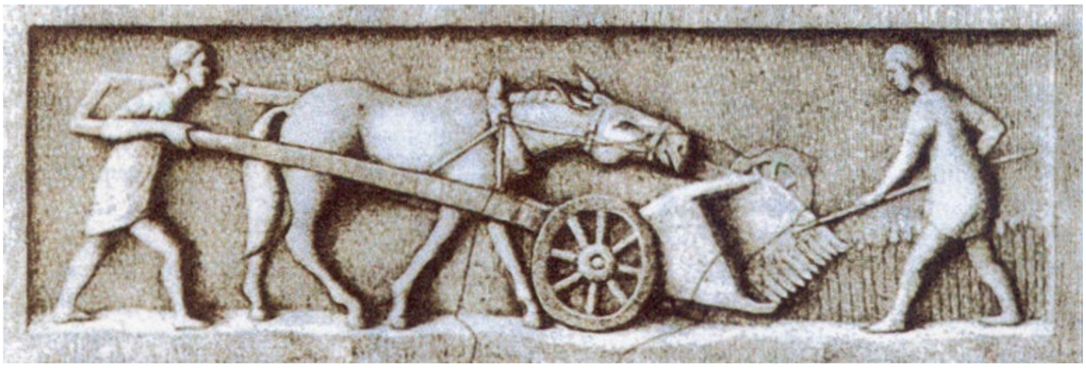

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Table of contents

Quality and quantity of winter wheat varieties in 22 years' time range

Katalin M. KASSAI[1] – Ákos TARNAWA[1] – Ferenc H. NYÁRAI[1] – Zsolt SZENTPÉTERI[1] – Adnan ESER[1] – Hajnalka KATÓ[1] – Márton JOLÁNKAI[1]

1:Szent István University, Institute of Crop Production, Páter Károly u. 1., 2100 Gödöllő, Hungary, E-mail: Jolankai.Marton@mkk.szie.hu

Abstract: The Nagygombos experiments of the Szent István University, Gödöllő, Hungary have always been a testing area for various agronomic technologies as well as a research site for evaluating the performance of crop varieties. The present paper is intended to give an overview of 18 winter wheat *Triticum aestivum* L. varieties tested during the time range between 1996 and 2018. All of the varieties were studied under similar agronomic conditions, each of them for min 3 years in a series of a polyfactorial replicated field trial. The 120 kg/ha N plant nutrition applications of the respective crop years were processed in the evaluation. Amount of grain yield, protein %, wet gluten content and farinographic values of the varieties examined were compared. The results obtained suggest, that most of the varieties had a rather high variation concerning yield figures, however protein and farinographic indicators proved to be more stable characteristics. Wet gluten content was influenced mainly by the crop year. The study may support a conclusion that certain varieties have shown a higher stability in technological quality manifestation regardless to the amount of their grain yield. Alföld 90, Yubileynaya 50, Mv Magdaléna and Mv Toldi varieties proved to be the best quality varieties in this research series.

Keywords: wheat, quality, quantity, long term trial

Received 08 November 2019, Revised 23 March 2020, Accepted 25 March 2020

Introduction

Environmental conditions are highly influential regarding crop quality and quantity performance. Grain yield and yield quality of winter wheat *Triticum aestivum* L. is highly influenced by the meteorological conditions of the given crop year, especially the amount and distribution of precipitation and the actual temperature (GRIMWADE ET AL 1996, GYŐRI 2008, PEPÓ 2010). Crop yield and grain quality can also be influenced by agronomic applications. Plant nutrition in general and N topdressing in particular should be considered as the most effective treatments within the technologies of winter wheat production. The amount of nitrogen and the timing and distribution of the application have an impact on wheat quality, especially on the protein production of the crop (GYŐRI 2006, PEPÓ 2010). Wheat varieties may have different responses to agroecological impacts (VIDA ET AL 1996; MESTERHÁZY 2019). Varietal differences are to be evaluated in long term trials to reduce the impact of variable crop year effects (KISMÁNYOKY AND RAGASITS 2003).

Materials and Methods

A wide range of high milling and baking quality winter wheat *Triticum aestivum* L. varieties were examined under identical agronomic conditions in a long term field trial. The small plot trials were run at the Nagygombos experimental field of the Szent István University, Crop Production Institute, Hungary. Soil type of the experimental field is chernozem (calciustoll). Annual precipitation of the experimental site belongs to the 550-600 mm belt of the Northern edges of the Hungarian Great Plain. Experiments were conducted in a split-plot design with four replications. The size of each plot was 10 m^2. Plots were sown and harvested by plot machines (standard Wintersteiger cereal specific experimental plot machinery series). Various identical agronomic treatments were applied to plots. Plant nutrition applica-

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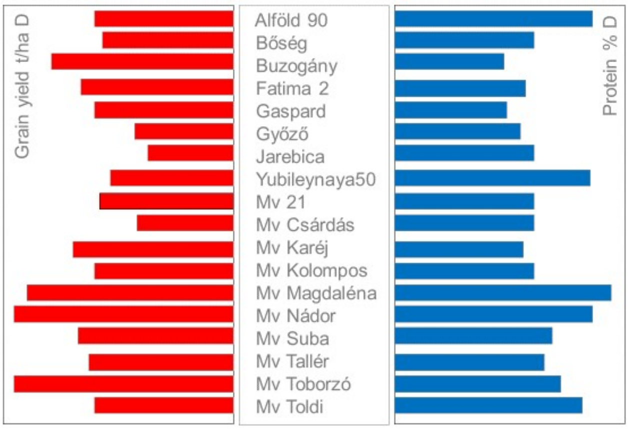
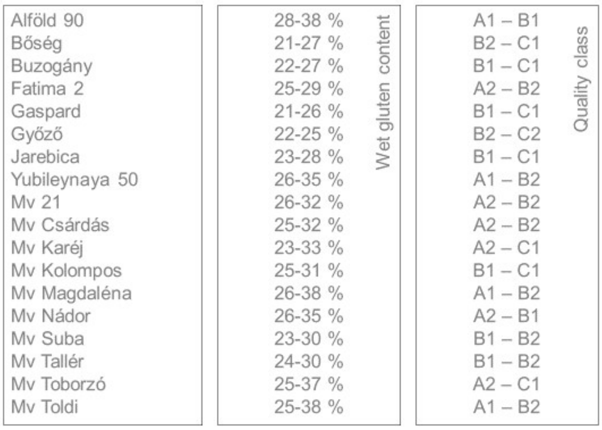


Figure 1. Grain yield and protein content of wheat varieties Nagygombos, 1996-2018.



	Wet gluten content	Quality class
Alföld 90	28-38 %	A1 – B1
Bőség	21-27 %	B2 – C1
Buzogány	22-27 %	B1 – C1
Fatima 2	25-29 %	A2 – B2
Gaspard	21-26 %	B1 – C1
Győző	22-25 %	B2 – C2
Jarebica	23-28 %	B1 – C1
Yubileynaya 50	26-35 %	A1 – B2
Mv 21	26-32 %	A2 – B2
Mv Csárdás	25-32 %	A2 – B2
Mv Karéj	23-33 %	A2 – C1
Mv Kolompos	25-31 %	B1 – C1
Mv Magdaléna	26-38 %	A1 – B2
Mv Nádor	26-35 %	A2 – B1
Mv Suba	23-30 %	B1 – B2
Mv Tallér	24-30 %	B1 – B2
Mv Toborzó	25-37 %	A2 – C1
Mv Toldi	25-38 %	A1 – B2

Figure 2. Wet gluten content and quality classes of wheat varieties Nagygombos, 1996-2018.

tions were done in single and combined treatments. N topdressing variants were applied by single and repeated topdressings representing 6 levels: 0, 80, 80+40, 120, 120+40 and 160 kg/ha N in single and split applications. All plots were sown with identical series of wheat varieties for studying their performance in relation with agronomic impacts. The present paper is intended to give an overview of 18 winter wheat *Triticum aestivum* L. varieties tested during the time range between 1996 and 2018. All of the varieties were studied under similar agronomic conditions, each of them for min 3 years in a series of a polyfactorial replicated field trial. The 120 kg/ha N plant nutrition applications of the respective crop years were processed in the evaluation for both yield and quality parameters. Wheat grain quality parameters: protein and wet gluten contents were determined from grain samples, as well as quality characteristics at the Research Laboratory of the SIU Crop Production Institute, and RET Regional Knowledge Centre laboratories according to Hungarian and EU standards (MSZ 1998; EK 2000, HORVÁTH 2014). During the examined period levels of fusarium head blight (*Fusarium graminearum*) infection were detected in the wheat trials.

Results

During the 22 years of the experimental series many varieties had been studied in the trials (JOLÁNKAI ET AL 2018). In this paper only those are introduced which were tested for minimum 3 years at the Nagygombos site. Amount of grain yield, protein %, wet gluten content and farinographic values of the varieties examined were compared. The results obtained suggest, that most of the varieties had a rather high variation concerning yield figures, however protein and farinographic indicators proved to be more stable characteristics. Wet gluten content was influenced mainly by the crop year.

Figure 1 provides information on grain yield and protein content of the varieties. Buzogány, Mv Magdaléna, Mv Nádor, Mv Toborzó were the highest yielding varieties during the examined years. The study may support a conclusion that certain varieties have shown a higher stability in quality manifestation regardless to the amount of their grain yield. *Figure 2* presents information on the quality ranges of the varieties summarising the wet gluten content and the baking quality groups. Alföld 90, Yubileynaya 50, Mv Magdaléna and Mv Toldi varieties proved to be the best quality varieties in this research series.

During the examined period fusarium head blight infection had been monitored in all years. According to the data presented in *Figure 3* it can be stated, that except few extreme years the Nagygombos experimental site was not exposed to severe fusarium head blight infections. There were two peaks with high infection: 1997 to 1999 was a strong infection period followed by an almost 10 years' free period of Fusarium sp. The next peak occurred in 2010. Both high infections were escorted by extreme weather conditions – like water flood during the pre harvest vegetation period.

Discussion

According to the results obtained it should be emphasized that the quantity and quality parameters of any crop varieties are to be examined in polyfactorial long term trials that may provide the researcher identical conditions to exclude unfavourable factors and buffer the crop year effects.

The authors are sorry to inform the LOTEX participants that in 2018 year the Nagygombos experimental site of the SIU Crop Production Institute had to be terminated. *Figure 4* attached presents a satellite photo of the experimental site from 2016. We do hope, that preserving the main blocks of the experimental design, once the trial can be restarted again.

Acknowledgements

The authors are indebted to all those, who supported the experimental work during the past periods – so the earlier OMFB, OTKA, NKFP, TEMPUS, COPERNICUS, VKSZ fundings as well as that of the present NVKP 16-1-2016-0016 project.

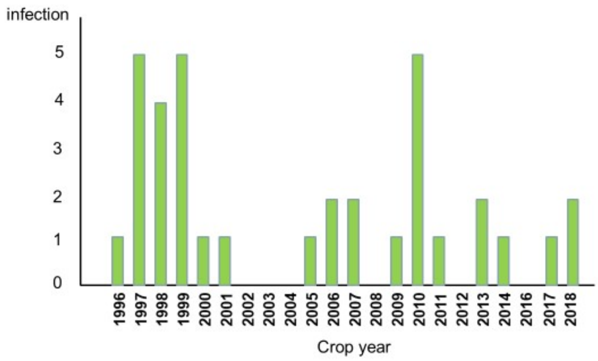


Figure 3. Occurence of fusarium head blight infection in wheat trials Nagygombos, 1996-2018.

Figure 4. Satellite photo of the Experimental site of the SIU Crop Production Institute Nagygombos, 2016.

References

Grimwade, B., Tatham, A.S., Freedman, R.B., Shewry, P.R., Napier, J.A. (1996): Comparison of the expression patterns of wheat gluten proteins and proteins involved in the secretory pathway in developing caryopses of wheat. Plant Molecular Biology. 30:1067–1073. https://doi.org/10.1007/BF00020817

Győri, Z. (2006): A trágyázás hatása az őszi búza minőségére. Agrofórum. 17(9):14-16.

Győri, Z. (2008): Complex evaluation of the quality of winter wheat varieties. Cereal Research Communications. 36(2):1907-1910.

Horváth, Cs. (2014): Storage proteins in wheat (*Triticum aestivum* L.) and the ecological impacts affecting their quality and quantity, with a focus on nitrogen supply. Columella - Journal of Agricultural and Environmental Sciences. 1(2):57-75. https://doi.org/10.18380/SZIE.COLUM.2014.1.2.57

Jolánkai, M., Tarnawa, Á., Nyárai, H.F., Szentpétery, Z., Kassai, M.K. (2018): Agronomic benefits of long term trials. Columella Columella - Journal of Agricultural and Environmental Sciences. 5(1):27-30. https://doi.org/10.18380/SZIE.COLUM.2018.5.1.59

Kismányoky, T., Ragasits, I. (2003): Effects of organic and inorganic fertilization on wheat quality. Acta Agronomica Hungarica, 51(1):47-52. https://doi.org/10.1556/AAgr.51.2003.1.6

Mesterházy, Á. (2019): Kalászos fuzárium járvány, 2019. Helyzetkép és tennivalók. Hungarian Academy of Sciences, Section of Agricultural Sciences. Scientific lecture.

MSZ 6383:1998, 824/2000/EK Wheat quality standards.

Pepó, P. (2010): Adaptive capacity of wheat (*Triticum aestivum* L.) and maize (*Zea mays* L.) crop models to ecological conditions. Növénytermelés. 59. Suppl. 325-328.

Vida, Gy., Bedő, Z., Jolánkai, M. (1996): Agronómiai kezeléskombinációk őszi búzafajták sütőipari minőségére gyakorolt hatásának elemzése főkomponens-analízissel. Növénytermelés. 45(6):453-462.

Comparative study of two different neem-derived pesticides on *Meloidogyne incognita* under *in vitro* and pot trials under glasshouse conditions

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Abstract: Two different neem-derived plant protection products i.e neem leaf extract and a commercial product containing 1% azadirachtin were used to study their effects on *M. incognita* under *in vitro* and pot trails under glasshouse conditions. In the *in vitro* studies, highest concentration (1%) of neem leaf extract resulted in more than 90% mortality in J2 whereas the commercial product did not differ significantly in mortality compared to control. In the pot trials under glasshouse conditions, fresh shoot weight and number of fruits of both the landraces did not differ significantly for different treatments. Zeck scale was found to be the best for evaluation of gall index compared to other scales i.e Garabedian and Van Gundy scale and Mukhtar et al. scale. Gall index decreased in all the treatments compared to positive control and 0.1% azadirachtin was significantly different from the control. This shows that neem-derived pesticides can reduce the galling of roots and can help control *M. incognita* infestation with proper planning and implementing the treatments.

Keywords: neem leaf extract, azadirachtin, root-knot nematodes, biological control, tomato

Received 11 November 2019, Revised 05 March 2020, Accepted 30 March 2020

Introduction

More than 90 root-knot nematode (*Meloidogyne*) species are known worldwide (Hunt & Handoo 2009), from which 23 species are present in Europe (Wesemael et al. 2011). Root-knot nematodes are responsible for decreasing yields (Bernard et al. 2017) of nearly every cultivated crop in the world (Sasser, 1980). They have a wide range of host plants: with different sensitivity, but they are able to infect the roots of vegetables (Anwar et al. 2007), medicinal and culinary plants (Walker 1995; El-Sherif et al. 2012), ornamental plants (Dabaj and Jenser, 1990; den Nijs et al. 2004) and weeds as well (Rich et al. 2008).

If once their appearance is noticed in a field, their total eradication is an almost impossible task (Briar et al. 2016). It is especially so recently due to the restricted use of soil disinfecting chemicals (Briar et al. 2016). Moreover, certain species, like *Meloidogyne incognita* has several biological races with different pathogenicity and host plant preferences (Khan and Khan 1991). Consequently, mixed natural populations of *Meloidogyne* species can break the resistance of Meloidogyne-resistant varieties of crops (Eddaoudi et al. 1997; Tzortzakakis et al. 2016).

Neem as a pesticide is used for centuries in Asia and has known to possess several beneficial plant protective properties such as antifeedant, repellent, antifungal (Schmutterer 1988) and nematicidal (Nile et al. 2017; Yadav et al. 2018). Javed et al. (2007) investigated the efficacy of different neem formulations on *Meloidogyne javanica* (Treub, 1885) Chitwood, 1949 on tomato. They found that crude extracts of neem cake and leaves reduced the severity of the nematode infestation both under *in vitro* circumstances and in plants under glasshouse conditions. However, in the case of pure azadirachtin which is a refined neem product, neither the

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immobilisation of nematodes nor increased mortality was observed under in vitro conditions. Similar results were obtained by Khanna and Kumar (2006) when they tested five different neem formulations against *M. incognita in vitro*. Out of the five different neem formulations tested, neem seed kernel extract and Econeem, a commercial product consisting of Azadirachta A and B, gave the highest juvenile mortality (73-77%) whereas the other formulations i.e Nimbecidine, NeemAzal T/S and Neem Gold were comparatively less effective.

During a study conducted by Lynn et al. (2010) on the effects of azadirachtin and neem based formulations to control sweet potato whitefly and *M. incognita*, they observed a reduction in the development of both the whiteflies and root-knot nematodes and recommended that the soil-based application would be the best to control both leaf-sucking and soil pests. Sahu et al. (2018) compared the efficacy of different oil cakes in pot culture experiment with tomato. It was concluded that the neem cake applied at a rate of 100 g/m2 increased the morphological characteristics of tomato and significantly reduced the number of root galls, thus it is considered a most promising management option against *M. incognita* infecting tomato. Singh et al. (1980) also found significant reduction in the abundance of different plant parasitic nematodes and fungi by coating the tomato seeds with oil cakes of *Ricinus communis* L., *Brassica campestris* L, *Azadirachta indica*, *Madhuca indica* and *Arachis hypogaea* L. Similar results were obtained by Siddiqui and Alam (1987) with seed dressing method using neem and Persian lilac (*Melia azedarach* L.) extracts to control the plant-parasitic nematodes *M. incognita* and *Rotylenchulus reniformis* Linford et Oliveira.

The objective of this study was to test two different neem-derived products i.e. traditional aqueous neem leaf extract and a commercial product of azadirachtin, for their nematicide effect against *M. incognita in vitro* and in pot experiments. We wanted to compare the traditional water extract which can be easily prepared without any processing locally (being cost effective and easily available in nature) and the commercial product which is much more expensive to the farmers and growers. In addition, we also compared the different *M. incognita* infestation scales to get a better understanding about the severity of infestation.

Materials and Methods

Preparation of aqueous neem leaf extracts (NLE)

Pre-air dried neem leaves were obtained from the local market situated in Mumbai Sub-urban area, Konkan Division, Maharashtra, India. The method of Doshi et al. (2018) was followed with modified working concentrations. For *in vitro* studies, a stock concentration of 5% was prepared by suspending 5 g of air-dried neem leaf powder in 100 ml distilled water. It was filtered through muslin cloth and was centrifuged at 5000 rpm for 5 mins to remove the debris and leaf particles. Working concentrations of 0.01, 0.05, 0.1, 0.5 and 1% were prepared from the stock solution with distilled water.
For glasshouse trials, a stock solution of 20% was made by adding 200 g of neem leaf powder to 1000 mL of distilled water. It was followed by the same procedure as *in vitro* to get a clear solution. Working concentrations of 1, 10 and 20% were prepared from the stock solution with distilled water.

Preparation of azadirachtin (NAZ)

NeemAzal T/S (Trifolio-M GmBH) which contains 1% azadirachtin and is a registered product in the EU was used for preparation of azadirachtin. The methodology of Doshi et al. (2018) was followed with modified concentrations. For *in vitro* studies, the following working concentrations were applied: 0.0001, 0.0003, 0.0005, 0.001 and 0.01% all in distilled water.

In pot trials, the working concentrations were increased to 0.001, 0.01 and 0.1% with a stock solution of 0.1% which is prepared by dissolving 100 mL of the product in 1000 mL distilled water.

M. incognita inoculum

Second stage juveniles (J_2) of *M. incognita* were obtained from egg masses previously collected from the infected Hungarian determinate tomato landrace cv. 'Dányi' grown in the greenhouse. In order to dissolve the gelatinous matrix and release the eggs, the egg masses were shaken by hand for 2 mins in 0.2% sodium hypochlorite (NaOCl) solution, then they were washed with tap water until the smell of NaOCl was removed. The eggs were suspended in tap water and kept at 24 ± 1°C in dark for hatching. After 14 days, the hatching of the eggs and viability of J_2 were checked under a dissecting microscope with transmitting illumination at a 40x magnification. Only moving and viable J_2 were picked up and were collected using a Pasteur pipette in a glass bottle with tap water and were stored in dark at 20°C ± 1°C for 24 hrs before using for the experiments.

Experiment 1: In vitro effect of neem-derived products on M. incognita (J_2)

A total of eight samples of each concentrations and control were applied. The entire experiment was performed in vitro in flat-bottom 96-well microplates (Kartell S.p.A., Italy) in three repetitions. Five J_2-s were put into each well with 60 μl of distilled water using a micropipette. Then 200 μl of different neem leaf extract or azadirachtin concentrations and 200 μl distilled water was added in the microplate wells as treatments and negative control respectively. Microplates were incubated at room temperature (25 °C) in dark for 24 hours. Nematode mortality was checked under dissecting microscope at 40× after 24 hours. In order to check the motility of nematodes as a sign of viability, pH was dropped by adding 10 μl of 5% lactic acid, a modification of the procedure described by Ciancio (1995). A maximum mortality of 20% in control was considered as a criterion for the validity of the tests (Kiss et al. 2018).

Experiment 2: Effects of neem-derived products on M. incognita infestation under glasshouse conditions

One Hungarian determinate tomato landrace. 'Dányi' (RCAT057829) and a Hungarian indeterminate tomato landrace 'Ceglédi' (RCAT030275) were chosen for this experiment. For potting material, horticultural soil and sand in the ratio of 1:1 (henceforth called as 'mixture') was used. After filling the pots with the mixture, approximately 20 g of *M. incognita* infested soil was added in the middle by making a ditch followed by planting of 1-month old tomato plants. The average temperature recorded during the experiment in the glasshouse was between 25 - 28°C and the relative humidity was between 55-60%. For positive infected control (henceforth called as positive control), only inoculation was done but no treatment was performed. Each treatment was replicated 5 times for both the landrace. The plants were watered daily. The first treatment was done by adding 50 ml of the different concentrations of neem derivatives by soil drenching method after 7 days from planting. In the case of negative control, plants were potted just with the mixture and watered with the rest of the plants. Plants were watered only after the treatment to help spread and mix everywhere in the pots. The treatments were repeated once per week on every 7th day after the previous treatment, for a period of 6 weeks altogether. Experiments were terminated 9 weeks after the setup. Gall index was measured using three different scales by Zeck (1971), Garabedian and Van Gundy

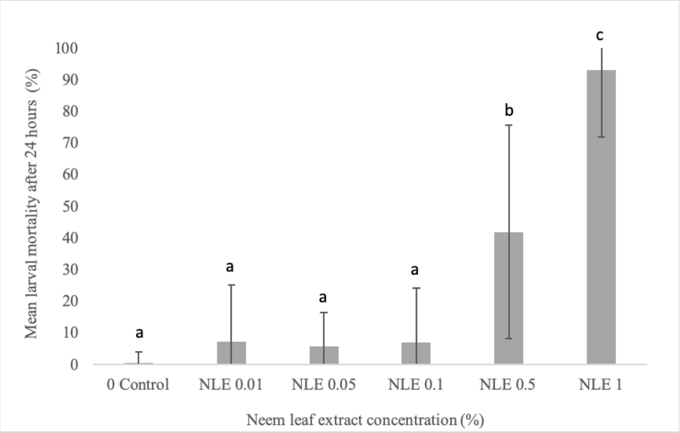

Figure 1. Mortality effect (%) of different concentrations of neem leaf extract (NLE) (%) on *Meloidogyne incognita* J_2 larvae under in vitro conditions after 24 hours. Different letters represent significant difference at 95% confidence level ($p \leq 0.05$). Data are the mean mortality values of 3 replications of the whole experiment, i.e. 24 replicates.

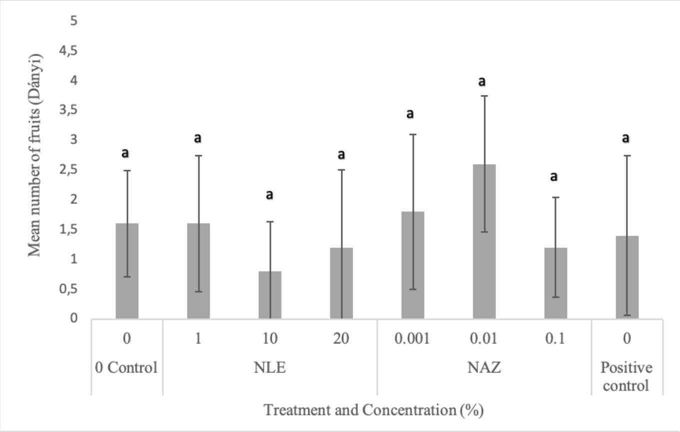

Figure 2. Mean number of fruits produced by *Meloidogyne incognita* infested 'Dányi' determinate tomato landrace after treatment with different neem leaf extract (NLE) and azadirachtin (NAZ) concentrations. Same letters indicate no significant difference at 95% confidence level ($p<0.05$). Data is average of five individual plants per treatment.

(1983) and Mukthar et al. (2013). Morphological characteristics such as fresh shoot weight and number of fruits were measured and recorded.

Data analyses

In the case of Experiment 1, post-hoc Tukey's test was performed after arcsine square root transformation of the data. In the case of Experiment 2, post-hoc Tukey's test was used in R software (R Core Team 2017) for all the three scales. With this approach, a more complete picture from root damage was given. Graphs and tables were made in excel sheet. In addition, we used post-hoc Welch test followed by Tukey's test to compare the two tomato landraces with respect to the root damage caused by *M. incognita* depending on three different scales and to select the best scales for evaluation.

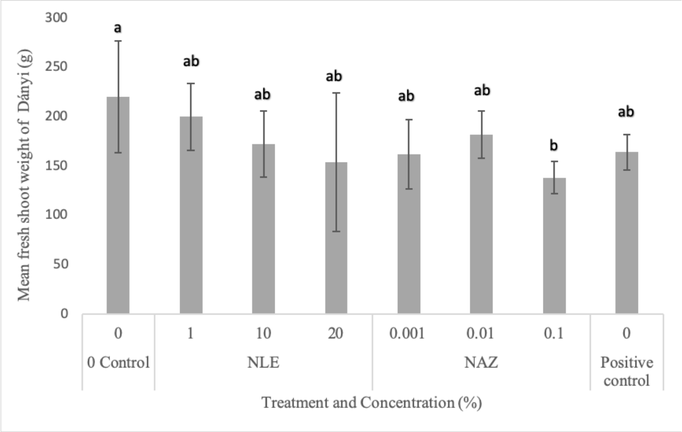

Figure 3. Mean shoot fresh weight in grams of *Meloidogyne incognita* infested 'Dányi' determinate tomato landrace after treatment with different neem leaf extract (NLE) and azadirachtin (NAZ) concentrations. Different letters represent significant difference at 95% confidence level ($p \leq 0.05$). Data is replicate of five individual plants per treatment.

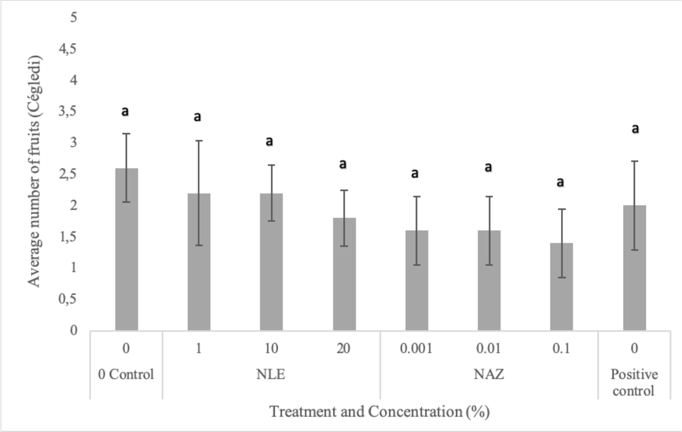

Figure 4. Mean number of fruits produced by *Meloidogyne incognita* infested 'Ceglédi' indeterminate tomato landrace after treatment with different neem leaf (NLE) extract and azadirachtin (NAZ) concentrations. Same letters indicate no significant difference at 95% confidence level ($p<0.05$). Data is average of five individual plants per treatment.

Results

Experiment 1: Effect of neem-derived products on *M. incognita* second stage juveniles (J_2)

The mortality effect of different concentrations of azadirachtin (NAZ) and neem leaf extract (NLE) on mortality of *M. incognita* J_2 larvae under in vitro conditions was demonstrated. In case of NAZ, the mortality of the larvae was inconsistent, wherein numerically the highest mortality was found at the lowest concentration i.e 0.0001% followed by 0.003% and not at the highest concentration of 0.01% as it would have been expected. However, all these mortality values were quite low with no significant differences (Table 1). In case of NLE, it is evident from Figure 1 that higher concentration of NLE yielded in higher mortality. Mortality

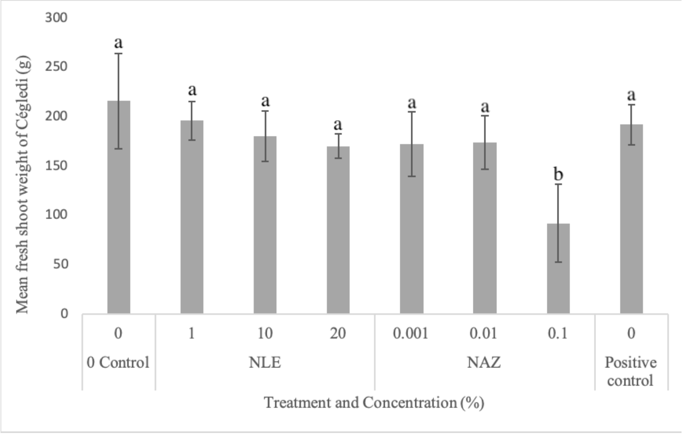

Figure 5. Mean shoot fresh weight in grams of *Meloidogyne incognita* infested ‘Ceglédi’ determinate tomato landrace after treatment with different neem leaf extract (NLE) and azadirachtin (NAZ) concentrations. Different letters represent significant difference at 95% confidence level ($p \leq 0.05$). Data is average of five individual plants per treatment.

Table 1. Different azadirachtin (NAZ) concentrations tested for mortality of *Meloidogyne incognita* J_2 larvae after 24 hours. Same letters indicate no significant difference at 95% confidence level ($p<0.05$). *Data are the mean mortality values of 3 replications of the whole experiment i.e. 24 replicates.

Treatment	Concentration (%)	*Per cent juvenile mortality after 24 hours (mean ± SD)
Control	0	0.69 ± 3.40 a
Azadirachtin (NAZ)	0.0001	10.97 ± 4.83 a
	0.001	4.58 ± 2.40 a
	0.003	9.26 ± 4.41 a
	0.005	6.37 ± 2.44 a
	0.01	6.98 ± 2.11 a

in the case of the two highest concentrations of NLE, i.e. 0.5% and 1% was significantly higher ($p < 0.05$) as compared to azadirachtin in Table 1.

Experiment 2: Effects of neem-derived products on *M. incognita* infestation in pot experiment under glasshouse conditions

Neither in the case of Dányi (Fig 2) nor ’Ceglédi’ (Fig 4) tomato landraces was there any significant difference in the number of fruits with respect to different treatments and concentrations. Further evaluation such as yield could had been possible as we did not wait for the fruits to ripen.

Having said that, azadirachtin (NAZ) 0.1% showed lower fresh shoot weight with a significant difference in both ’Dányi’ (Fig 3) and ’Ceglédi’ (Fig 5) varieties with respect to 0 control. Apart from this difference, there was no significant difference between the other treatments. All the three scales showed significant difference as compared to non-infected control. In the case of both ’Dányi’ and ’Ceglédi’, Zeck scale proved the strongest next to the scales of Mukhtar et al.

Table 2. Average root damage caused by *Meloidogyne incognita* on two Hungarian landraces tomato, the determinate 'Dányi' and the indeterminate 'Ceglédi' depending on three scales: Zeck, Garabedian and Van Gundy and Mukhtar et al. (p-value: Welch test, confidence interval (CI) 95%: 95% confidence level).

Tomato landraces	Dányi		'Ceglédi'	
M. incognita infection -/+	-	+	-	+
Replications	5	34	5	34
Teck scale (0-10)				
mean ± CI 95%	0 ± 0	4.53 ± 0.60	0 ± 0	5.32 ± 0.40
p-value	$4.8*10^{-14}$		$1.69*10^{-23}$	
Garabedian and Van Gundy scale (0-5)				
mean ± CI 95%	0 ± 0	2.21 ± 0.34	0 ± 0	2.53 ± 0.37
p-value	$2.64*10^{-14}$		$7.95*10^{-15}$	
Mukhtar et al. scale (0-6)				
mean ± CI 95%	0 ± 0	4.06 ± 0.54	0 ± 0	4.62 ± 0.48
p-value	$5.12*10^{-16}$		$2.31*10^{-19}$	

Table 3. Average root damage caused by *Meloidogyne incognita* on Hungarian determinate tomato landrace ''Dányi", depending on three scales: Zeck, Garabedian and Van Gundy and Mukhtar et al. receiving the following treatments: 0.001, 0.01 and 0.1% of NeemAzal T/S and 1, 10 and 20% of neem leaf extract. ANOVA post-hoc Welch test followed by Tukey's test was performed. Different letters indicate significant difference at 95% confidence level (p<0.05).

Treatments	Concentration (%)	Zeck (0-10)	Garabedian and Van Gundy (0-5)	Mukhtar et al. (0-6)
Negative control	0	0 ± 0 a	0 ± 0 a	0 ± 0 a
Positive control	0	5.8 ± 0.96 c	2.8 ± 0.96 b	5.6 ± 0.78 c
NeemAzal T/S	0.001%	5.2 ± 0.73 c	2.4 ± 0.78 b	4.4 ± 1 bc
	0.01%	4.6 ± 1.47 bc	2.6 ± 1 b	4.4 ± 1 bc
	0.1%	2 ± 0.8 ab	1.25 ± 0.44 ab	2 ± 0.72 ab
Neem leaf extract	1%	5.4 ± 0.48 c	2.4 ± 0.48 b	4.2 ± 0.96 bc
	10%	5 ± 1.52 bc	2.4 ± 0.78 b	4.4 ± 1.33 bc
	20%	3.2 ± 2.09 bc	1.4 ± 1 ab	3 ± 1.96 c

and Garabedian and Van Gundy (Table 2).

In the case 'Dányi' landrace, values of the root damage were inconsistent, since the values of Zeck and Mukhtar et al. scales of NeemAzal T/S 0.1% concentration were significantly different from positive control, however, the scale of Garabedian and Van Gundy said the opposite. Moreover, according to the Garabedian and Van Gundy scale, the 20% concentration of neem leaf extract

Table 4. Average root damage caused by *Meloidogyne incognita* on indeterminate Hungarian tomato landraces 'Ceglédi', depending on three scales: Zeck, Garabedian and Van Gundy and Mukhtar et al. receiving the following treatments: 0.001, 0.01 and 0.1% of NeemAzal T/S and 1, 10 and 20% of neem leaf extract. ANOVA post-hoc Welch test was performed followed by Tukey's test. Different letters indicate significant difference at 95% confidence level (p<0.05).

Treatments	Concentration (%)	Zeck (0-10)	Garabedian and Van Gundy (0-5)	Mukhtar et al. (0-6)
Negative control	0	0 ± 0 a	0 ± 0 a	0 ± 0 a
Positive control	0	6.4 ± 0.48 c	3.4 ± 0.48 c	5.2 ± 0.73 c
NeemAzal T/S	0.001%	5.6 ± 0.48 bc	2.6 ± 0.48 bc	5.4 ± 0.78 bc
	0.01%	5.8 ± 0.73 bc	3.4 ± 1.47 c	5 ± 1.07 c
	0.1%	4 ± 1.24 b	1.4 ± 0.78	2.6 ± 0.78 ab
Neem leaf extract	1%5 ± 1.52 bc	2.4 ± 0.78 bc	4.8 ± 1.57 bc	
	10%	5.6 ± 0.48 bc	2.6 ± 0.48 bc	5.2 ± 0.96 bc
	20%	4.75 ± 0.84 bc	1.75 ± 0.84 ac	4 ± 1.01 ac

was similar to the negative control, but Zeck and Mukhtar et al. scales showed differences (Table 3).

In the case of 'Ceglédi' landrace, concentrations of neem leaf extract did not differ from positive control, according to all the three scales. On the other hand, 0.1% of NeemAzal T/S was significantly lower than only *M. incognita* infected treatment (Table 4).

Discussion

Although Khan et al. (1974) attributed to the toxicity of neem formulations to azadirachtin, it is evident from our *in vitro* experiment results that neem leaf extract showed better nematicidal property. Azadirachtin did not show any significant difference in the nematicidal activity which was reported by Javed et al. (2008) and Ntalli et al. (2009). Our results contradict the study of Grandison (1992), where he could not observe any effect of neem seed on J_2 larvae of *M. javanica*. But our results are in line with Abo-Elyousr et al. (2010) and Agbenin et al. (2005) as they both concluded that the neem leaf extracts were lethal to *Meloidogyne* larvae. In accordance with our results, previous investigations by several different researchers have shown 70% - 100% mortality using aqueous extracts of neem formulations as mentioned by Javed et al. (2008). This might be due to the array of different phytotoxins and chemical compounds which might work individually or synergistically, and which are water soluble (Nile et al. 2017). It could not be found which compound was responsible for the 90% and higher mortality in the case of neem leaf extracts in our study, but according to Qamar et al. (1989), kaemptro and myricetin could be the chemical compounds responsible for nematicidal activity in neem leaf extracts.

As seen in the results, in the case of 0.1% azadirachtin (NAZ), fresh shoot weight for both the landraces was lower and significantly different compared to 0 control. This is probably because the roots were adversely affected by the emulsifier used to dissolve the commercial product containing azadirachtin (i.e if the azadirachtin concentration is 0.1%, then the concentration of the emulsifier is 10%). According to the Hungarian approval document of azadirachtin, the maximum concentration of the applied spray mixture could be 0.003% against glasshouse whitefly (*Trialeurodes vaporariorum* Westwood 1856) in protected tomato (04.2/4878-1/2012. Nébih 2018), but there is no further information about the maximum concentration that can be used.

The results of the glasshouse experiment are in accordance with Agbenin et al. (2005) who used 20% fresh neem leaf extract weekly for 8 weeks on tomato plants (Roma VF) against *M. incognita*, and treatment did not differ from untreated control. According to Kankam and Sowley (2016), neem leaf powder applied to the root zone of chili pepper plants resulted the lowest root gall index next to neem seed powder and neem cake.

In the laboratory experiment, when *M. incognita* larvae came in contact continuously to the leaf extracts or product solutions, leaf extracts have stronger lethal effect. By contrast, under glasshouse conditions with weekly application, neem leaf extracts did not show the same lethal effects on the *M. incognita* larvae as compared to the laboratory conditions. As a conclusion, neem leaf extracts could be more effective against *M. incognita* with continuous and timely application either by drip irrigation or soil drenching.

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References

Abo-Elyousr, K. A., Khan, Z., El-Morsi Award, M., Abedel-Moneim, M.F. (2010): Evaluation of plant extracts and *Pseudomonas* spp. for control of root-knot nematode, *Meloidogyne incognita* on tomato. Nematropica, 40:289–299.

Agbenin, N.O., Emechebe, A.M., Marley, P.S., Akpa, A.D. (2005): Evaluation of nematicidal action of some botanicals on *Meloidogyne incognita in vivo* and *in vitro*. Journal of Agriculture and Rural Development in the Tropics. 106(1)29–39.

Anwar, S.A., Zia, A., Hussain, M., Kamran, M. (2007): Host suitability of selected plants to *Meloidogyne incognita* in the Punjab. Pakistan International Journal of Nematology. 17:144–150.

Bernard, G.C., Egnin, M., and Bonsi, C. (2017): The impact of plant-parasitic nematodes on agriculture and methods of control. In Nematology - concepts, diagnosis and control, Shah, M.M., and Mahamood, M. Eds., InTech, Rijeka, Croatia, pp. 121–151 http://dx.doi.org/10.5772/intechopen.68958

Briar, S.S., Wichman, D., and Reddy, G.V.P. (2016): Plant-parasitic nematode problems in organic agriculture. In Nandwani, D. ed., Organic farming for sustainable agriculture, Sustainable

Development and Biodiversity 9, Springer International Publishing Switzerland, pp. 107–122 https://doi.org/10.1007/978-3-319-26803-3_5

Ciancio, A. (1995): Observations on the nematicidal properties of some mycotoxins. Fundamental & Applied Nematolology. 18:451–454

Dabaj, K.H., Jenser G. (1990): Host plants of *Meloidogyne hapla* and *M. incognita* in two botanical gardens in Hungary. Nematologia Mediterranea. 18:135–137.

den Nijs, L.J.M.F., Brinkman, H., van der Sommen, A.T.C. (2004): A Dutch contribution to knowledge on phytosanitary risk and host status of various crops for *Meloidogyne chitwoodi* Golden et al. 1980 and *M. fallax* Karssen, 1996: an overview. Nematology, 6(3):303–312.

Doshi, P., Mészárosné Póss, A., Tóth, F., Szalai, M., Turóczi, G. (2018): Effect of neem-derived plant protection products on the isopod species *Porcellionides pruinosus* (Brandt, 1833). ZooKeys. 801:415–425. https://doi.org/10.3897/zookeys.801.25510

Eddaoudi, M., Ammati, M., Rammah, A. (1997): Identification of the resistance breaking populations of *Meloidogyne* on tomatoes in Morocco and their effect on new sources of resistance. Fundamental & Applied Nematolology. 20(3):285–289.

El-Sherif, A.G., Refaei, A.R., El-Naggar, M.E., Hefny, M.M. (2012): Host suitability of some medicinal plants to *Meloidogyne incognita* infection under greenhouse conditions. Journal of Plant Protection and Pathology. 3(7):693–700.

Garabedian, S., Van Gundy, S.D. (1983): Use of avermectins for the control of *Meloidogyne incognita* on tomato. Journal of Nematology. 15(4):503–510.

Grandison, G. (1992): The investigation of chemical derivatives from neem (*Azadirachta indica*) as environmentally safe method of control of plant-parasitic nematodes. In: Schmutterer, H. (Ed.), The Neem Tree. VCH, Weinheim, Germany, pp. 140.

Hunt, D.J., Handoo, Z.A. (2009): Taxonomy, identification and principal species. In Root-knot nematodes, Perry, R.N., Moens, M. (szerk.), CABI Publishing: Wallingford, UK, pp. 55–97.

Javed, N., Gowen, S.R., Inam-ul-Haq, M., Anwar, S.A. (2007): Protective and curative effect of neem (*Azadirachta indica*) formulations on the development of root-knot nematode *Meloidogyne javanica* in roots of tomato plants. Crop protection. 26:530–534. https://doi.org/10.1016/j.cropro.2006.05.003

Javed, N., Gowen, S.R., El-Hassan, S.A., Inam-ul-Haq, M., Shahina, F., Pembroke, B. (2008): Efficacy of neem (*Azadirachta indica*) formulations on biology of rootknot nematodes (*Meloidogyne javanica*) on tomato. Crop Protection. 27:36–43. https://doi.org/10.1016/j.cropro.2007.04.006

Khan, A.M., Alam, M.M., Ahmad, R. (1974): Mechanism of the control of plant parasitic nematodes as a result of the application of oil cakes to the soil. Indian Journal of Nematology. 4:93–96.

Khan, A.A., Khan, M.W. (1991): Race composition of *Meloidogyne incognita* and *M. arenaria* populations in vegetable fields in Uttar Pradesh. Supplementary to Journal of Nematology. 23(4S):615–619.

Khanna, A.S., Kumar, S. (2006): In vitro evaluation of neem-based nematicides against *Meloidogyne incognita*. Nematologia Mediterranea. 34:49–54.

Kankam, F., Sowley E.N.K. (2016): Evaluation of neem (*Azadirachta indica* L.) products for the control of root-knot nematode of chilli pepper (*Capsicum annum* L.). Archives of Phytopathology and Plant Protection, 49(5-6):111–119.https://doi.org/10.1080/03235408.2016.1157379

Kiss, L.V., Hrács, K., Nagy, P.I., Seres, A. (2018): Effects of zinc oxide nanoparticles on *Panagrellus redivivus* (Nematoda) and *Folsomia candida* (Collembola) in various test media. International Journal of Environmental Research. 12:233–243. https://doi.org/10.1007/s41742-018-0086-y

Lynn, O.M., Song, W.G., Shim, J.K., Kim, J.E., Lee, K.Y. (2010): Effects of Azadirachtin and Neem-based Formulations for the Control of Sweetpotato Whitefly and Root-knot Nematode. Journal of Korean Society and Applied Biological Chemistry. 53(5):598–604. https://doi.org/10.3839/

jksabc.2010.092

Mukhtar, T., Kayani, M.Z., Hussain, M.A. (2013): Response of selected cucumber cultivars to *Meloidogyne incognita*. Crop Protection. 44:13–17.

Nébih. Available online: https://novenyvedoszer.nebih.gov.hu/Engedelykereso/DocumentHandler.ashx?documentId=8a8082a8463a37810146f77dbaba6f26&documentName=NeemAzalTS_mod_kiskult_20140429.pdf (accessed on 22 September 2019).

Nile, A.S., Nile, S.H., Keum, Y.S., Kim, D.H., Venkidasamy, B., Ramalingam, S. (2017): Nematicidal potential and specific enzyme activity enhancement potential of neem (*Azadirachta indica* A. Juss.) aerial parts. Environmental Science and Pollution Research. 25(5):4204–4213. https://doi.org/10.1007/s11356-017-0821-5

Ntalli, N.G., Menkissoglu-Spiroudi, U., Giannakou, I.O., Prophetou-Athanasiadou, D.A. (2009). Efficacy evaluation of a neem (*Azadirachta indica* A. Juss) formulation against root-knot nematodes *Meloidogyne incognita*. Crop Protection. 28:489–494. https://doi.org/10.1016/j.cropro.2009.01.011

Qamar, F. M., Safed, M., Kapadia, Z., Seema, NN., Badar, Y. (1989): Nematicidal properties of crude extracts of some indigenous plants, Part I. Pakistan Journal of Science and Industrial Research. 32:600–602

R Core Team. (2017): R: A Language and Environment for Statistical Computing. https://www.R-project.org/

Rich, J.R., Brito, J.A., Kaur, R., Ferrell, J.A. (2008): Weed species as hosts of *Meloidogyne*: A review. Nematropica. 39:157–185

Sasser, J.N. (1980): Root-knot nematode: A global menace to crop production. Plant Disease. 198(64):36–41. https://doi.org/10.1094/PD-64-36

Sahu, S., Patra, M.K., Dash, B. (2018): Management of Root Knot Nematode (*Meloidogyne incognita*) in Tomato (cv. Pusa ruby) using Different Oil Cakes. International Journal of Current Microbiology Applied Science. 7(3):2527–2532.

Siddiqui, M.A., Alam, M.M. (1987): Efficacy of seed dressing with extracts of neem and persian lilac against *Meloidogyne incognita* and *Rotylenchulus reniformis*. Nematologia Mediterranea. 15:399–403

Singh, S.P., Ahmad, M., Khan, A.M., Saxena, S.K. (1980): Effect of seed treatments with certain oilcakes or nematicides on the growth of tomato and on rhizosphere population of nematodes and fungi. Nematologia Meditteranea. 8:193–198

Schmutterer, H. (1988): Potential of azadirachtin containing pesticides for integrated pest control in developing and industrialized countries. Journal of Insect Physiology. 34:713–719

Tzortzakakis, E.A., Vieira dos Santos, M.-C., and Conceição, I. (2016): An update on the occurrence of resistance-breaking populations of root-knot nematodes (*Meloidogyne* spp.) on resistant tomato in Greece with six new records from Crete. Hellenic Plant Protection Journal. 9. 60–65. https://doi.org/10.1515/hppj-2016-0007

Walker, J.T. (1995): Garden herbs as hosts for southern root-knot nematode [*Meloidogyne incognita* (Kofoid & White) Chitwood, race 3]. Horticultural Science. 30(2):292–293. https://doi.org/10.21273/HORTSCI.30.2.292

Wesemael, W.M.L., Viaene, N., Moens, M. (2011): Root-knot nematodes (*Meloidogyne* spp.) in Europe. Nematology. 13(1):3–16. https://doi.org/10.1163/138855410X526831

Yadav, S., Patil, J., Kumar, A. (2018): Bio-nematicidal effect of *Azadirachta indica*, against *Meloidogyne incognita* in tomato. International Journal of Chemical Studies. 6(3):2757–2761.

Zeck, W.M. (1971): Ein Bonitierungsschema zur Feldauswertung von Wurzelgallenbefall. Pflanzenschutz – Nachrichten Bayer. 24(1):144–147.

Complementation of wild strawberry (*Fragaria vesca* L.) *SPATULA* (*FvSPT*) and *SPIRAL* (*FvSPR*) genes in *Arabidopsis thaliana*

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Abstract: This study assessed the function of genes involved in wild strawberry (*Fragaria vesca* L.) fruit development and maturation to better understand the mechanism of non-climacteric fruit ripening. *SPATULA* (*FvSPT*) and *SPIRAL* (*FvSPR*) genes of *Fragaria vesca* displayed differential expression between the green and red ripening stages. *SPT*, which encodes a bHLH transcription factor, was characterized in *Arabidopsis thaliana* L. where its recessive mutation caused degenerative carpel and fruit development. The *spt* mutant of *A. thaliana* had shorter, smaller, and wider spatula-shaped siliques than the wild type. *SPT* was expressed throughout the development of marginal and transmission tract tissues, confirming its role in regulating the growth of these tissues. Two *A. thaliana SPIRAL* genes, *SPR1* and *SPR2*, are required for directional control of cell elongation. Recessive mutations in either of these genes decreased anisotropic growth of endodermal and cortical root cells and etiolated hypocotyls and caused right-handed helical growth in epidermal cells. The strawberry *SPATULA* (*FvSPT*) and *SPIRAL* (*FvSPR*) genes were amplified and *spt* and *spr* mutant *A. thaliana* plants were transformed with *FvSPT::pGWB401*, *FvSPR1-1::pGWB401* and *FvSPR1-2::pGWB401* vector constructs. Silique length and seed number/silique in the *A. thaliana spt* mutant were effectively complemented by *FvSPT* whereas *spr* was almost fully complemented by *FvSPR1-2*, but not by *FvSPR1-1*.

Keywords: *bHLH* gene; spatula shape-silique; helical root growth; *spr* and *spt* mutants

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Introduction

Fruits are of two ripening types, climacteric, such as in tomato, apple, or banana, or non-climacteric, such as in wild strawberry (*Fragaria vesca* L.), grape, or orange. Climacteric ripening is accompanied by enhanced ethylene production, but this phenomenon cannot be observed in non-climacteric fruit (Chen et al. 2018). However, this categorization is not too stringent, and several studies have reported the regulatory function of ethylene in controlling gene expression during non-climacteric maturation (Li et al. 2016; Megías et al. 2016; Kou and Wu 2018; Tadiello et al. 2018). Investigating and assessing the genes involved in strawberry ripening can contribute to a better understanding of the non-climacteric process in this fruit crop. *FaSPT* (*FaSPATULA*; GeneBank accession no. *AY679615*) is one of the genes that displayed altered expression during strawberry ripening (Balogh et al. 2005; Tisza et al. 2010).

Recessive mutations of the *SPATULA* (*SPT*) gene in *Arabidopsis thaliana* L. (*spt1* and *spt2*) cause degenerative carpel development and a transmission tract within the style and septum is absent (Alvarez and Smyth 1999). These phenomena are accompanied by inhibited growth and a decrease in the number of ovules. Anatomical gaps caused by rips can mostly be observed in carpel tips and the stigma. In *spt* mutants, the transmission tract and style within the septum bring about an extracellular matrix. Despite this anatomical deformation, fertilization can take place, but at a low frequency. Siliques of *spt* mutants are smaller, broader in the

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center and terminus than wild type (WT) siliques, and their shape is spatula-like (Alvarez and Smyth 1998). The *SPT* gene encodes a basic-helix-loop-helix (bHLH) transcription factor that is continuously expressed in the marginal tissues of developing carpels, where it is also likely responsible for their further growth (Bowman and Smyth 1999). Heisler et al. (2001) examined the transcription factors that influenced *SPT* expression in *A. thaliana* and showed that *CRABS CLAW* and *AGAMOUS* genes, which contribute to carpel development (Alvarez and Smyth 2002; Lee et al. 2005), did not impact *SPT* expression, and that *SPT* played a role in flower organogenesis. *SPT* is a homologue of the phytochrome-interacting factor (PIF) which regulates seed dormancy (Josse et al. 2011). Groszmann et al. (2011) found similarity between *SPT* and *ALCATRAZ* (*ALC*) genes, claiming that both were essential in flower and fruit development, and that *A. thaliana alc* mutants could be successfully complemented with *35S::SPT* vectors. Zumajo-Cardona et al. (2017) isolated *paleo*, *SPT* and *ALC* genes from different plants and examined their gene expression and conserved regions, and also performed phylogenetic analyses, noting that these genes may play a role in early floral organ development and specification in *Bocconia frutescens* L. Makkena and Lamb (2013) investigated the role of *SPT* in the regulation of root meristem development in strawberry where its expression increased as fruit ripening progressed, but decreased in response to wounding, auxin and ethylene. In strawberry, RNAi-based gene silencing of *SPT* retarded fruit development (Tisza et al. 2010).

Members of the *SPIRAL* (*SPR*) gene family encode small proteins that regulate the organization of microtubules by affecting cell growth and elongation (Furutani et al. 2000; Nakajima et al. 2004). Members of the *SPR* gene family in *A. thaliana* are classified into two main categories, *spr1* and *spr2*, and five subgroups of *spr1*, *spr1-1* to *spr1-5* (Nakajima et al. 2006). *A. thaliana* plants harbouring a mutant *SPR* gene develop roots with characteristic helical growth. Epidermal cell rows of roots of spr mutants in *A. thaliana* are twined resulting in left-handed helical growth, and cortical cells of etiolated *spr* hypocotyls showed microtubule arrays with irregular orientations (Furutani et al. 2000). The *SPR2* gene codes for a protein that binds to a plant-specific microtubule (Shoji et al. 2004). Mutations in the *SPR2* gene may result in right-handed helical growth in hypocotyls, petioles and petals (Furutani et al. 2000; Buschmann et al. 2004). Using cDNA-AFLP, Balogh et al. (2005) identified the *FaSPR* gene (C11M32M003) from cultivated strawberry (*Fragaria* × *ananassa* Duch.). Polgári et al. (2010) analysed the cDNA-AFLP fragment and the full-length cDNA (AY695666) of *FaSPR,* showing over 60% homology at the nucleotide level with two gene groups in *A. thaliana* and other plants.

The complementation test is a very efficient tool for functional genomic analysis. In the plant kingdom, the model plant *A. thaliana*, with its well-known genome, has plenty of natural and induced mutants, which are used to prove similar or analogous functions of genes isolated from different organisms (Groszmann et al. 2011).

Our aim was to functionally characterize the *Fragaria vesca SPATULA* (*FvSPT*) and *SPIRAL* (*FvSPR*) genes. To achieve this, we carried out a complementation analysis using *FvSPT* (XM_004287975; LOC101290893), *FvSPR1-1* (XM_004297177; LOC01307108) and *FvSPR1-2* (XM_004299243; LOC10130 9836) constructs within the pGWB401 vector and transformed *A. thaliana* Columbia mutants *spt* and *spr*. An understanding of the functionality of *FvSPT* and *FvSPR* genes would allow for their use in transgenic constructs for postharvest applications.

Materials and Methods

After sowing seeds *ex vitro* in soil, they were incubated at 4°C for 4 days, then placed in the dark. After 4 days, they were put in a 22°C climate chamber (Binder KBWF 240, Tuttlingen, Germany) and grown under an 8-h photoperiod at a photosynthetic photon flux density (PPFD) of 37 μmol m^{-2} s^{-1} provided by Biolux tubes (Osram L58W, Markham, Canada). When the first flowers appeared (14-16 days after seedling emergence; Smyth et al. 1990), they were cut. Plantlets were then grown under a 16-h photoperiod at a PPFD of 37 μmol m^{-2} s^{-1} and at 22°C. Plant material was grown at Szent István University.

FaSPT, FaSPR1-1 and *FaSPR1-2* genes (coding sequences), which were identified with cDNA-AFLP (Balogh et al. 2005; Tisza et al. 2010), together with their promoters, were applied in the complementation tests. The homology was analysed with ClustalO (https://www.ebi.ac.uk/Tools/msa/clustalo/) between *FvSPR1-1* (XM_004297177; LOC01307108), *FvSPR1-2* (XM_004299243; LOC101309836), *AtSPR1-2* (BT024676), *FvSPT* (XM_00428 7975 and AY679615) and *AtSPT* (BT026462). For primer design and *in silico* analysis of the promoter regions, we used the "*Fragaria vesca* Whole Genome v2.0a1 assembly & annotation" from GDR (http://www.rosaceae.org). Genomic DNA was isolated from 100 mg of fresh plant tissue of in-house *Fragaria vesca* L. cv. Rügen using NucleoSpin Plant II kit (Macherey-Nagel, Düren, Germany) following the manufacturer's protocol. The *SPT* gene and its promoter (6600 bp), as well as the *SPR1-1* and *SPR1-2* genes and their promoters (9647 bp and 2443 bp, respectively) were amplified with the GoTaq Long PCR Master Mix (Promega, Madison, WI, USA). A total of 100 ng of genomic DNA was used as a template in a 50 μL PCR mix. The PCR mixture consisted of 25 μL volume of GoTaq Long PCR Master Mix (2×), and 40 pmol of each primer. The PCR conditions were 95°C for 2 min followed by 35 cycles at 95°C for 30 s, 65°C for 7 min. Cycling was followed by a final incubation at 72°C for 10 min. PCR products were separated by electrophoresis on 1.0% agarose gels in 1× TAE buffer (Sambrook et al. 1989) and were detected by fluorescence under UV light (302 nm) after staining with 0.1% ethidium bromide. A molecular marker of 1 Kb Plus DNA Ladder (ThermoFisher Scientific, Carlsbad, CA, USA) was used. The PCR products were purified with Wizard® SV Gel and the PCR Clean-Up System (Promega). Purified PCR fragments were ligated into a pDONR221 entry vector (Life Technologies, Carlsbad, CA, USA). The pGWB401 vector (Nakagawa et al. 2007; Tanaka et al. 2011) was used to establish plant transformation constructs containing full-length genomic clones of *FvSPT, FvSPR1-1* and *FvSPR1-2* genes (i.e., containing promoters and coding sequences). *A. thaliana spt* and *spr* mutants of Columbia (Col), purchased from the Eurasian Arabidopsis Stock Centre NASC (http://arabidopsis.info/), were grown under an 8-h photoperiod at a PPFD of 37 μmol m^{-2} s^{-1} provided by Biolux tubes (Osram L58W, Markham, Canada), and at 22°C in a climatic chamber (Binder KBWF 240, Tuttlingen, Germany). The *spr1-2* Col mutant (NASC ID: N6547) has defective directional cell elongation, abnormal cortical microtubule function and exhibits right-handed helical growth in roots, which are caused by the *SPR1-2* allele (At1g69230; GenBank: BT26462) mutation by sequence tagged T-DNA insertion line. The *spt* Col mutant (NASC ID: N857133) has a T-DNA insertion in the *SPT* gene (At4g36930; GenBank: BT024676) on chromosome 4, position 17414295 on TAIR10.

Genetic transformation of *spr1-1/spr1-2* and *spt* mutants was carried out when secondary

Table 1. Primer names and sequences applied in RT-qPCR.

Primer name	Position of primer	Sequence (5′ – 3′)	Amplicon length
FvSPR1	forward	TGCAGATGGCTCAACTCAA	280 bp
	reverse	ACCTGGGAAAGGGTGGAGTA	
FvSPR2	forward	TGTATGAATTACGTAACCAT	178 bp
	reverse	TTCTCTTTCGACACTCGTC	
FvSPT	forward	ACTATTTAAAATTAAAAGAA	197 bp
	reverse	ATTAGGAAATCCACTCAGACA	
FvGAPDH	forward	AGGTTGTGCTGGTAATGGAA	218 bp
	reverse	ATTGCAGTGGTGGATACCTT	

flowering (about 1 month after seedling formation) started. In-house *Agrobacterium tumefaciens* GV3101 strain was used for floral dip transformation (Clough and Bent 1998), which was repeated when the tertiary flowers appeared after 1-2 weeks. Seeds were harvested after about 6 weeks then sown in soil. Transformants were selected by treating plants with kanamycin solution (Duchefa, Haarlem, The Netherlands) (Xiang et al. 1999). Specifically, two-leaf *A. thaliana* plantlets were sprayed consecutively with 100, 200 then 400 mg/mL kanamycin for 3 days, 1 week and 2 weeks, respectively after sowing seeds. Plants that survived the third spray were analysed by PCR with a Phire Plant Direct PCR Kit (ThermoFisher Scientific, Carlsbad, CA, USA). The PCR mixture consisted of 10 μL of Phire Plant PCR Buffer (2×), 40 pmol of each primer, 0.4 μL of Phire Hot Start II DNA Polymerase and 0.5 μL of diluted plant tissue. The PCR conditions were 98°C for 5 min followed by 40 cycles at 98°C for 5 s, 60°C for 5 s and 72°C for 20 s. Cycling was followed by a final incubation of 72°C for 1 min. PCR products were separated by electrophoresis based on the same protocol that was used for promoter PCR. The PCR-positive T_1 individuals were grown in a climatic chamber until T_4. The T_3 and T_4 individuals were analysed with RT-qPCR for quantification of the transgene expression and determination of transgene copy number (Fletcher et al. 2014). Vector construction and genetic transformation were conducted at Szent István University.

The T_3 and T_4 plants with one transgene copy were examined; we observed and measured their habit, roots and siliques (three biological and technical replicates), and the number of seeds and siliques (three biological and technical replicates) was determined and compared to wild type (WT) and mutant Col plants. Data was analysed statistically in SPSS version 22 (SPSS Inc., IBM Corp., Armonk, NY, USA). Following mean separation by ANOVA using Windows Microsoft Excel (2017), statistical significance was determined using Tukey's multiple range test (P<0.001). Statistical analyses were carried out at the University of Debrecen, IAREF, Research Institute of Nyíregyháza.

To prove the transcription of the *FvSPT, FvSPR1-1* and *FvSPR1-2* genes, total RNA was isolated from *F. vesca* (cv. Rügen) and the transformant *A. thaliana* plants. Total RNA was applied to RT-qPCR with primers designed for the exon-exon junction of *FvSPT, FvSPR1-1* and *FvSPR1-2*, as well as the *GAPDH* housekeeping gene (primer sequences are listed in Table 1). We calculated the transformation efficiency based on the number of transformants/number of florets dipped in the transformation (floral dip) solution.

Silique length of T_3 and T_4 progeny (80 siliques per generation, three biological replicates) was measured and seeds were counted under a SMZ-161-BL stereomicroscope (Motic; Hong Kong, China). RT-qPCR and microscopic analyses were carried out at the University of Debrecen, IAREF, Research Institute of Nyíregyháza.

Results and Discussion

In this study, we isolated the *F. vesca FvSPT*, *FvSPR1-1* and *FvSPR1-2* genes (Balogh et al. 2005; Polgári et al. 2010), which showed altered expression in the course of fruit ripening, and introduced them into *A. thaliana spt* and *spr1-2* mutants with the objective of trying to complement mutated functions. Using *in silico* analysis for the promoter regions and genes, we determined the *FvSPT*, *FvSPR1-1* and *FvSPR1-2* genes and their promoters based on homology with the At1g69230 (Figure 1) and At4g36930 (Figure 2) genes in *F. vesca* genomic sequences (Shulaev et al. 2011) and GDR data (http://www.rosaceae.org). Homology was 84.03%, 69.45% and 74.24% between *FvSPT* (XM_004287975) and *AtSPT* (At4g36930), *FvSPR1-1* (XM_004297177) and *AtSPR1-2* (At1g69230), and *FvSPR1-2* (XM_004299243) and *AtSPR1-2* (At1g69230), respectively. We amplified the *FvSPT* (6600 bp), *FvSPR1-1* (9647 bp) and *FvSPR1-2* (2443 bp) genes, including their promoters. After constructing the *FvSPT::pGWB401*, *FvSPR1-1::pGWB401* and *FvSPR1-2::pGWB401* vector constructs, we confirmed, using colony PCR, that the vectors carried the inserts. The *A. thaliana spt* and *spr* mutants (60 plants/line) were transformed with the vector constructs. In germinated plants that survived three-step kanamycin selection (3 days, one week and two weeks after germination) with 100 mg/mL, 200 mg/mL and 400 mg/mL, respectively, we confirmed that the plants carried the *FvSPT*, *FvSPR1-1* and *FvSPR1-2* genes by applying direct PCR with the specific gene of interest using PCR primers for the putative transformed plants and RT-qPCR. Average transformation efficiency for the three genes was 0.38% with only one transformation at secondary flowering. When redefining the transformation efficiency as the number of transformants/number of seeds set and we used two transformation processes (when secondary and tertiary flowers appeared), then transformation efficiency was much higher (7.6%). After these plants developed until 6 weeks, it was possible to compare the phenotype of the WT Col silique (Figure 3A), the *FvSPT*-complemented plants (Figure 3B), and the *spt* mutant (Figure 3C).

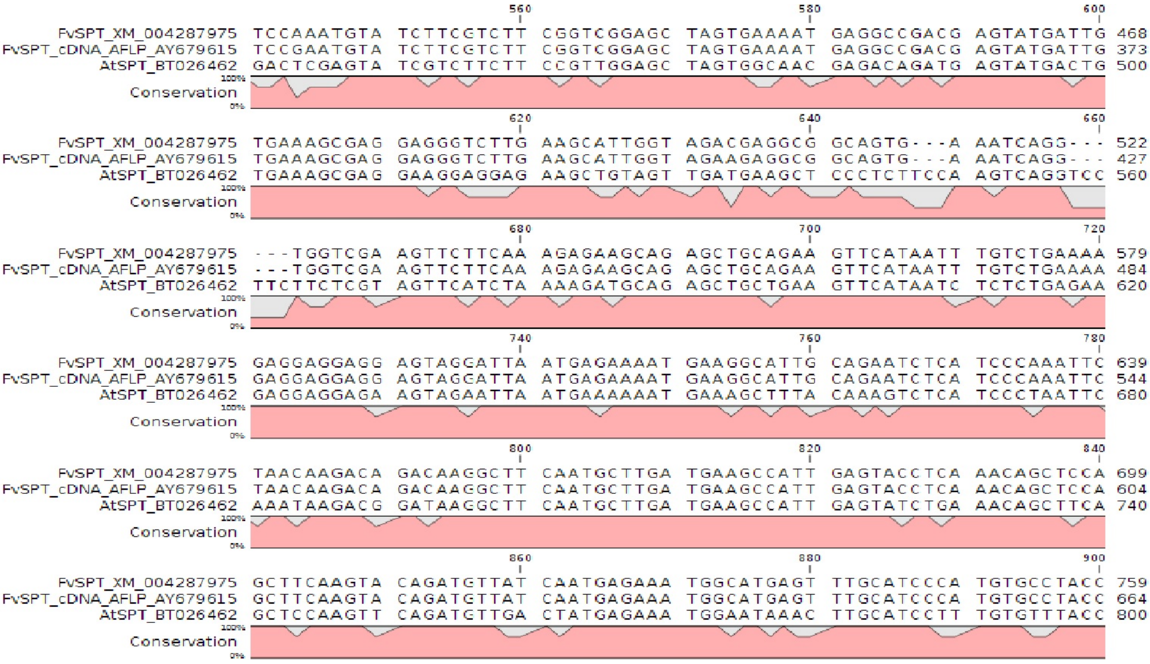
The *spt* mutant plants were significantly shorter (Figure 4C) and had shorter siliques (Figure 5) than WT (Figure 4A). The successfully complemented *FvSPT* transformant was significantly taller than the *spt* mutant (Figure 4D), while its non-malformed silique resembled that of WT (Figure 3), demonstrating that the *FvSPT* gene was able to effectively compensate for the missing silique-related function of *spt*.

When silique length (from an average of 12 siliques/plant) of 80 plants of WT, *spt* and *spr* mutants and complemented Col mutants were compared, the *spt* mutant displayed significantly shortest silique length (3.8 mm), while WT as well as the *FvSPT/FvSPR1-1/FvSPR1-2*-complemented plants had significantly longer siliques (12.8-13.3 mm). Relative to the *spr* mutant, silique length was significantly increased only in the *FvSPR1-1*-complemented plants (Figure 5).

The *spt* mutant produced fewest

```
                                                           140                            160                            180
                                                             |                              |                              |
FvSPR1_1_XM_004297177 TGGAGTCAGC AGCGGTGGGG GTCAGAGTTC CTTGGACTAT CTTTTTGGGA GTGGAGAGGC 180
      FvSPR_AY695666 TGGAGTCAGC AGCGGTGGGG GTCAGAGTTC CTTGGACTAT CTTTTTGGGA GTGGAGGGGA 134
   AtSPR1_2_BT024676 TGGAGTTAGT GCAGGTGGAG GACAAAGTTC TTTGGGATAT CTTTTTGGGA GCGGAGAGGC 68
FvSPR1_2_XM_004299243 TGGAGGGAGT AGTGGAGGGG GAGGCAGTTC TTTGGGCTAT CTTTTTGGAG GTGGAGAAGA 152
        Conservation
                                                           200                            220                            240
                                                             |                              |                              |
FvSPR1_1_XM_004297177 ---TCCAAAG CCAAGAACAA ACAATGTCCA AGCTGCCCCA AAGGAAGTGC AGGCTGTGAA 237
      FvSPR_AY695666 ---TCCAAAG CCAAGAACAA ACAATGTCCA AGCTGCCCCA AAGGAAGTGC AGGCTGTGAA 191
   AtSPR1_2_BT024676 ---TCCAAAG CTA------- ---------- -GCAGCCGTT AACAAAACTC CAGCTGAAAC 107
FvSPR1_2_XM_004299243 AGGTCAGAAT CCTGCCCCCA AAAAGGTTGT AACTCCC--- ---AAGGTTC AGGTGGAAGT 206
        Conservation
                                                           260                            280                            300
                                                             |                              |                              |
FvSPR1_1_XM_004297177 TGAACCTACT CCCAAACCAA CGGCTTCTCC TCCCCCTCCC AAACCTGAAC CAGTAGATAT 297
      FvSPR_AY695666 TGAACCTACT CCCAAACCAA CTGCTTCTCC TCCCCCTCCC AAACCTGAAC CAGTAGATAT 251
   AtSPR1_2_BT024676 TGAGTCTTCT GCTCATGCTC CACCTACTCA AGCTGCTGCT GCAAACG--- CTGTTGATAG 164
FvSPR1_2_XM_004299243 TGAACCTACT CCTAAGCCTA CTGCTGCTGC ACCACCACCT G--------- CGGAAGTGAT 257
        Conservation
                                                           320                            340                            360
                                                             |                              |                              |
FvSPR1_1_XM_004297177 CACCAAGCAG ATTCCTGCTG GTATCCATAG TACTTCTACA AATAACTACT TGCGTGCAGA 357
      FvSPR_AY695666 CACCAAGCAG ATTCCTGCTG GTATCCATAG TACTTCTACA AATAACTACA TGCGTGCAGA 311
   AtSPR1_2_BT024676 CATCAAACAA GTTCCTGCTG GTCTCAATAG CAACTCTGCA AACAATTACA TGCGTGCAGA 224
FvSPR1_2_XM_004299243 CAATAAGGAG ACTCCAGCAG GTGTTCCTGG GAACACTTCA AACAACTACT TTAGAGCTGA 317
        Conservation
```

Figure 1. Homology between *FvSPR1-1* (XM_004297177), *FvSPR* (AY695666), *AtSPR1-2* (BT024676) and *FvSPR1-2* (XM_004299243).



```
                                                                560                            580                            600
                                                                  |                              |                              |
       FvSPT_XM_004287975 TCCAAATGTA TCTTCGTCTT CGGTCGGAGC TAGTGAAAAT GAGGCCGACG AGTATGATTG 468
FvSPT_cDNA_AFLP_AY679615 TCCGAATGTA TCTTCGTCTT CGGTCGGAGC TAGTGAAAAT GAGGCCGACG AGTATGATTG 373
          AtSPT_BT026462 GACTCGAGTA TCGTCTTCTT CCGTTGGAGC TAGTGGCAAC GAGACAGATG AGTATGACTG 500
             Conservation
                                                                620                            640                            660
                                                                  |                              |                              |
       FvSPT_XM_004287975 TGAAAGCGAG GAGGGTCTTG AAGCATTGGT AGACGAGGCG GCAGTG---A AATCAGG--- 522
FvSPT_cDNA_AFLP_AY679615 TGAAAGCGAG GAGGGTCTTG AAGCATTGGT AGAAGAGGCG GCAGTG---A AATCAGG--- 427
          AtSPT_BT026462 TGAAAGCGAG GAAGGAGGAG AAGCTGTAGT TGATGAAGCT CCCTCTTCCA AGTCAGGTCC 560
             Conservation
                                                                680                            700                            720
                                                                  |                              |                              |
       FvSPT_XM_004287975 ---TGGTCGA AGTTCTTCAA AGAGAAGCAG AGCTGCAGAA GTTCATAATT TGTCTGAAAA 579
FvSPT_cDNA_AFLP_AY679615 ---TGGTCGA AGTTCTTCAA AGAGAAGCAG AGCTGCAGAA GTTCATAATT TGTCTGAAAA 484
          AtSPT_BT026462 TTCTTCTCGT AGTTCATCTA AAAGATGCAG AGCTGCTGAA GTTCATAATC TCTCTGAGAA 620
             Conservation
                                                                740                            760                            780
                                                                  |                              |                              |
       FvSPT_XM_004287975 GAGGAGGAGG AGTAGGATTA ATGAGAAAAT GAAGGCATTG CAGAATCTCA TCCCAAATTC 639
FvSPT_cDNA_AFLP_AY679615 GAGGAGGAGG AGTAGCATTA ATGAGAAAAT GAAGGCATTG CAGAATCTCA TCCCAAATTC 544
          AtSPT_BT026462 GAGGAGGAGA AGTAGAATTA ATGAAAAAAT GAAAGCTTTA CAAAGTCTCA TCCCTAATTC 680
             Conservation
                                                                800                            820                            840
                                                                  |                              |                              |
       FvSPT_XM_004287975 TAACAAGACA GACAAGGCTT CAATGCTTGA TGAAGCCATT GAGTACCTCA AACAGCTCCA 699
FvSPT_cDNA_AFLP_AY679615 TAACAAGACA GACAAGGCTT CAATGCTTGA TGAAGCCATT GAGTACCTCA AACAGCTCCA 604
          AtSPT_BT026462 AAATAAGACG GATAAGGCTT CAATGCTTGA TGAAGCCATT GAGTATCTGA AACAGCTTCA 740
             Conservation
                                                                860                            880                            900
                                                                  |                              |                              |
       FvSPT_XM_004287975 GCTTCAAGTA CAGATGTTAT CAATGAGAAA TGGCATGAGT TTGCATCCCA TGTGCCTACC 759
FvSPT_cDNA_AFLP_AY679615 GCTTCAAGTA CAGATGTTAT CAATGAGAAA TGGCATGAGT TTGCATCCCA TGTGCCTACC 664
          AtSPT_BT026462 GCTCCAAGTT CAGATGTTGA CTATGAGAAA TGGAATAAAC TTGCATCCTT TGTGTTTACC 800
             Conservation
```

Figure 2. Homology between *FvSPT* (XM_004287975), *FvSPT* cDNA (AY679615) and *AtSPT* (BT026462).

seeds/silique (7.7), while WT Col produced the most (47.7) (Figure 6). The spr mutant, which had significantly shorter siliques than the WT (Figure 5), also developed significantly fewer seeds (37.5) than WT (47.7). The number of seeds in *FvSPT*-

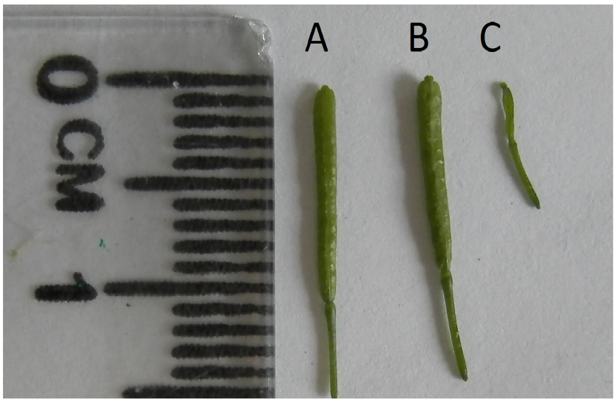


Figure 3. Siliques of *Arabidopsis thaliana* Columbia wild type (A); *FvSPT*-complemented Columbia *spt* mutant (B) and Columbia *spt* mutant (C) from 6-week-old plants.

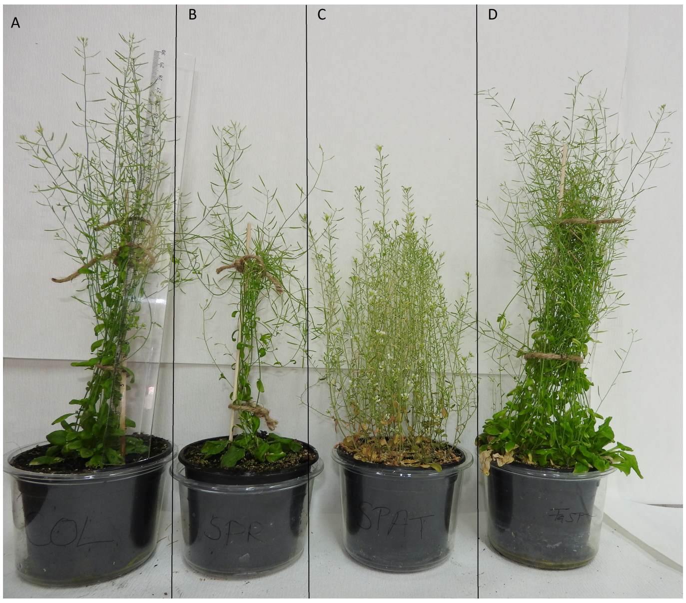


Figure 4. Habit of 8-week-old plants of *Arabidopsis thaliana* Columbia wild type (A), *spiral* mutant, *spr* (B), *spatula* mutant, *spt* (C) and *FvSPT1-2*-complemented (D).

complemented siliques was, as expected, significantly higher than the *spt* mutant, but significantly lower than the WT control (Figure 6). Similarly, the number of seeds in *FvSPR1-1*- and *FvSPR1-2*-complemented siliques was, also as expected, significantly higher than the *spr* mutant, but significantly lower than the WT control (Figure 6). The *FvSPR* complementation was not as pronounced as the *FvSPT* complementation, but the trait (number of seeds/silique) was still complemented, nonetheless. Despite these differences, *FvSPT*-, *FvSPR1-1*- and *FvSPR1-2*-complemented genotypes displayed the same phenotype as the WT control. As one example, see the comparison between WT and *FvSPT1-2*-complemented plants in Figure 4.

A contrast of the phenotypes of *FvSPR1-1*- and *FvSPR1-2*-complemented, WT and *spr* mutant plants can be seen in Figure 7 and

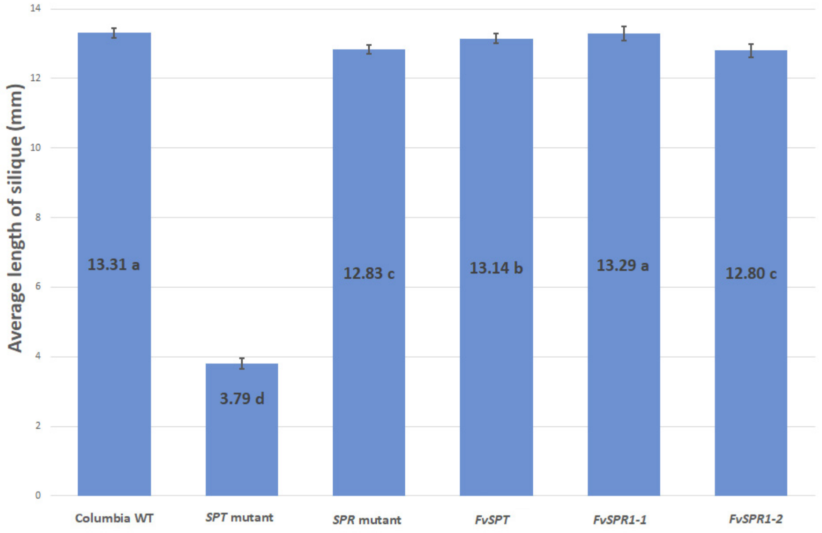


Figure 5. Average length of silique (mm). Col WT: Columbia wild type; *spt* mutant: *spatula* mutant Col; *spr* mutant: *spiral* mutant Col; *FvSPT*: *SPATULA* gene of strawberry (*Fragaria vesca* L.) complemented *A. thaliana* Col; *FvSPR1-1*: *SPIRAL1-1* gene of strawberry complemented *A. thaliana* Col; *FvSPR1-2*: *SPIRAL1-2* gene of strawberry complemented *A. thaliana* Col. Different letters within blue bars indicate significant differences with Col WT based on one-way ANOVA (Tukey's multiple range test; $P<0.001$); 80 plants/experiment/line and three biological replicates.

Figure 8. The *spr* mutation could only be restored by *FvSPR1-2* (Figure 7). In the case of *FvSPR1-1* plants, similar helical roots developed as in the *spr* mutants. There are three recessive *A. thaliana spr* mutants, *spr1-1, spr1-2* and *spr1-3* (Nakajima et al. 2006). We used the *spr1-2* mutant in our experiment, so this could theoretically only be complemented by *FvSPR1-2*, and not by *FvSPR1-1* (*FvSPR1-1*-complemented plants continued to have helical roots, i.e., the mutant phenotype was not corrected), indicating that *FvSPR1-1* does not have the same function.

To show the expression of *FvSPT*, *FvSPR1-1* and *FvSPR1-2* genes, we isolated total RNA from the transformants and confirmed the transcription of these genes by RT-qPCR. The primers designed for exon-exon junctions amplified 146 bp and 265 bp on the cDNA and gDNA, respectively. The *A. thaliana GAPDH* gene was used as the reference, generating a 130 bp fragment. RT-qPCR results prove that the *FvSPT::pGWB401*, *FvSPR1-1::pGWB401* and *FvSPR1-2::pGWB401* constructs functioned in the *AtSPT*-complemented *A. thaliana* plants.

Our experimental results attested that *FaSPT* and *FaSPR* genes isolated from octoploid *F.* × *ananassa* by cDNA-AFLP (Balogh et al. 2005), show sequence similarity not only to *A. thaliana AtSPT*, *AtSPR1-1* and *AtSPR1-2*, but also as well as with diploid strawberry (*F. vesca*) *FvSPT*, *FvSPR1-1* and *FvSPR1-2*, but they also have the ability to complement the *A. thaliana* mutant phenotype (*spt* and *spr1-2* mutant Columbia). Similarly to the result of Heisler et al. (2001), in which the WT *AtSPT2* allele complemented the *At-spt2* mutation, *FvSPT* had the same effect, confirming the same functional ability of this strawberry-derived gene. The literature indi-

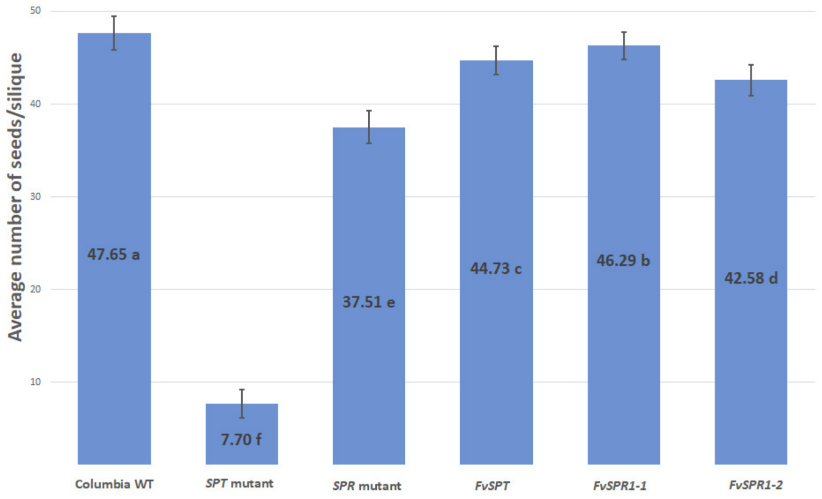


Figure 6. Average number of seeds/silique. Col WT: Columbia wild type; *spt* mutant: *spatula* mutant Col; *spr* mutant: *spiral* mutant Col; *FvSPT*: *SPATULA* gene of *Fragaria vesca* complemented *A. thaliana* Col; *FvSPR1-1*: *SPIRAL1-1* gene of *F. vesca* complemented *A. thaliana* Col; *FvSPR1-2*: *SPIRAL1-2* gene of *F. vesca* complemented *A. thaliana* Col. Different letters within blue bars indicate significant differences with Col WT based on one-way ANOVA (Tukey's multiple range test; $P<0.001$); 80 plants/experiment/line and three biological replicates.

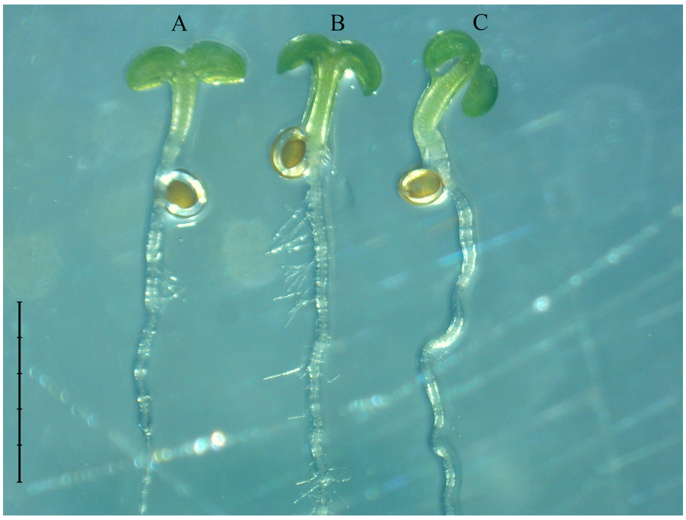


Figure 7. Roots of *FvSPR1-2* complemented *Arabidopsis thaliana* Columbia (A), Columbia wild type (B) and *spiral* mutant (*spr*) of *A. thaliana* Columbia (C) plants after 1 week (Scale bar: 5 mm).

cates that mutant *SPR1-1*, *SPR1-2* and *SPR1-3* genes cause the same abnormal root malformation symptoms in *A. thaliana* (Furutani et al. 2000). We showed, however, that only *FvSPR1-2* was able to restore the dysfunctional *spr1-2*.

Figure 8. Roots of *spiral* mutant of *Arabidopsis thaliana* Columbia (A), Columbia wild type (B) and *FvSPR1-2*-complemented Columbia (C) plants after 2 weeks (Scale bar: 5 mm).

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References

Alvarez, J., Smyth, D.R. (1998): Genetic pathways controlling carpel development in *Arabidopsis thaliana*. Journal of Plant Research 111: 295-298. https://doi.org/10.1007/BF02512187

Alvarez, J., Smyth, D.R. (1999): *CRABS CLAW* and *SPATULA*, two *Arabidopsis* genes that control carpel development in parallel with *AGAMOUS*. Development 126: 2377-2386.

Alvarez, J., Smyth, D.R. (2002): *CRABS CLAW* and *SPATULA* genes regulate growth and pattern formation during gynoecium development in *Arabidopsis thaliana*. International Journal of Plant Sciences 163(1): 17-41. https://doi.org/10.1086/324178

Balogh, A., Koncz, T., Tisza, V., Kiss, E., Heszky, L. (2005): Identification of ripening-related genes in strawberry fruit by cDNA-AFLP. International Journal of Horticultural Science 11(4): 33-41. DOI: https:/doi.org/10.31421/IJHS/11/4/602

Bowman, J.L., Smyth, D.R. (1999): *CRABS CLAW*, a gene that regulates carpel and nectary development in *Arabidopsis*, encodes a novel protein with zinc finger and helix-loop-helix domains. Development 126: 2387-2396.

Buschmann, H., Fabri, C.O., Hauptmann, M., Hutzler, P., Laux, T., Lloyd, C.W., Schäffner, A.R. (2004): Helical growth of the *Arabidopsis* mutant *tortifolia1* reveals a plant-specific microtubule-associated protein. Current Biology 14: 1515-1521.https://doi.org/10.1016/j.cub.2004.08.033

Chen, Y., Grimplet, J., David, K., Castellarin, S.D., Terol, J., Wong, D.C., Gambetta, G.A. (2018): Ethylene receptors and related proteins in climacteric and non-climacteric fruits. Plant Science 276: 63-72. https://doi.org/10.1016/j.plantsci.2018.07.012

Clough, S.J., Bent, A.F. (1998): Floral dip: a simplified method for *Agrobacterium*-mediated transformation of *Arabidopsis thaliana*. The Plant Journal 16: 735-743. https://doi.org/10.1046/j.1365-313x.1998.00343.x

Fletcher, S.J. (2014): qPCR for quantification of transgene expression and determination of transgene copy number. In: Fleury, D., Whitford, R. (ed.) Crop Breeding (pp. 213-237). Humana Press, New York, NY. https://doi.org/10.1007/978-1-4939-0446-4_17

Furutani, I., Watanabe, Y., Prieto, R., Masukawa, M., Suzuki, K., Naoi, K., Hashimoto, T. (2000): The *SPIRAL* genes are required for directional control of cell elongation in *Arabidopsis thaliana*. Development 127: 4443-4453.

Groszmann, M., Paicu, T., Alvarez, J.P., Swain, S.M., Smyth, D.R. (2011): *SPATULA* and *ALCATRAZ*, are partially redundant, functionally diverging bHLH genes required for *Arabidopsis* gynoecium and fruit development. The Plant Journal 68: 816-829. https://doi.org/10.1111/j.1365-313X.2011.04732.x

Heisler, M.G.B., Atkinson, A., Bylstra, Y.H., Walsh, R., Smyth, D.R. (2001): *SPATULA*, a gene that controls development of carpel margin tissues in *Arabidopsis*, encodes a bHLH protein. Development 128: 1089-1098.

Josse, E., Gan, Y., Torrent, J., Stewart, K., Gilday, A.D., Jeffree, E.C., Vaistij, F., García, J.F., Nagy, F., Graham, I.A. (2011): A *DELLA* in disguise: *SPATULA* restrains the growth of the developing *Arabidopsis* seedling. The Plant Cell 23: 1337-1351.https://doi.org/10.1105/tpc.110.082594

Kou, X., Wu, M. (2018): Characterization of climacteric and non-climacteric fruit ripening. In: Guo, Y. (ed.) Plant Senescence (pp. 89-102). Humana Press, New York, NY. https://doi.org/10.1007/978-1-4939-7672-0_7

Lee, J.Y., Baum, S.F., Alvarez, J., Patel, A., Chitwood, D.H., Bowman, J.L. (2005): Activation of *CRABS CLAW* in the nectaries and carpels of *Arabidopsis*. The Plant Cell 17(1): 25-36. https://doi.org/10.1105/tpc.104.026666

Li, L., Lichter, A., Chalupowicz, D., Gamrasni, D., Goldberg, T., Nerya, O., Porat, R. (2016): Effects of the ethylene-action inhibitor 1-methylcyclopropene on postharvest quality of non-climacteric fruit crops. Postharvest Biology and Technology 111: 322-329. https://doi.org/10.1016/j.postharvbio.2015.09.031

Makkena, S., Lamb, R.S. (2013): The bHLH transcription factor *SPATULA* regulates root growth by controlling the size of the root meristem. BMC Plant Biology 13(1): 1. https://doi.org/10.1186/1471-2229-13-1

Megías, Z., Martínez, C., Manzano, S., García, A., del Mar Rebolloso-Fuentes, M., Valenzuela, J. L. and Jamilena, M. (2016): Ethylene biosynthesis and signaling elements involved in chilling injury and other postharvest quality traits in the non-climacteric fruit of zucchini (*Cucurbita pepo*). Postharvest Biology and Technology 113: 48-57. https://doi.org/10.1016/j.postharvbio.2015.11.001

Nakagawa, T., Kurose, T., Hino, T., Tanaka, K., Kawamukai, M., Niwa, Y., Toyooka K., Matsuoka, K., Jinbo, T., Kimura, T. (2007): Development of series of gateway binary vectors, pGWBs, for realizing efficient construction of fusion genes for plant transformation. Journal of Bioscience and Bioengineering 104: 34-41. https://doi.org/10.1263/jbb.104.34

Nakajima, K., Furutani, I., Tachimoto, H., Matsubara, H., Hashimoto, T. (2004): *SPIRAL1* encodes a plant-specific microtubule-localized protein required for directional control of rapidly expanding *Arabidopsis* cells. The Plant Cell 16: 1178-1190. https://dx.doi.org/10.1105%2Ftpc.017830

Nakajima, K., Kawamura, T., Hashimoto, T. (2006): Role of the *SPIRAL1* gene family in anisotropic growth of *Arabidopsis thaliana*. Plant Cell Physiology 47: 513-522. https://doi.org/10.1093/pcp/pcj020

Polgári, D., Kalapos, B., Tisza V., Kovács, L., Kerti, B., Heszky, L., Kiss, E. (2010). *In silico* analysis of a putative *SPIRAL* gene related to strawberry ripening. Acta Agronomica Hungarica 58: 267-272. https://doi.org/10.1556/AAgr.58.2010.3.9

Sambrook, J., Fritsch, E.F., Maniatis, T. (1989): Molecular Cloning: A Laboratory Manual (No. Ed. 2). Cold Spring Harbor Laboratory Press, NY, USA.

Shoji, T., Narita, N.N., Hayashi, K., Asada, J., Hamada, T., Sonobe, S., Hashimoto, T. (2004): Plant-specific microtubule-associated protein *SPIRAL2* is required for anisotropic growth in *Arabidopsis*. Plant Physiology 136: 3933-3944. https://doi.org/10.1104/pp.104.051748

Shulaev, V., Sargent, D.J., Crowhurst, R.N., Mockler, T.C., Folkerts, O., Delcher, A.L., Jaiswal, P., Mockaitis, K., Liston, A., Mane, S.P., Burns, P., Davis, T.M., Slovin, J.P., Bassil, N., Hellens, R.P., Evans, C., Harkins, T., Kodira, C., Desany, B., Crasta, O.R., Jensen, R.V., Allan, A.C., Michael, T., P., Setubal, J.C., Celton, J.M. (2011): The genome of woodland strawberry (*Fragaria vesca*). Nature Genetics 43: 109-116. https://doi.org/10.1038/ng.740

Smyth, D.R., Bowman, J.L., Meyerowitz, E.M. (1990): Early flower development in *Arabidopsis*. The Plant Cell 2(8): 755-767. https://doi.org/10.1105/tpc.2.8.755

Tadiello, A., Busatto, N., Farneti, B., Delledonne, M., Velasco, R., Trainotti, L., Costa, F. (2018): The interference of the ethylene perception machinery leads to a re-programming of the fruit quality-related transcriptome and induces a cross-talk circuit with auxin in apple. Acta Horticulturae 1206: 69-74. https://doi.org/10.17660/ActaHortic.2018.1206.10

Tanaka, Y., Nakamura, S., Kawamukai, M., Koizumi, N., Nakagawa, T. (2011): Development of a series of gateway binary vectors possessing a tunicamycin resistance gene as a marker for the transformation of *Arabidopsis thaliana*. Bioscience, Biotechnology and Biochemistry 75: 804-807. https://doi.org/10.1271/bbb.110063

Tisza, V., Kovács, L., Balogh, A., Heszky, L., Kiss, E. (2010): Characterization of *FaSPT,* a *SPATULA* gene encoding a bHLH transcriptional factor from the non-climateric strawberry fruit. Plant Physiology and Biochemistry 48: 822-826. https://doi.org/10.1016/j.plaphy.2010.08.001

Xiang, C., Han, P., Oliver, D.J. (1999): In solium selection for *Arabidopsis* transformants resistant to kanamycin. Plant Molecular Biology 17: 59-65. https://doi.org/10.1023/A:1007588001296

Zumajo-Cardona, C., Ambrose, B.A., Pabón-Mora, N. (2017): Evolution of the *SPATULA/ALCATRAZ* gene lineage and expression analyses in the basal eudicot, *Bocconia frutescens* L. (Papaveraceae). EvoDevo 8(1): 5. https://doi.org/10.1186/s13227-017-0068-8

How do Central European forest stands respond to climate change - Review

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Abstract: Current global climate change alters the behaviour of species, and this can be also witnessed when investigating the species composition, the structure and the processes of different ecosystems. Today a growing number of researches deal with the climatic exposures of forests, mostly focusing on the responses of the dominant climate susceptible tree species and the direct and indirect impacts of climate change experienced in forests. During literature review we looked for publications investigating the experienced impacts of climate change in our region including responses witnessed in growth, mortality and regeneration capacity of tree species. From different climatic exposures the impacts of increased CO_2 level, nitrogen deposition, milder winters and more droughty and drier summers can be experienced most in our region. Publication's statements on the changes of tree species and forest stands have been grouped and summarized according to the characteristic exposures. Based on the literature data it can be stated that climate change variously alters the tree species composition, mixture ratio and diversity of forest stands and the outcomes of other forest dynamic processes through vitality, production and competitiveness of different tree species.

Keywords: climatic exposure; deciduous forests; climate sensitive tree species; forest dynamics; beech; oak; weather extremities

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Introduction

In the last 30-40 years atmosphere, oceans and surface of the Earth became warmer significantly increasing the average temperature values of the last 1400 years. In addition, the carbon and nitrogen cycles of ecosystems also substantially changed (Galloway 2004, IPCC 2014). At the same time, average global surface temperature values vary to a great extent, which can be experienced not only at the decade level but even on an annual basis. According to the weather data recorded since 1901, the average amount of precipitation increased in the temperate zone of northern hemisphere, but its distribution within the year considerably changed compared to the former period (IPCC 2014). Since 1950 the frequency of weather extremities has also changed. Most importantly, the number of colder weather extremities decreased, while the occurrence of extreme warm weather conditions increased, and we also had to face a growing number of heavy rainfall events in more regions (IPCC 2014, IPCC 2019). According to the different CO_2 emission scenario based climate model simulations, these weather conditions are expected to get more extreme in the future. What is more, there are some regions (e.g. the North Pole) which respond much faster than other regions of Earth. It is expected that due to global warming heat waves are getting longer and more frequent (IPCC 2014), making it even more difficult for the living creatures to survive the dry periods (Allen et al 2010). According to the recorded weather facts (the extremely hot and dry periods), 2018 can be considered a new reference year regarding hot droughts in Europe (Buras et al. 2019).

Regional datasets and tendencies are in line with the global changes, and can provide more accurate data at the local level (Szelepcsényi 2014, Dobor et al. 2015, 2016, Spinoni 2015). Regarding Europe as a whole,

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the Mediterranean Region is the most threatened area, since not only the more frequently appearing droughts but also the increasing risk and occurrence of forest fires are expected there (Lindner 2010). At the same time, huge regional and local differences can be witnessed when analysing climate change. Hlásny et al. (2014) draw the attention to the "hotspots" of the Carpathian region, which may be highly vulnerable to climate change due to the loss of biodiversity. Climate models predict stronger Mediterranean weather characteristics, more frequent weather extremities and significant increase of the average temperature (even by 4°C) in Hungary by the end of the century (Krüzselyi et al. 2011, Spinoni et al. 2015). Based on the different model simulations, it is expected that there will be less cold weather extremities but more warm ones, and drought periods will be also longer (Pieczka et al. 2011). Although there are some uncertainties regarding the amount and the distribution of precipitation, it seems that the amount of rainfall will change, its intensity is expected to increase, and the winter months will be the wettest ones (Bartholy et al. 2011, Horányi et al. 2011, Bihari et al. 2018).

For the natural ecosystems climate change will appear in the form of climatic exposures. According to the definition of the Intergovernmental Panel on Climate Change (IPCC), climatic exposure means "the nature and degree to which a system is exposed to significant climatic variations" (Füssel & Klein 2006). The various species respond to climatic exposures differently due to their unique sensitivity levels. This leads to complex and diverse outcomes affecting the species composition and the productivity of forest ecosystems (Lindner et al. 2010).

The most important questions to be answered by this review are the following:

Regarding growth, mortality and regeneration capacity of the trees living in the natural forests of Hungary ...

- What are the major characteristics of climate change (experienced in the last decades) in our region?
- How will these changes – as climatic exposures – affect the native tree species and the natural forest stands of our region?

Materials and Methods

During literature review we focused on those papers, which were investigating the impacts of the major climate factors changed in recent decades and influencing the behaviour of trees, stands and processes of natural deciduous forest ecosystems in our Pannonian region and surroundings. We searched for publications focusing on the impacts of the following climatic exposures: increasing of atmospheric carbon-dioxide and nitrogen deposition; summer drought stress; milder winters, longer vegetation season and earlier onset of spring and shift of temperature and precipitation's regime. Articles presenting the impacts of the specific climate exposure through the examples of Central European, temperate zone and climate zonal forests of Hungary had priority. Those professional articles have been taken into account that investigating directly the already experienced impacts of exposures.

The statements of the selected papers were tagged and grouped according to the main types of climatic exposures: A) increasing global atmospheric CO_2 concentration; B) increasing reactive atmospheric Nitrogen (Nr) deposition; C) more frequent mild winters, earlier onset of spring and the extension of the growing season; D) increasing temperature with no water limitation; E) Increasing summer temperature and drought stress and F) indirect effects of increasing summer temperature and drought stress. Afterwords the impact statements were ordered, grouped and summarized with no weighting, however the number of references shows a higher degree of agreement.

Results

The main impacts of climatic exposure on trees and natural forest stands have been arranged, assessed and summarized in Table 1.

Table 1. Thematic overview of the main impacts of climatic exposure on trees and natural forest ecosystems caused by the recent climate change – summarized results of a review.

Climatic Exposure	Impact on Forests	References
A) Increasing global atmospheric CO_2 concentration by 15-20 ppm / decade since 1980 (NOAA 2019)	Increased growth of young tree stands if no other limitation factors (i.e. light, water, nutrient) occur, but mature forest trees (oak, hornbeam) generally did not show growth enhancement in response to elevated CO_2 level, partly except of beech.	Asshoff et al. (2006), DeLucia et al. (1999), Hickler et al. (2015), Laubhann et al. (2009), Norby et al. (2005)
B) The reactive atmospheric Nitrogen ($N_{reactive}$) deposition almost tripled the natural level by the early 1990s. According to the projected trend it is to double more by 2050 Galloway et al. (2008)	Increase of $N_{reactive}$ deposition can enhance ecosystem productivity (mostly in N-limited regions) through fertilization and decreases biodiversity (i.e. herb layer) through acidification and eutrophication.	Fowler et al. (2013), Galloway et al. (2008) Fowler et al. (2013), Galloway et al. (2004), Sybryn et al. (2018)
	Nitrogen deposition generally shows a significant positive impact on tree growth, although the N and the C cycles of the ecosystems are complex and coupled processes. Too much nitrogen slows down forest growth.	Etzold et al. (2020), Komarov et al. (2012), Laubhann (2009)
C) More frequent mild winters, earlier onset of spring and the extension of the growing season. The average advance of spring was 2-4 days/decade between 1951 and 2000 in our region. An increase of mean annual temperature by 1°C led to the extension of the growing season by 5. days	Spring warming has a strong impact, but the combination of winter and spring warming has the greatest impact on earlier budding (beech, birch and oak).	Ahas et al. (2002), Fu et al. (2012), Menzel et al. (2006), Chmielewski & Rötzer (2001)
	Indirect impact: warm temperatures can decrease the winter mortality of forest pest insects, thereby shortening generation time (generalization is difficult because of the various species-specific responses)	Pureswram et al. (2018)
	Pest insect populations can react fast to the favourable periods and condition deterioration of their host trees.	Csóka et al. (2018), Hirka et al. (2018), Mátyás et al. (2018)

Table 1. *Continued.* Thematic overview of the main impacts of climatic exposure on trees and natural forest ecosystems caused by the recent climate change – summarized results of a review.

Climatic Exposure	Impact on Forests	References
D) Increasing temperature with no water limitation Trend: Significant warming tendencies are dominant at the period of 1961-2001, in the Carpathian Basin (Pongracz & Bartholy, 2006).	Increasing growth (basal area) of larger beech and pedunculate oak trees, but beech turned to decline since the 1960s due to the decreasing relative summer humidity in the Atlantic climate. Stable stand volume increment of mixed mountain forests, however significant changes in the growth dynamics at the species level: spruce dropped, fir rose, beech did not change.	Kint et al. (2012), Laubhann et al. (2009), Hilmers et al. (2019)
E) Increasing summer temperature and drought stress Trend: Heat waves in the Carpathian region between 1961-2010: four out of seven heat events occurred after 2000 (Spinoni et al. 2015).	Forest growth declined during drought, especially during more severe droughts in the drier climates. Beech is the most drought sensitive tree species compared to sycamore, Norway maple, sessile oak and common ash. Its growth decreases in the driest stands (less precipitation than 600 mm/year) since about the 1980s. Decreasing productivity by increasing aridity for beech, hornbeam, sessile oak and Turkey oak.	Árvai et al. (2018), Führer et al. (2011), Führer et al. 2016), Gálos & Führer (2018), Gleason et al. (2017), Horváth & Mátyás (2014), Mátyás et al. (2018) Sáenz-Romero et al. (2019), Spathelf et al. (2014), Zimmermann et al. (2015)
F) Indirect effects of increasing summer temperature and drought stress	Change of dominance among trees species, beech was more competitive but less drought tolerant than oak until annual precipitation fell below 540 mm in a 100-year-old mixed forest.	Mette et al. (2013)
	Drought induced self-thinning (mortality) of sessile oak stands in Hungary	Berki et al. (2014), Berki et al. (2016), Herczeg et al. (2018), Árvai et al.

Table 1. *Continued.* Thematic overview of the main impacts of climatic exposure on trees and natural forest ecosystems caused by the recent climate change – summarized results of a review.

Climatic Exposure	Impact on Forests	References
F) *continued* Indirect effects of increasing summer temperature and drought stress	Expanding distribution area at leading edge of manna ash tree due to good natural regeneration capacity on drier sites	Molnar & Czúcz (2009)
	Reduction of macroclimatically suitable areas for beech and sessile oak in the following decades near the xeric distributional limits	Czúcz et al. (2011), Salamon-Albert et al. (2016), Illés (2018)

Discussion

Increasing CO$_2$ and atmospheric Nitrogen deposition can enhance tree growth and ecosystem productivity

Although increasing atmospheric CO$_2$ levels and reactive Nitrogen deposition increase forest productivity at many places, researches revealed that such responses are site, age and species specific. According to the researches investigating young hardwood and pine stands, trees treated with air enriched with CO$_2$ grew more intensively that the surrounding ones; and their net primary production (NPP) also significantly increased together with their fine-root. In drought years there was no difference between the growth intensity of the treated trees and those of the control group (surrounding trees without any CO$_2$ treatment). However, in the long run decreased production has been observed, which could be traced back to the limited access to minerals (DeLucia et al. 1999). Other outdoor CO$_2$ chamber experiments also found that younger tree stands reached higher NPP values, exceeding the reference value with 23% on the average (with significant standard deviation) (Norby et al, 2005). It has been verified at more locations that water supply has a greater impact on the growth intensity of the different tree species. Usually, higher CO$_2$ concentration level leads to increased productivity but in many cases the effects of CO$_2$ have been overestimated because at areas with water deficiency less growth can be observed, or decline can be experienced (Hickler et al. 2015). Asshoff et al. (2006) investigated the changes of the sum of the basal area of 5 dominant European tree species in old forest stands. They found that in the investigated 4 years long period increased CO$_2$ level did not lead to the enhanced production of the tree species. Although beech reached outstanding growth levels in the first year, this growth advantage disappeared due to the drought experienced in the third year. During the research also phenological variables (e.g. budding and defoliation) have been analysed, which turned to be species specific, but could not be linked to the increased CO$_2$ levels.

“By now, food and energy production of [industry] increased the rate of anthropogenic Nitrogen creation tenfold compared to the value of the late 19th century.” (Gal-

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loway 2004, Galloway 2008, Sybryn et al. 2018) Komarov and his colleagues (2012) modelled the changes of productivity according to two different climate scenarios and forestry inventory data in three forest sites in Russia under continental climate. Due to the growing level of N-deposition increased productivity can be expected besides the spread of deciduous tree species in the mixed tree stands (Komarov et al. 2012). N-deposition increased the growth of all the investigated dominant tree species (beech, sessile oak, pedunculate oak, fir and spruce) in European forests (Laubhann et al. 2009).

Milder winters and the earlier onset of spring cause less winter mortality among forest pest insects, and accelerate budding

Milder winters and the earlier onset of spring can be witnessed all over Europe leading to the lengthening of the vegetation period and the growing risk of late frost damages. Milder winter weather facilitates the survival of phytophagous insects, consequently larger populations and increased insect damages can be witnessed in the vegetation period (Pureswarm et al. 2018, Csóka et al. 2018). In the absence of the critical winter periods the range of insects also changes enabling the conquest of new territories (Pureswarm et al. 2018, Csóka et al. 2018). The average advance of the summer half year was 2-3 days/decade in Europe. The phenological characteristics of the species also responded to this in accordance with the earlier spring temperature increase. On the other hand, defoliation takes place one day later/decade on the average (Menzel et al. 2006). Based on the data of the European phyto-phenological database, budding starts 4 weeks earlier in Western and Central Europe compared to the reference period (1951-1998) of the database. Differences can be observed in the timing of spring phenological events in the mountainous regions, which is mostly the result of the diverse microclimatic patterns and the different altitude levels (Ahas et al. 2002) Temperature manipulation experiments have been used for investigating the phenological events of beech, oak and birch seedlings in winter and spring periods through the simulation of warming. Budding started earlier in the case of all the three tree species when spring warming has been simulated, while winter warming seemed to have no effects at all (Fu et al. 2012). Milder winter weather facilitates the survival of phytophagous insects, consequently larger populations and increased insect damages can be witnessed in the vegetation period. (Csóka et al. 2018, Hirka et al. 2018). In the absence of the critical winter periods the range of insects also changes enabling the conquest of new territories (Pureswarm et al. 2018, Mátyás et al. 2018).

Altered growth dynamics of trees due to increasing temperature

The connection between more environmental variables (e.g. average temperature, precipitation, carbon-dioxide level and nitrogen level) and the growth of stand forming tree species (beech, sessile oak, pedunculate oak, spruce and fir) have been investigated in Europe. Increasing average temperature had a positive impact on the growth of all the tree species, except for spruce (Laubhann et al. 2009). In Austrian mixed forest stands – consisting of pine and deciduous trees – higher average temperature combined with lower precipitation levels did not alter the productivity of the trees. In the mixed forests researched by Hilmers and his colleagues the diminishing population of spruce was counterbalanced by the increased production of other tree species (e.g. beech and silver fir) (Hilmers et al. 2019). Similarly, the basal area growth of temperate tree species has been investigated in areas under Atlantic climate between 1901 and 2008. According to the data, the productivity of pedunculate oak continuously increases, while in the case of beech productivity started to decrease from the 1960s due to the drop in relative humidity values during the summer periods in the Atlantic regions (Kint et al. 2012).

Increasing drought stress decreases productivity, and can induce higher mortality among the more susceptible tree species

The characteristic exposures of climate change include the more frequent occurrence of warmer and drier summers and longer drought periods accompanied with heat waves. The intensity of growth significantly decreased during drought periods, especially at arid and semi-arid locations. In regions with sufficient water supply competition had a greater impact on production. This could be also observed in drought periods when the impact of aridity has not been enhanced by high temperature. (Gleason et al, 2017). By using the aridity index, calculations have been made on the future changes of the habitat conditions and the production of the different tree species in Central Europe. From the analysed weather variables late spring temperature and total annual precipitation turned to be of special importance. Since the amount of available water affects the production of organic matter, decreased productivity, worse health status and lower density values can be expected in the case of some of the species at areas with high forestry aridity index (FAI) values (Salamon et al. 2016, Führer et al. 2011, Gálos & Führer 2018, Sáenz-Ronero et al. 2019). In Hungary, the spread, the ecological needs and the climate sensitivity of more stand forming tree species have been investigated, and based on the results their future has been predicted for the next decades according to the expected climatic changes. These forests and tree species are located close to the xeric edge of the temperate deciduous forest zone in the Carpathian Basin, therefore some of the currently dominating tree species (beech, sessile oak and Turkey oak) may disappear from huge areas, and the structure and the characteristic of the forests may fundamentally change (Spathelf et al. 2014). A further finding of the researches is that habitats suitable for beech will be limited to smaller areas by 2050, and the ideal habitats meeting the ecological needs of beech – being a stand forming species today – will virtually disappear from Hungary by the end of the century a (Czúcz et al. 2011, Illés 2018). Beech is susceptible to aridity, and this can be also witnessed in the changes of dominance relations. Drier and warmer weather forecasted in Central Europe decreases the competitiveness of beech, consequently the ratio of drought tolerant tree species (e.g. maples and ashes) and the dominance of sessile oak are expected to increase (Mette et al. 2013, Zimmermann et al. 2015, Árvai et al. 2018). From the indicator species signalling drier climate manna ash can be highlighted. Data on its historical and recent spread and the latest related observations have been summarized by Molnár and Czúcz (2009). According to their research, the spreading and the increasing ratio of the species can be witnessed in the forest stands of the North Hungarian Mountains where habitat conditions were not so ideal for manna ash earlier (Molnár & Czúcz 2009).

Conclusions

Climatic exposure caused by climate change is a complex process showing regional and local patterns. The major changes – increasing CO_2 concentration and atmospheric Nitrogen deposition, more frequent mild winters and earlier onset of spring, higher temperatures, and increasing summer drought stress – cause direct impacts, which sometimes counterbalance each other. Climatic exposure mostly alters growth dynamics, vitality and competitiveness of the trees, which are highly specific due to the ecological and trait profile and various sensitivity of different tree species. The more frequent appearance of forest pest insects and the new invaders may cause severe indirect impacts on trees. We found it during the literature review that in the majority of the cases tree specific growth and mortality related changes can lead to considerable shift in density and mixture ratio of trees in the stand. Much less evidences are available on the close relation between climate exposure and success of tree regeneration. It is clear that besides climate exposure the establishment and the regeneration of the trees also greatly depend on several other factors (i.e. canopy closure, propagulum sources, competition relationships, browsing effect of the stock of game, etc.) in the natural forest stands. Finally, we can conclude that investigating and exploring changes in tree specific growth and mortality as the possible outcomes of climate exposure are promising fields of research.

References

Ahas, R., Aasa, A., Menzel, A., Fedotova, V. G., Scheifinger, H. (2002): Changes in European spring phenology. Int. J. Climatol., 22: 1727-1738. https://doi.org/10.1002/joc.818

Allen, C. D., Macalady, A. K., Chenchouni, H., Bachelet, D., McDowell, N., Vennetier, M. and Cobb, N. (2010): A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. Forest Ecology and Management, 259 (4): 660-684. https://doi.org/10.1016/j.foreco.2009.09.001

Aubin, I., L. Boisvert-Marsh, H. Kebli, D. McKenney, J. Pedlar, K. Lawrence, E. H. Hogg, Y. Boulanger, S. Gauthier, C. Ste-Marie (2018): Tree vulnerability to climate change: improving exposure-based assessments using traits as indicators of sensitivity. Ecosphere 9(2): article e02108 https://doi.org/10.1002/ecs2.2108

Árvai M., Morgós A., Kern Z. (2018): Growth-climate relations and the enhancement of drought signals in pedunculate oak (Quercus robur L.) tree-ring chronology in Eastern Hungary, https://doi.org/10.3832/ifor2348-011

Asshoff, R., Zotz, G., Körner, C. (2006): Growth and phenology of mature temperate forest trees in elevated CO2. Global Change Biology, 12: 848-861. https://doi.org/10.1111/j.1365-2486.2006.01133.x

Bartholy J., Bozó L., Haszpra L. (2011): Klímaváltozás 2011 – Klímaszcenáriók a Kárpát-medence térségére, Magyar Tudományos Akadémia és az Eötvös Loránd Tudományegyetem Meteorológiai Tanszéke, Budapest, 2011, p. 287.

Berki I., Rasztovits E., Móricz N., Kolozs L. (2016): The Role of Tree Mortality in Vitality Assessment of Sessile Oak Forests. South-East Europea Forestry 7 (2): 91-97. https://doi.org/10.15177/seefor.16-14

Berki I., Rasztovits E., Móricz N., Kolozs L. (2014): Erdőállományok egészségi állapotának értékelése – egy új megközelítés. Erdészettudományi Közlemények 4(2): 149-155. https://doi.org/10.17164/EK.2018.013

Berki, I., Móricz, N., Rasztovits, E., Gulyás, K., Garamszegi, B., Horváth, A., Balázs, P. Lakatos, B. (2018): Mortality and accelerating growth in sessile oak sites. Bulletin of Forestry Science, 8(1):119-130. (in Hungarian) https://doi.org/10.17164/EK.2018.008

Bihari, Z., Babolcsai, G., Bartholy, J., Ferenczi, Z., Gerhát-Kerényi, J., Haszpra, L., Homoki-Ujváry, K., Kovács, T., Lakatos, M., Németh, Á., Pongrácz, R., Putsay, M., Szabó, P., Szépszó, G. (2018): Éghajlat [Climate in Hungarian] In: Kocsis, K., Horváth, G., Keresztesi, Z., Nemerkényi, Zs. (eds): Magyarország nemzeti atlasza 2. kötet. Természeti környezet [Natural Environment, National Atlas of Hungary], MTA CSFK Földrajztudományi Intézet, Budapest, pp. 58-69., 12 p.

Buras A., Menzel A. (2019): Projecting Tree Species Composition Changes of European Forests for 2061–2090 Under RCP 4.5 and RCP 8.5 Scenarios. Front. Plant Sci. 9:1986. https://doi.org/10.3389/fpls.2018.01986

Chmielewski, F-M., Rötzer T. (2001): Response of tree phenology to climate change across Europe, Agricultural and Forest Meteorology 108(2): 101-112. https://doi.org/10.1016/S0168-1923(01)00233-7

Czúcz, B., Gálhidy, L., Mátyás, Cs. (2011): Present and forecasted xeric climatic limits of beech and sessile oak distribution at low altitudes in Central Europe. Annals of Forest Science, 68(1), 99–108. https://doi.org/10.1007/s13595-011-0011-4

Csóka, G., Csókáné Hirka, A., Csepelényi, M., Szőcs, L., Molnár, M., Tuba, K., Hillebrand, R., Lakatos, F. (2018): Erdei rovarok reakciói a klímaváltozásra (esettanulmányok). Erdészettudományi Közlemények, 8 (1). pp. 149-162. ISSN 2062-6711 https://doi.org/10.17164/EK.2018.010)

Dobor, L., Barcza, Z., Hlásny, T., Havasi, Á., Horváth, F., Ittzés, P., Bartholy, J. (2015): Bridging the gap between climate models and impact studies: the FORESEE Database. Geosci. Data J. 2:1–11. https://doi.org/10.1002/gdj3.22

Etzold, S., Ferretti, M., Reinds, G.J., Solberg, S., Gessler, A., Waldner, P., Schaub, M., Simpson, D., Benham, S., Hansen, K., Ingerslev, M., Jonard, M., Karlsson, P.E., Lindroos, A., Marchetto, A., Manninger, M., Meesenburg, H., Merilä, P., Nöjd, P., ... De Vries, W. (2020): Nitrogen deposition is the most important environmental driver of growth of pure, even-aged and managed European forests. Forest Ecology and Management, 458: 117762 (13 pp.). https://10.1016/j.foreco.2019.117762

Fowler, D., Coyle, M., Skiba, U., Sutton, M. A., Cape, J. N., Reis S., Sheppard L.J., Jenkins A., Grizzetti, B., Galloway, J.N., Vitousek, P., Leach, A., Bouwman, A.F., Butterbach-Bahl, K., Dentener, F., Stevenson, D., Amann, M., and Voss, M. (2013): The global nitrogen cycle in the twenty-first century. Philosophical Transactions of the Royal Society B, 368 (1621), 20130164, https://doi:10.1098/rstb.2013.0164

Fu YH, Campioli M, Deckmyn G, Janssens IA. (2012): The Impact of Winter and Spring Temperatures on Temperate Tree Budburst Dates: Results from an Experimental Climate Manipulation. PLoS ONE 7(10):e47324. https://doi.org/10.1371/journal.pone.0047324

Führer, E., Horváth, L., Jagodics, A., Machon, A., Szabados, I. (2011): Application of a new aridity index in Hungarian forestry practice. IDŐJÁRÁS - Quarterly Journal of the Hungarian Meteorological Service, 115(3), 205–216

Führer, E., Edelényi, M., Jagodics, A., Jereb, L., Horváth, L., Kern, Z. … Pödör, Z. (2016): Az időjárás hatása egy időskorú bükkös évenkénti körlap-növekedésére. Erdészettudományi Közlemények, 6(1–2), 61–78. https://doi.org/10.17164/EK.2016.006

Füssel, H., Klein, R.J.T. Climate Change Vulnerability Assessments: An Evolution of Conceptual Thinking. Climatic Change 75, 301–329 (2006). https://doi.org/10.1007/s10584-006-0329-3

Galloway, J.N., Dentener, F.J., Capone, D.G. et al. (2004): Nitrogen cycles: past, present, and future. Biogeochemistry 70: 153-226. https://doi.org/10.1007/s10533-004-0370-0

Galloway, J.N., Townsend, A.R., J.W. Erisman, J.W., Bekunda, M., Cai, Z., Freney, J.R., Martinelli, L.A., Seitzinger, S.P., and Sutton, M.A. (2008): Transformation of the nitrogen cycle: recent trends, questions, and potential solutions. Science, 320(5878), 889-892. https://doi.org/10.1126/science.1136674

Gálos B., Führer E. (2018): A klíma erdészeti célú előrevetítése. Erdészettudományi Közlemények 8(1): 43-55. ISSN 2062-6711 https://doi.org/10.17164/EK.2018.003

Gleason, K.E., Bradford, J.B., Bottero, A., D'Amato, A.W., Fraver, S., Palik, B.J., Battaglia, M. A., Iverson, L., Kenefic, L., and Kern, C.C. (2017): Competition amplifies drought stress in forests across broad climatic and compositional gradients. Ecosphere 8(7): e01849.10.1002/ecs2.1849 https://doi.org/10.1002/ecs2.1849

Herceg, A., Kalicz, P., Kisfaludi, B. Gribovszki, Z. (2018): A Thornthwaite-type water balance model for the analysis of the hydrological impact of climate change. Bulletin of Forestry Science, 8(1): 73-92. (in Hungarian) https://doi.org/10.17164/EK.2018.005

Hickler, T., Rammig, A., Werner, C. Curr(2015): Modelling CO2 impact on forest productivity, Forestry Rep (2015)1/69. https://doi.org/10.1007/s40725-015-0014-8

Hilmers, T., Avdagić A., Bartkowicz L., Bielak K., Binder F., Bončina A., Dobor L., Forrester D.,I., Hobi M.,L., Ibrahimspahić A., Jaworski A., Klopčič M., Matović B., Nagel T., A., Petráš R., Rio del M., Stajić B., Uhl E., Zlatanov T., Tognetti R., Pretzsch H. (2019): The productivity of mixed mountain forests comprised of *Fagus sylvatica*, *Picea abies*, and *Abies alba* across Europe, Forestry: An International Journal of Forest Research 92(5): 512–522. https://doi.org/10.1093/forestry/cpz035

Hlásny, T., Mátyás Cs., Seidl R., Kulla L., Merganičova K., Trombik J., et al. (2014): Climate change increases the drought risk in Central European forests: What are the options for adaptation? Forestry Journal (Lesnicki Casopis) 60: 5–18. https://doi.org/10.2478/forj-2014-0001

Hirka, A., Pödör, Z., Garamszegi, B. Csóka, Gy. (2018): 50 years trends of the forest drought damage in Hungary (1962-2011). Bulletin of Forestry Science 8(1):11-25. (in Hungarian) https://doi.org/10.17164/EK.2018.001

Horányi, A., Szépszó, G., Bartholy, J., Pongrácz, R.(2011): Az éghajlati modellek korlátai. In: Klímaváltozás – 2011: Klímaszcenáriók a Kárpát-medence térségére

Horváth, A., Mátyás, Cs. (2014): Növedékcsökkenés előrevetítése egy bükk származási kísérlet alapján. Erdészettudományi Közlemények, 4(2): 91-99.

Illés, G. (2018): Predicting the climate change induced yield potential changes of sessile oak stands. Bulletin of Forestry Science, 8(1): 105-118. (in Hungarian) https://doi.org/10.17164/EK.2018.007

IPCC (2014): Climate Change 2014: Impacts, Adaptation, and Vulnerability. Part A: Global and Sectoral Aspects. Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Field, C.B., V.R. Barros, D.J. Dokken, K.J. Mach, M.D. Mastrandrea, T.E. Bilir, M. Chatterjee, K.L. Ebi, Y.O. Estrada, R.C. Genova, B. Girma, E.S. Kissel, A.N. Levy, S. MacCracken, P.R. Mastrandrea, and L.L.White (eds.)]. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, 1132 pp.

IPCC, 2019: Summary for Policymakers. In: Climate Change and Land: an IPCC special report on climate change, desertification, land degradation, sustainable land management, food security, and greenhouse gas fluxes in terrestrial ecosystems [P.R. Shukla, J. Skea, E. Calvo Buendia, V. Masson-Delmotte, H.- O. Pörtner, D. C. Roberts, P. Zhai, R. Slade, S. Connors, R. van Diemen, M. Ferrat, E. Haughey, S. Luz, S. Neogi, M. Pathak, J. Petzold, J. Portugal Pereira, P. Vyas, E. Huntley, K. Kissick, M. Belkacemi, J. Malley, (eds.)]. In press.

Kint, V., Aertsen, W., Campioli, M. et al. (2012): Radial growth change of temperate tree species in response to altered regional climate and air quality in the period 1901–2008. Climatic Change (2012) 115: 343. https://doi.org/10.1007/s10584-012-0465-x

Kinzig, A. P., Ryan, P., Etienne, M., Allison, H., Elmqvist, T. Walker, B. H. (2006): Resilience and regime shifts: assessing cascading effects. Ecology and Society 11(1), 20. http://www.ecologyandsociety.org/vol11/iss1/art20/

Komarov, A. S. and Shanin, V. N. (2012): Comparative analysis of the influence of climate change and nitrogen deposition on carbon sequestration in forest ecosystems in European Russia: simulation modelling approach, Biogeosciences 9:4757–4770, https://doi.org/10.5194/bg-9-4757-2012

Krüzselyi, I., Bartholy, J., Horányi, A., Pieczka, I., Pongrácz, R., Szabó, P., Szépszó, G., Torma, Cs., (2011): The future climate characteristics of the Carpathian Basin based on a regional climate model mini-ensemble. Advances in Science and Research 6, 69–73 https://doi.org/10.5194/asr-6-69-2011

Laubhann, D., Sterba, H., Reinds, G. J., De Vries, W. (2009): The impact of atmospheric deposition and climate on forest growth in European monitoring plots: An individual tree growth model, Forest Ecology and Management, 258(8): 1751-1761. https://doi.org/10.1016/j.foreco.2008.09.050.

Lindner, M., Maroschek, M., Netherer, S., Kremer, A., Barbati, A., Garcia-Gonzalo J., Seidl, R., Delzon, S., Corona, P., and Kolström, M. (2010): Climate change impacts, adaptive capacity, and vulnerability of European forest ecosystems, Forest Ecology and Management, Vol 259 (4) pp: 698-709 https://doi.org/10.1016/j.foreco.2009.09.023

Maes L. S., Perring P. M., Vanhellemont M., Depauw L., Bulcke J., Brūmelis G., Brunet J., Decocq G., Ouden J., Härdtle W., Hédl R., Heinken T., Heinrichs S., Jaroszewicz B., Kopecký M., Máliš F., Wulf M., Verheyen K. (2019): Environmental drivers interactively affect individual tree growth across temperate European forests, https://doi.org/10.1111/gcb.14493

Mátyás, Cs., Führer E., Berki I., Csóka Gy., Drüszler Á., Lakatos F., Móricz N. (2010): Erdők a szárazsági határon. Klíma-21 Füzetek 61:84-97 Mátyás Cs., Berki I., Bidló A., Csóka Gy., Czimber K., Führer E., Gálos B., Gribovszki Z., Illés G., Hirka A., Somogyi Z. (2018): Sustainability of Forest Cover under Climate Change on the Temperate-Continental Xeric Limits, FORESTS 9: (8) p. 489. https://doi.org/10.3390/f9080489

Menzel, A., Sparks, T. H., Estrella, N., Koch, E., Aasa, A., Ahas, R. , Alm-Kübler, K. , Bissolli, P. , Braslavská, O. , Briede, A. , Chmielewski, F. M., Crepinsek, Z. , Curnel, Y. Dahl, Å. , Defila, C. , Donnelly, A. , Filella, Y. , Jatczak, K. , Måge, F. , Mestre, A. , Nordli, Ø. , Peñuelas, J. , Pirinen, P. , Remišová, V. , Scheifinger, H. , Striz, M. , Susnik, A. , Van Vliet, A. J., Wielgolaski, F. , Zach, S. And Zust, A. (2006): European phenological response to climate change matches the warming pattern. Global Change Biology, 12: 1969-1976. https://doi.org/10.1111/j.1365-2486.2006.01193.x

Mette, T., Dolos, K., Meinardus, C., Bräuning, A., Reineking, B., Blaschke, M., Pretzsch, H. Beierkuhnlein, C., Gohlke, A., and Wellstein C. (2013): Climatic turning point for beech and oak under climate change in Central Europe. Ecosphere 4(12):145. https://doi.org/10.1890/ES13-00115.1

Molnár, Cs., Czúcz B. (2009): A virágos kőris (*Fraxinus ornus* L.) terjedése és mai termőhelyei Az Északi-középhegységben, Bot. Közlem. 96(1–2): 71–81. 2009

Nagel, TA, Iacopetti, G, Javornik, J, et al. Cascading effects of canopy mortality drive long-term changes in understorey diversity in temperate old-growth forests of Europe. J Veg Sci. 2019; 30: 905– 916. https://doi.org/10.1111/jvs.12767

NOAA (2019): Trends in atmospheric carbon dioxide – Annual mean global carbon dioxide growth rate. https://www.esrl.noaa.gov/gmd/ccgg/trends/gl_gr.html, accessed in 13th of November, 2019

Norby, R. J., DeLucia, E. H., Gielen, B., Calfapietra, C., Giardina, C. P., King, J. S., Ledford, J., McCarthy, H. R., Moore, D. J. P., … and Oren, R. (2005): Forest response to elevated CO_2 is conserved across a broad range of productivity. PNAS 102 (50): 18052-18056; https://doi.org/10.1073/pnas.0509478102

Pieczka I., Pongrácz R., Bartholy J.,Kis A., Miklós E.(2011): A szélsőségek várható alakulása a Kárpát-medence térségében az ENSEMBLES projekt eredményei alapján

Pureswaran, D.S., Roques, A. Battisti, A. Curr Forestry Rep (2018) 4: 35. https://doi.org/10.1007/s40725-018-0075-6

Pongrácz, R., Bartholy, J. (2006): Tendency analysis of extreme climate indices with special emphasis on agricultural impacts http://www.cbks.cz/sbornikStrecno06/prispevky/Sekcia_2/S2-8.pdf

Salamonné, A. É., Lőrincz, P., Pauler, G., Bartha, D., Horváth, F. (2016): Drought Stress Distribution Responses of Continental Beech Forests at their Xeric Edge in Central Europe. FORESTS, 7 (298). pp. 1-16. ISSN 1999-4907 https://doi.org/10.3390/f7120298

Sáenz-Romero C, Kremer A, Nagy L, Újvári-Jármay É, Ducousso A, Kóczán-Horváth A, Hansen JK, Mátyás C. 2019. Common garden comparisons confirm inherited differences in sensitivity to climate change between forest tree species. PeerJ 7:e6213 https://doi.org/10.7717/peerj.6213

Spathelf P., Maaten E., Maaten-Theunissen M., Campioli M., Dobrowolska D. (2014): Climate change impacts in European forests: the expert views of local observers, Annals of Forest Science (2014) 71:131–137 https://doi.org/10.1007/s13595-013-0280-1

Spinoni, J. , Lakatos, M., Szentimrey, T. , Bihari, Z. , Szalai, S. , Vogt, J. and Antofie, T. (2015): Heat and cold waves trends in the Carpathian Region from 1961 to 2010. Int. J. Climatol.35:4197-4209. https://doi.org/10.1002/joc.4279

Szelepcsényi, Z., Breuer, H., Sümegi, P. (2014). Analysis of projected climate change in the Carpathian Basin region based on Holdridge life zone system. Theoretical and Applied Climatology, 16: 13642.

Zimmermann, J., Hauck, M., Dulamsuren, C. et al. (2015): Climate Warming-Related Growth Decline Affects *Fagus sylvatica*, But Not Other Broad-Leaved Tree Species in Central European Mixed Forests. Ecosystems 18:560-572. https://doi.org/10.1007/s10021-015-9849-x

Effect of water supply and season on the productivity and carotenoid content of cherry type processing tomato

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Abstract: The weather, the growing conditions and the genetic background of the varieties determine the efficiency of processing tomato growing. During years Strombolino a cherry type tomato hybrid with determinate growth habit was investigated to show the effect of unirrigated and regularly irrigated treatments on the yield quantity and phytonutrients content under open field conditions. Too much water (precipitation + irrigation) was disadvantaged for the productivity and yield quality of cherry tomato hybrid because the amount of diseased yield increased while the soluble solids content (°Brix) and lycopene content of tomato fruit decreased. In the mildly wet year, irrigation had positive effect on the marketable yield, the β-carotene and zeaxanthin content of tomato fruit but the ratio of diseased fruit increased. In the dry years smaller weighed fruits were produced but the regular irrigation significantly increased the marketable yield, °Brix, β-carotene and cis lycopene content of fruit.

Keywords: cherry tomato; irrigation; yield; phytonutrients

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Introduction

Frequently occurance of high temperature and low precipitation due to global warming (Spinoni et al. 2015) decreases the productivity of vegetables crops grown in open field conditions and unfavourably influence unfavourably the yield quality (Bisbis et al. 2018). The damage caused by drought can be mitigated using irrigation technology, but the efficiency of irrigation was influenced by the time and dose of irrigation (Helyes et al. 2018), the water use efficiency of the varieties (Nemeskéri et al. 2015, 2018) and the weather of the years (Molnár et al. 2012).

In recent years, the focus of research has been on the bioactive substances of different vegetable species, especially tomatoes (Duc et al. 2017; Pék et al. 2012 and 2013). Tomato is one of the most water-demanding crops. Water deficiency affects the physiological processes, growth and yield of tomato. Nevertheless, the water deficit can benefit the tomato quality due to the accumulation of high level of soluble solids affecting the taste, aroma and water content of fruit (Barbagallo et al. 2013; Klunklin and Savage 2017). The amount of marketable yield, the soluble solids (°Brix) and lycopene content of fruit are important parameters for the preparation of various tomato purée in the processing industry (Berki et al. 2014). Nevertheless, the soluble solids content of tomato fruit depends on the variety, growing and weather conditions (Helyes and Varga 1994; Sass-Kiss et al. 2005; Pék et al. 2019). Under good water supply conditions, the tomato varieties produced high yield with low Brix value but the Brix yield per hectare increased (Pék et al. 2015). Lycopene content of tomato fruits depends on the variety and environmental conditions including temperature, and water supplies (Brand et al. 2006; Helyes et al. 2006; Petrović et al. 2019).

Tomato is classified as a functional food because of high levels of lycopene and carotenoids that are responsible for the red colour of the fruits (Rocha et al. 2013; Díaz et al. 2020). Its consumption helps prevent chronic diseases such as cancer and cardio-

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vascular diseases and it has favourable effects on the human health due to the phytonutrients of fresh fruits such as phenolic compounds, flavonoids, carotenoids and vitamins (Agarwal and Rao 2000). Lycopene comprisess 64-80% of the carotenoids depending the varieties in addition the amount of β-carotene, lutein and zeaxanthin is remarkable in the tomato (Lugasi et al. 2004; Chaudhary et al. 2018; Helyes 2014). Lutein and zeaxanthin is considered to retard the development of eye diseases such as age-related macular degeneration (Frede et al. 2017) and they have antioxidant properties because they take part in the scavenging of free radicals in the cells (Steiner et al. 2018). The accumulation of phytonutrients in tomato is influenced by the genotype, thefruit ripening and environmental factors. An increase in lycopene content of cherry type tomato varieties was detected using deficit irrigation but it decreased in large fruited cultivars (Dumas et al. 2003). Although there have been a number of studies conducted on the effect of irrigation on the yield of tomato the knowledge about the responses of cherry type tomato to water deficiency under field condition is scarce.

The aim of the present study was to investigate the effect of water supply on the productivity, the distribution of yield and phytonutrients of small sized processing cherry tomato.

Materials and Methods

Experimental design

During four years (from 2012 to 2015) the productivity and yield quality of early ripening Strombolino F_1 processing cherry tomato was investigated under regularly irrigated and non-irrigated conditions in open field experiments at the Experimental Farm of the Institute of Horticulture of Szent István University, Gödöllő. Tomato seedlings have been propagated in greenhouse and the four-week-old seedlings were transplanted ton the field on 8 to 11 of May depending on the weather of the years. Tomato seedlings were planted in twin rows, with 40 cm spacing inside the row and 120 cm between adjacent twin rows. The space between the plants in the rows was 30 cm with a plant density of 4.2 plants m^{-2}. The irrigation treatments were arranged in a randomized complete block design with four repetitions. Regular irrigation (RI) was used to provide the optimal water demand of plants using meteorological data to determine the dose of irrigation as described by Helyes at al. (2018). In non-irrigated plots the plants were grown only under natural precipitation conditions. The irrigation method was drip irrigation and was performed three times per week and it was finished 19 days before the harvest.

Analysis of yield quantity and quality

Ten and twenty plants were selected and harvested from each repetition between the 8th and 11th of August depending on the year. After harvest the fruit yield of selected plants was weighed and classified. The marketable group contained the healthy red coloured tomato fruits. The healthy green coloured fruits were classified into the second group and the third group comprised of the diseased fruits.

Five tomato fruit of red marketable yield from each repetition was used to determine the content of total soluble solids (°Brix) using a Krüss DR201-95 handheld refractometer (A. Krüss Optronic GmbH, Hamburg, Germany). Identification and measurement of carotenoids was performed using HPLC analysis as described by Daood et al. (2014).

Data were evaluated by two-way analysis of variance (ANOVA) using SPSS 20.0 (IBM Hungary Ltd, Budapest, Hungary) Windows software. The average values of treatments were compared by Tukey test at $p < 0.05$.

Table 1. Total amount of precipitation and irrigated plots water during the growth of Strombolino F_1 cherry type processing tomato

	Years							
Water supply	2012		2013		2014		2014	
mm	until flowering	total	until flowering	total	until flowering	total	until flowering	total
Precipitation	118.6	219.4	144.9	166.2	129.5	380.7	116.5	175.6
Irrigation	159.9	337.0	169.8	351.7	184.5	447.7	154.8	438.1

Results

On the basis of precipitation shown in Table 1, the weather was dry in years 2013 and 2015 and it was mildly wet in 2012 and rainy in 2014. Processing tomato requires at least 400 mm rainfall for undisturbed growth (Battilani et al. 2012) that was ensured by regular irrigation in the dry 2015 year. During the growth of tomato, the distribution of precipitation was different in the years which influenced the fruits setting and fruit development. From planting to the flowering, the amount of precipitation was similar 116 and 145 mm, respectively, both in wet and dry years. Nevertheless, a significant difference in the precipitation between the years was detected during fruit development and fruit ripening; a lot of rain fell in the wet years, but it was 19 mm and 57 mm in the dry years of 2013 and 2015 respectively.

In mildly wet (2012) and moderate dry (2015) years the total yield was larger than in the very dry (2013) and rainy (2014) year. Nevertheless, the amount of marketable and green yield originating in the individual productivity of plants was determined by the water supply together with the year (Table 2). In mildly wet (2012) year, a high productivity of plants was shown by large number of fruits and fruit weight per plant. In this year the irrigation was event efficient; regular irrigation resulted in a significantly increase in the weight of red fruit and marketable yield, but the rate of diseased fruits was high. In the rainy (2014) year the plants used a large proportion of available water to develop their vegetative organs rather than for the yield production. This was proven by the low fertility which resulted in the fewer larger sized matured red and green tomato fruits. In a rainy year the irrigation had no impact on the weight of red tomato fruits, but it significantly increased the amount of diseased yield in comparison with the non-irrigated plants (Table 2.). In dry (2013 and 2015) years, the plants produced generally smaller sized red fruits than in wet years however the water supply had significant influence on the fertility of plants i.e. fruit number per plant (Table 2). The largest soluble solids content (°Brix) of tomato fruit was measured in the very dry 2013 year and it was the lowest in rainy 2014. Irrigation had a negative effect on soluble solids content (°Brix) of fruit in the moderate dry (2015) and mildly wet (2012) year. It can be said that in dry years regular irrigation significantly increased the weight of tomato fruits when compared with the non-irrigated plants however the extent of increase was related to the intensity of drought which affected the marketable yield.

Under non-irrigated conditions, independently of the years, a low marketable yield (15-21 t ha^{-1}) has been produced (Figure 1ab). In dry years, under non-irrigated conditions more uniform and healthy yield was detected than in wet years. In dry years use

Table 2. Water supply on the yield components, distribution of yield and soluble solids content (°Brix) of fruit of Strombolino F$_1$ processing tomato

Traits	Water supply	Wet years†		Wet years††	
		2012	2014	2013	2015
Fruit nr. plant^{-1}	NI	130.09a	93.31b	123.77a	76.01c
	RI	184.83a*	99.25c	84.32c*	149.10b*
Total fruit kg plant^{-1}	NI	0.93a	0.74b	0.73b	0.35c
	RI	1.89a*	0.89c*	0.74d	1.56b*
Red fruit weight g	NI	7.48b	8.85a	6.15c	5.05d
	RI	10.28a*	9.88a	9.63a*	10.78a*
Green fruit weight g	NI	6.15a	4.18b	1.48d	2.68c
	RI	2.63c*	4.05b	2.55c*	7.73a*
Diseased fruit weight g	NI	6.90a	6.75a	7.10a	4.38b
	RI	11.83a*	8.40c*	9.73b*	9.75b*
Total yield t ha^{-1}	NI	38.73a	30.80b	26.63c	21.90d
	RI	78.93b*	37.00d*	57.33c*	97.43a*
Marketable yield t ha^{-1}	NI	15.63c	14.65c	25.55a	18.53b
	RI	57.45b*	14.38c	53.93b*	85.27a*
Green yield t ha^{-1}	NI	9.85a	1.48c	0.38d	2.23b
	RI	1.03d*	1.48c	1.68b*	5.43a*
Diseased yield t ha^{-1}	NI	13.28a	8.20b	0.73d	1.13c
	RI	20.45a*	15.78b*	1.75d*	6.68c*
°Brix	NI	6.93a	4.60c	6.60b	7.35a
	RI	5.05b*	4.40c	6.03a	4.53c*

Value in the rows following different letters indicates the significant difference at $P<0.05$ level using Tukey test * significant difference between non-irrigation (NI) and regular irrigation (RI) in the year. †2012 mildly wet, 2014 rainy year, ††2013 very dry 2015 moderate dry year.

of regular irrigation increased the marketable red yield and low green and diseased yields were produced (Figure 1a). In wet years the relatively low marketable yield was due to the low fruit setting however the regular irrigation significantly increased the amount of diseased and green yields in comparison with the non-irrigation (Figure 1b).

It can be established that in dry years (2013 and 2015) the amount of marketable yield was increased by irrigation while the rate of diseased yield can be maintained at low level, but too much water (precipitation + irrigation) can decrease the marketable yield and increase the diseased yield of cherry tomato.

During the fruit development and ripening stages of tomato the water supply conditions, and temperature influence the synthesis of secondary metabolites and phytonutrient compounds (Table 3). The largest content of zeaxanthin and lowest of β-carotene in fruits was measured in the dry (2013) year. The regular irrigation resulted in an increase in the β-carotene and cis lycopene contents of fruits in the dry year however in the mildly wet (2012) year an increase in the accumulation of zeaxanthin and β-carotene could be detected. The finding showed that regular irrigation depending on the weather of the

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Table 3. Water supply on the phytonutrients content of Strombolino F_1 cherry type tomato

Phytonutrients $\mu g\ g^{-1}$	Water supply	Years 2012	2014	2013
Total carotenoids	NI	168.67a	121.16b	64.14c
	RI	159.94a	126.56b	56.05c*
β-carotene	NI	3.63a	1.63b	3.83a
	RI	5.75a*	2.95b*	4.17a
Lycopene	NI	150.20a	101.82b	53.51c
	RI	140.70a*	109.39b	46.01c*
cis lycopene	NI	8.02a	7.35a	2.03b
	RI	7.45b	9.05a*	3.10c*
Zeaxanthin	NI	0.39b	0.63a	0.37b
	RI	0.49a*	0.27b*	0.48a*

Value in the rows following different letters indicates the significant difference at $P<0.05$ level using Tukey test * significant difference between non-irrigation (NI) and regular irrigation (RI) in the year.

years enhanced the accumulation of zeaxanthin into the fruit, except in a very dry year, when it decreased.

It can be established that abundant water was unfavourable on the nutritional quality of cherry tomatoes because the soluble solids content (°Brix) and lycopene content of fruit decrease. In mildly wet year the effect of irrigation on the β-carotene and zeaxanthin content of fruit was favourable however the best nutritional quality of cherry tomato fruit including high β-carotene and cis lycopene could be reached in the dry years.

Discussion

During the reproductive stage of development of tomato, the water supply influence the fruits number and weight of plants which determine the final yield and its morphological distribution (Pék et al. 2019). The impact of water deficiency on the yield and quality depends on the genotype, the duration and intensity of water stress occurring during the fruit development stage (Riggi et al. 2008, Ripoll et al. 2014). It was shown that long-term water deficit decreased a greater extent the fresh mass and fruit diameter of cherry tomatoes than in large fruited ones but the reduction of fruit growth of cherry tomato could be compensated by improving fruit nutritional value (Petrović et al. 2019, Duc et al 2017). Others (Coyago-Cruz et al. 2019) also found that water stress reduced the fruit weight and number per cluster of cherry tomato (Coyago-Cruz et al. 2019). According to our results both water supply and years influence the yield components, yield fraction and accumulation of phytonutrients of Strombolino F_1 processing cherry tomato.

In mildly wet and moderate dry years the regular irrigation more efficiently improved the fruit setting (fruit number per plant) and the weight of fruits than in marginal wet and arid years (Table 2). Therefore, the total yield increased (78.83 and 97.38 t ha^{-1} respectively) in these years however the distribution of yield was different. Others (Bőcs et al. 2011) also found a strong positive effect of water supply on the marketable yield and average fruit weight of tomato with large fruit size was detected. The result concern-

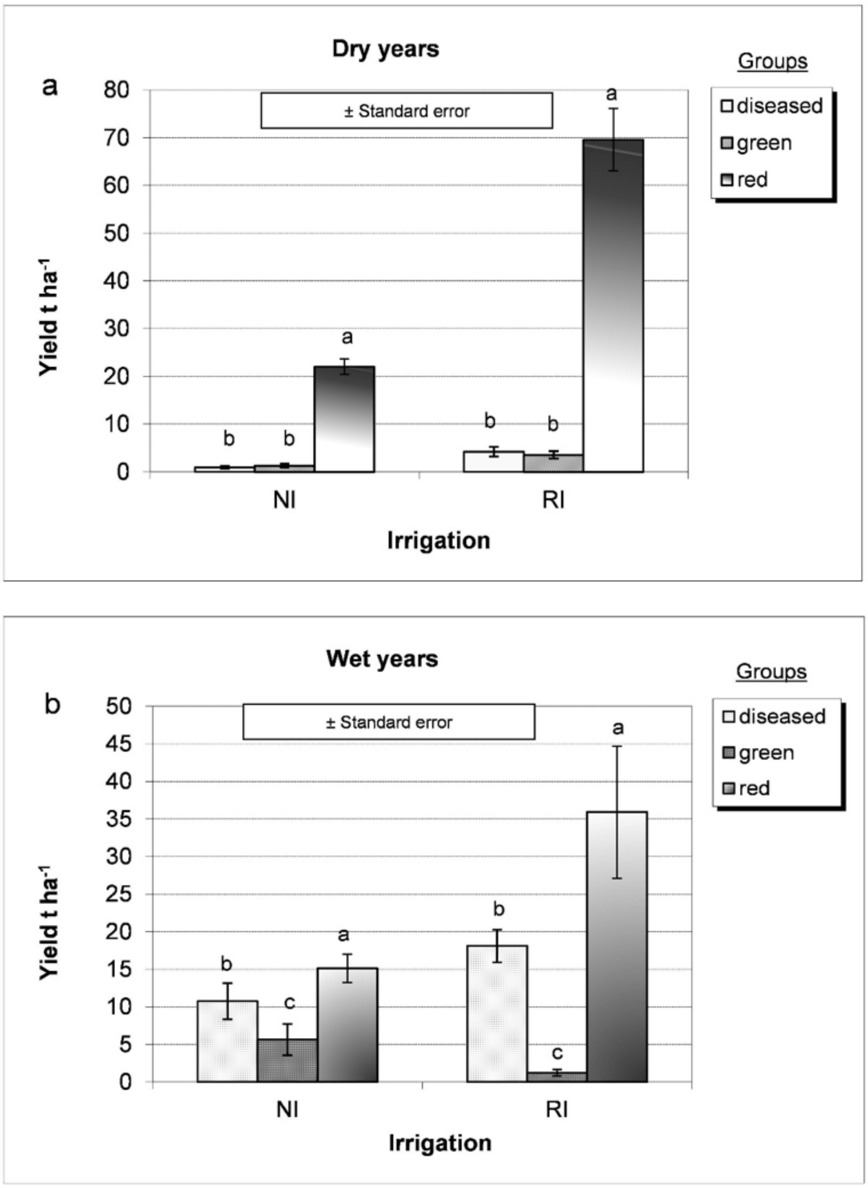

Figure 1. Effect of water supply on the yield quantity of Strombolino F_1 cherry tomato in dry (a) and wet (b) years. Means following different letters are significant difference at $P<0.05$ level using Tukey test NI=non-irrigated RI= regularly irrigated conditions

ing the cherry type tomato was similar to that of Bőcs et al (2011) but the effect of irrigation was modified by the weather of years. In rainy year the plants increased the green biomass rather than to the fruit development while the amount of diseased yield was increased. In dry years the irrigated plants produced healthier marketable yield than in rainy years.

Prior to harvest the irrigation and precipitation strongly influence the tomato yield quality (Helyes et al. 2012). During this period the soluble solids contents including sugars, amino acids, organic acids are accumulating into the tomato fruits to provide the flavour aroma and water content of fresh fruits. The soluble solids content (°Brix) of tomato fruit is determined by the genetic attributes of the varieties. A higher Brix value was found in cherry type tomato varieties than in traditional weighed fruit ones (Lapushner et al. 1990) however the weight of fruits can be changed by water supply conditions. Helyes et al. (2018) published that in a moderate dry year using regular irrigation the Brix value of Uno Rosso F_1 processing tomato with larger size fruit decreased by 52% in comparison with that of non-irrigated plants (8.0 °Brix). According to the results in the moderate dry (2015) year, under irrigated condition the decrease in the Brix value was lower (38%) for cherry type Strombolino F_1 tomato compared to the non-irrigated plants but it did not change in the very dry (2013) year (Table 2). Lycopene and β-carotene accumulate in the final ripening stage of tomato and are responsible for the deep-red colour and β-carotene for the orange colour of the fresh fruits (Brandt et al. 2006). During this period the effect of drought stress influences the lycopene content however the results concerning lycopene synthesis are rather contradictory. Riggi et al. (2008) showed that drought stress decreased the lycopene content of fruits but Sánchez-Rodriguez et al. (2012) pointed out that the moderate water stress induced an increase in the lycopene content of tomatoes. Nevertheless, lycopene content of fruits is determined by the genetic background of cultivars. Pék et al. (2014) reported that small-fruited cherry tomatoes had higher lycopene content than large-fruited cultivars. Petrović et al. (2019) also established that independently of the water supply conditions the fruit of cherry tomato had higher total carotenoids than in larger fruits mainly due to higher content of lycopene. They also reported that the effect of drought was more favourable on the accumulation of β-carotene than on lycopene of tomatoes. The results partly confirmed this statement; in very dry year, despite of irrigation lycopene content of fruits of Strombolino F_1 tomato did not change but it was the lowest in a rainy year.

Conclusions

Strombolino F_1 processing cherry tomato responded sensitively to water stress under different weather conditions. Under water abundance few and large weighed fruits per plant were produced that had low nutritional quality including low °Brix and lycopene while the diseased yield increased significantly. In a mildly wet year the good fertility of plant is further increased by irrigation therefore the marketable yield significantly increased and the fruit nutritional quality was improved with increasing F_1 and cis lycopene and less decreased °Brix value but lycopene content of fruit decreased significantly while the amount of diseased yield increased. Contrary to smaller weighed fruits that were produced in dry years, the marketable yield was increased significantly by irrigation and their nutritional quality improved due to rising °Brix, F_1 and cis lycopene content of fruit while diseased yield was minimal.

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References

Agarwal, S., Rao, A.V. (2000): Tomato lycopene and its role in human health and chronic diseases. Canadian Medical Association Journal. 163: 6. 739-744.

Barbagallo, R.N., Di Silvestro, I., Patanè, C. (2013): Yield, physicochemical traits, antioxidant pattern, polyphenol oxidase activity and total visual quality of field-grown processing tomato cv. Brigade as affected by water stress in Mediterranean climate. Journal of Science and Food Agriculture. 93: 6. 1449-57. https://doi.org/10.1002/jsfa.5913

Battilani, A., Prieto, H., Argerich, C., Campillo C., Cantore, V. (2012): Tomato; p. 192-198. In: Steduto, P., Hsiao, T. C., Fereres, E., Raes, D. (eds.), Crop yield response to water FAO irrigation and drainage paper 66. Food and Agriculture Organization of the United Nations.

Berki, M., H.G. Daood, and L. Helyes. 2014. The influence of the water supply on the bioactive compounds of different tomato varieties. Acta Alimentaria. 43: 21–28.

Bisbis, M.B., N. Gruda, and M. Blanke. 2018. Potential impacts of climate change on vegetable production and product quality – A review. Journal of Cleaner Production. 170: 1602–1620. https://doi.org/10.1016/j.jclepro.2017.09.224

Brandt, S., Pék, Z., Barna, É., Lugasi, A., Helyes, L. (2006): Lycopene content and colour of ripening tomatoes as affected by environmental conditions. Journal of the Science of Food and Agriculture. 86: 4. 568-572. https://doi.org/10.1002/jsfa.2390

Bőcs A., Pék Z., Helyes L. (2011): Simultaneous impact of the different water supply and year type on processing tomato yield. International Journal of Horticulture Science. 17: 1-2. 79-81.

Chaudhary, P., Sharma, A., Singh, B., Nagpal, A. (2018): Bioactivities of phytochemicals present in tomato. Journal of Food Science and Technology. 55: 8. 2833-2849. https://doi.org/10.1007/s13197-018-3221-z

Coyago-Cruz, E., Meléndez-Martínez, A.J., Moriana, A., Girón, I.F., Martín-Palomo, M.J., Galindo, A., Pérez-López, D., Torrecillas, A., Beltrán-Sinchiguano, E., Corell, M. (2019): Yield response to regulated deficit irrigation of greenhouse cherry tomatoes. Agricultural Water Management. 213: 1. 212-221. https://doi.org/10.1016/j.agwat.2018.10.020

Daood, H.G., Bencze, G., Palotas, G., Pek, Z., Sidikov, A., Helyes, L. (2014): HPLC Analysis of carotenoids from tomatoes using cross-linked C18 column and MS detection. Journal of Chromatography Science. 52: 9. 985-991. https://doi.org/10.1093/chromsci/bmt139

Díaz, L.D., V. Fernández-Ruiz, and M. Cámara. 2020. An international regulatory review of food health-related claims in functional food products labeling. J. Funct. Foods 68:103896. https://doi.org/10.1016/j.jff.2020.103896

Dumas, Y., Dadomo, M., Di Lucca, G., Grolier, P. (2003): Effects of environmental factors and agricultural techniques on antioxidant content of tomatoes. Journal of the Science of Food and Agriculture. 83: 369-382. https://doi.org/10.1002/jsfa.1370

Frede, K., Ebert, F., Kipp, A.P., Schwerdtle, T., Baldermann, S. (2017): Lutein activates the transcription factor Nrf2 in human retinal pigment epithelial cells. Journal of Agricultural and Food Chemistry. 65: 29. 5944-5952. https://doi.org/10.1021/acs.jafc.7b01929

Helyes, L., Bőcs, A., Nemeskéri, E. (2018): Víztakarékos öntözés hatása az ipari paradicsom termésmennyiségére és minőségére. Kertgazdaság. 50: 4. 3-9.

Helyes L., Dimény J., Pék Z., Lugasi A. (2006): Effect of maturity stage on content, color and quality of tomato (*Lycopersicon lycopersicum* (L.) Karsten) fruit. Int. Journal of Horticultural Science 12, (1): pp. 41-44.

Helyes, L., Lugasi, A., Pék, Z. (2012): Effect of irrigation on processing tomato yield and antioxidant components. Turkish Journal of Agriculture and Forestry 36:702-709. https://doi.org/10.3906/tar-1107-9

Helyes, L., Lugasi,, A. Daood,, H. G. Pék,, Z. (2014): The Simultaneous Effect of Water Supply and Genotype on Yield Quantity, Antioxidants Content and Composition of Processing Tomatoes. Not Bot Horti Agrobo,42, (1) pp. 143-149. https://doi.org/10.15835/nbha4219396

Helyes, L., Varga, Gy. (1994): Irrigation demand of tomato according to the results of three decades. Acta Horticulturae. 376: 323-328. https://doi.org/10.17660/ActaHortic.1994.376.44

Klunklin, W., Savage, G. (2017): Effect on Quality Characteristics of Tomatoes Grown Under Well-Watered and Drought Stress Conditions. Foods. 6: 8. 56. https://doi.org/10.3390/foods6080056

Lapushner, D., Bar, M., Gilboa, N., Frankel, R. (1990): Positive heterotic effects for °brix in high Solid F1 hybrid cherry tomatoes. Acta Horticulturae. 277: 207-212. https://doi.org/10.17660/ActaHortic.1990.277.23

Lugasi, A., Hóvári, J., Bíró, L., Brandt, S., Helyes, L. (2004): Élelmiszereink likopin tartalmát befolyásoló tényezők és a hazai lakosság likopin - bevitele. [Factors influencing lycopene content of foods and lycopene intake of Hungarian population] Magyar Onkológia. 48: 2. 131-136.

Molnár, K., Víg, R., Nemeskéri, E, Dobos, A. (2012): A vízellátottság és az évjárat hatása eltérő genotípusú csemegekukorica (*Zea mays* L. *convar. saccharata* Koern.) hibridek termőképességére. [Effect of water supply and year on the productivity of different sweet corn genotypes (*Zea mays* L. *convar. saccharata* Koern.)] Agrártudományi Közlemények. 50: 203-210.

Nemeskéri, E., Molnár, K., Dobos, A. Cs. (2015): Különböző tenyészidejű borsófajták (*Pisum sativum* L.) vízhasznosítása eltérő vízellátás alatt. [Water use efficiency of pea varieties (*Pisum sativum* L.) of different maturity in the case of different water supply] Növénytermelés. 64: 1. 57-76.

Nemeskéri, E., Molnár, K., Pék, Z., Helyes, L. (2018): Effect of water supply on water use related physiological traits and yield of snap beans in dry seasons. Irrigation Science. 36: 3. 143-158. https://doi.org/10.1007/s00271-018-0571-2

Petrović, I., Savić, S., Jovanović, Z., Stikić, R., Brunel, B., Sérino, S., Bertin, N. (2019): Fruit quality of cherry and large fruited tomato genotypes as influenced by water deficit. Zemdirbyste-Agriculture. 106: 2. 123-128 https://doi.org/10.13080/z-a.2019.106.016

Pék, Z., Daood, H., Gasztonyi Nagyné, M., Neményi, A., Helyes, L., (2013): Effect of environmental conditions and water status on the bioactive compounds of broccoli. Central European Journal of Biology, 8 (8) pp. 777-787. https://doi.org/10.2478/s11535-013-0172-7

Pék, Z., Daood, H., Nagyné, M.G., Berki, M., Tóthné, M.M., Neményi, A., Helyes, L. (2012): Yield and phytochemical compounds of broccoli as affected by temperature, irrigation, and foliar sulfur supplementation. HortScience, 47 (11), pp. 1646-1652. https://doi.org/10.21273/HORTSCI.47.11.1646

Pék, Z., Szuvandzsiev, P., Daood, H., Neményi, A., Helyes, L. (2014): Effect of irrigation on yield parameters and antioxidant profiles of processing cherry tomato. Central European Journal of Biology 9: 383-395. https://doi.org/10.2478/s11535-013-0279-5

Pék, Z., Szuvandzsiev, P., Neményi, A., Helyes, L. (2015): Effect of season and irrigation on yield parameters and soluble solids content of processing cherry tomato. Acta Horticulturae. 1081:197-202. https://doi.org/10.17660/ActaHortic.2015.1081.24

Pék, Z., Szuvandzsiev, P., Neményi, A., Tuan, L.A., Bakr, J., Nemeskéri, E., Helyes, L. (2019): Comparison of a water supply model with six seasons of cherry type processing tomato. Acta Horticulturae. 1233: 1. 41-46. https://doi.org/10.17660/ActaHortic.2019.1233.7

Riggi, E., Patane, C., Ruberto, G. (2008): Content of carotenoids at different ripening stages in processing tomato in relation to soil water availability. Australian Journal of Agricultural Research. 59: 348-353. https://doi.org/10.1071/AR07215

Ripoll, J., Urban, L., Staudt, M., Lopez-Lauri, F., Bidel, L., Bertin, N. (2014): Water shortage and quality of fleshy fruits-making the most of the unavoidable. Journal of Experimental Botany. 65: 4097-4117. https://doi.org/10.1093/jxb/eru197

Rocha, MC., Deliza, R., Corrêa, FM., do Carmo, MGF., Abboud, ACS. (2013): A study to guide breeding of new cultivars of organic cherry tomato following a consumer-driven approach.

Food Research International. 51: 265-273. https://doi.org/10.1016/j.foodres.2012.12.019

Sánchez-Rodríguez, E., Ruiz, J.M., Ferreres, F., Moreno, D.A. (2012): Phenolic profiles of cherry tomatoes as influenced by hydric stress and rootstock technique. Food Chem. 134: 775-782. https://doi.org/10.1016/j.foodchem.2012.02.180

Sass-Kiss, A., Kiss, J., Milotay, P., Kerek, M.M., Tóth-Markus, M. (2005): Differences in anthocyanin and carotenoid content of fruits and vegetables. Food Research International. 38: 8-9.1023-1029. https://doi.org/10.1016/j.foodres.2005.03.014

Spinoni, J., S. Szalai, T. Szentimrey, M. Lakatos, Z. Bihari, A. Nagy, Á. Németh, T. Kovács, D. Mihic, M. Dacic, P. Petrovic, A. Kržič, J. Hiebl, I. Auer, J. Milkovic, P. Štepánek, P. Zahradnícek, P. Kilar, D. Limanowka, R. Pyrc, S. Cheval, M.V. Birsan, A. Dumitrescu, G. Deák, M. Matei, I. Antolovic, P. Nejedlík, P. Štastný, P. Kajaba, O. Bochnícek, D. Galo, K. Mikulová, Y. Nabyvanets, O. Skrynyk, S. Krakovska, N. Gnatiuk, R. Tolasz, T. Antofie, and J. Vogt. 2015. Climate of the Carpathian Region in the period 1961-2010: Climatologies and trends of 10 variables. Internatioanl Journal of Climatology. 35: 1322–1341. https://doi.org/10.1002/joc.4059

Steiner, B.M., McClements, D.J., Davidov-Pardo, G. (2018): Encapsulation systems for lutein: A review. Trends in Food Science and Technology. 82:71-81. https://doi.org/10.1016/j.tifs.2018.10.003

Effect of tillage and fertiliser treatments on yield of maize (*Zea mays* L.) hybrids

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Abstract: This research was conducted in a spilt-plot design at the University of Debrecen Látókép Research Station, site (N 47°33’ E 21°27’) in 2015 and repeated in 2016. There were three main plots, each 1.0 ha in size which represents the tillage treatments: moldboard plowing (MT), strip tillage (ST) and ripper tillage (RT). Maize hybrids, Loupiac (FAO 380) and Armagnac(FAO 490) were sown at 80,000 plants ha^{-1} with a row spacing of 76 cm in the main plots which were subdivided to accommodate three fertiliser treatments (N_0 kg ha^{-1} (control); N_{80} kg ha^{-1}; N_{160} kg ha^{-1}) with four replications. The hybrids were harvested at the end of the growing cycle with a Sampo 2010 plot harvester and the grain moisture content was computed at 15% moisture to arrive at the final yield. The findings revealed RT produced the highest yield of 10.37 t ha^{-1}, followed by MT and ST with 10.22 and 9.60 t ha^{-1} respectively. There was no significant difference($p>0.05$) in yield between the RT and MT treatments. However, both the RT and MT were found to be statistically significant ($p<0.05$) when compared to ST treatment. In 2015, a relatively dry year, yield of ST plots were not significantly different compared to MT and RT plots. A positive interaction between tillage and fertilisation was evident, with higher yield variation (CV=40.07) in the non-fertilised (N_0) tillage plots, compared to those which received the N_{80} and N_{160} kg ha^{-1} treatments (CV=22.42). Fertilizer application greatly increased the yield of maize and accounted for 43% of yield variances. The highest yield (11.88 t ha^{-1}) was obtained with N_{160} kg ha^{-1} treatment, followed by N_{80} kg ha^{-1} (10.83 t ha^{-1}), while the lowest yield (7.48 t ha^{-1}) was recorded in the nonfertilised plots(N_0 kg ha^{-1}). Year effect was highly significant with vast variation in yield between the two years, ranging from 8.36 t ha^{-1} in 2015 to 12.43 t ha^{-1} in 2016 for the same set of agrotechnical inputs. In 2016, higher yield was obtained with increase fertiliser dosage due to favourable growing condition which allowed for better fertiliser utilisation. However, with 2015 being a relatively dry year there was no yield increasing effect with higher fertiliser dosage (N_{160} kg ha^{-1}). Loupiac (FAO 380) was the better performing hybrid, with a yield of 11.09 t ha^{-1} compared Armagnac (FAO 490) with 10.60 t ha^{-1} . The adaptability traits of the two hybrids appears very similar, since the yield differential between the two hybrids was almost constant (0.48 vs 0.49) in both years , despite the vast variation in weather condition.

Keywords: maize; mouldboard; strip tillage; ripper tillage; fertiliser; hybrid; year effect

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Introduction

In an ever-changing world, improving and achieving sustainable yield requires continuous evaluation of production technologies in order to determine the best combination of inputs which will optimise yield for a given situation. Maize (*Zea mays* L.), is major grain crop in Hungary, cultivated on approximately one million hectares. Besides being an excellent feed source, maize is also a cheap source of energy and raw material for industry (Nagy, 2006a). Annual production over the last decade ranged from 4.8 to 9.3 million tons, with significant fluctuation in yield (Hungarian Central Statistical Office, 2018).

Year effect significantly influences the size of yield of maize and in particular, the amount and distribution of precipitation during the growing season, coupled with the temperature during the winter (Nagy, 2003; Széles et al. 2013). Harmonization of the agroecological (weather and soil), biological bases and agrotechnical factors (crop rotation, nutrient supply, soil cultivation, sowing, plant care, irrigation, harvest, etc.) is critical for optimization and reduction of fluctuation in the yield of maize (Nagy, 2003; Berzsenyi,

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2010; Berzsenyi et. al. 2011; Sárvári and Bene, 2012; Pepó and Csajbók, 2013).

Tillage is central to the agrotechnical factors which modifies soil structure by changing its physical properties, such as soil moisture content, bulk density and penetration resistance. These changes in soil physical properties, as a result of different tillage practices, influenced seedling emergence, plant population density, root distribution and crop yield (Khan et al., 2001; Iqbal, et al., 2013; Khurshid et al., 2006; Rashidi and Keshavarzpour, 2007).

The effects of basic soil tillage are largely modified yearly by the various levels of water supply (Nagy, 2006a). Under extremely dry conditions the final yield of maize was significantly affected by the soil tillage system (Kristo et al., 2013; Memon et al., 2012). Soil tillage systems had different effects on the preservation of the soil moisture contents, which significantly affected maize yield (Simić et al. 2009) and the most important goals of tillage include preserving the favourable soil attributes and alleviating circumstances leading to detrimental processes (Birkás, 2010).

In Hungary, changing climatic condition and increase frequency of drought years are paving way for alternative tillage methods which can conserve on soil moisture, minimise soil erosion and optimize yield (Birkás, 2015). Mouldboard plowing has been the common tillage practice in Hungary in maize production technology. It provides good depth and improves the water storage capacity of the soil (Nagy,2006a). However, this tillage practice offers minimal soil surface protection with crop residue and has high potential for moisure loss and soil erosion (Birkás, 2010).

Strip tillage (ST) and ripper tillage (RT) are two alternatives which have been identified for evaluation in this research. Unlike the moldboard plow , strip and ripper implements do not completely invert the soil and leaves a higher percentage of the soil surface covered by crop residue. The potential for erosion and moisture loss is therefore considerably less than that of moldboard plowing. Besides, ripper tillage reportedly loosens more soil, compared to conventional and zero tillage and allows for greater aeration and water retention capacity, which are favourable for plant growth (Memon et al. 2012).

Rátonyi et al.(2014) reported higher moisture retention in the soil profile of ST and RT in Hungary, compared to the conventional MT tillage and posited that maize yield in ST treatment can reach similar level as yields of maize in conventional MT treatment on chernozem soil in Hungary. On sites with root-restricting soil layers, deep tillage effects were 20% higher than at sites without such layers (Kuhlmann et al., 1989; Kirkegaard et al., 2007). Successful deep tillage requires soil water content to be below the plastic limit from the topsoil to the maximum tillage depth (Borchert, 1975; Eck and Unger, 1985). In addition to appropriate selection of tillage operations, the improvement in average yield per hectare can be obtained if soil fertility is maintained through proper dose, application method and use of organic and inorganic fertilizers (Mermon et al. 2012). Under favourable conditions fertilisation could improve yield up to 50%, however in excessively dry years, it does not have any yield increasing effect (Pepó, 2007).

The findings of this research will add to the body of knowledge gained from similar research and will serve as an effective tool to analyse trends, generate model and predict the most suitable crop production technologies which can be applied, in order to reduce fluctuation in yields and achieve production sustainability.

Table 1. Monthly temperature(°C) for the examined period (2015-2016).

Year	Temperature (°C)											
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
2015	1.69	2.00	6.84	11.19	16.10	20.31	23.38	23.33	17.77	10.44	7.06	2.61
2016	-0.64	5.99	7.15	12.88	16.35	21.15	22.50	20.76	18.55	9.86	5.26	-0.38
30-year mean	-2.6	0.2	5.0	10.7	15.8	18.7	20.3	19.6	15.8	10.3	4.5	-0.2

Materials and Methods

The experiment was conducted at the Látókép Research Station Site (N 47°33’ E 21°27’) of the University of Debrecen in 2015 and repeated in 2016. The soil type was calcareous chernozem soil, consisting of 11% sand, 65% silt and 24% clay in the upper soil layers , with a near neutral pH value (pH_{KCl}=6.46). It has a humus content of 2.8% and humus depth of approximately 80 cm, with good water holding capacity.

The experiment was set up in a split-plot design with three main plots, each 1.0 ha in size (37 × 272 m) which represents the tillage treatments: Moldboard plowing (MT) – 30 cm depth; Strip tillage (ST) – 30 cm depth; Ripper tillage (RT) – 45 cm depth. The main plots were subdivided to accommodate three fertiliser treatments (N_0 kg ha^{-1} (control); N_{80} kg ha^{-1}; N_{160} kg ha^{-1}) with four replications.

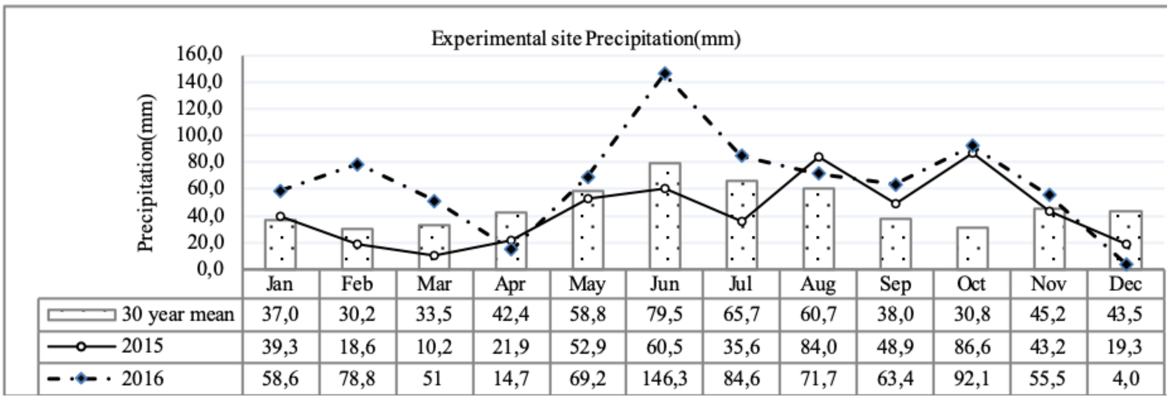
Maize hybrids, Loupiac (FAO 380) and Armagnac (FAO 490) were sown with 80,000 plants ha^{-1} with a row spacing of 76 cm. The crop was harvested at the end of the growing cycle with a Sampo 2010 plot harvester and the grain moisture content was adjusted to 15% moisture to arrive at the final yield. Yield data were analyzed using IBM SPSS-26.0 and treatment means were compared using the Fisher’s least significant difference (LSD) test (p<0.05).

Monthly temperature (Table 1) and precipitation (Figure 1) and were recorded for the period 2015-2016 and compared to the 30 year mean. Precipitation during the vegetative period (April to September) was 303.8 mm and 449.9 mm for 2015 and 2016 respectively, against a 30 year mean of 345.1 mm.

Results and Discussion

Cropyear interaction was highly significant with vast variation in yield between the two years, ranging from 8.36 t ha^{-1} in 2015 to 12.43 t ha^{-1} in 2016 for the same set of agrotechnical inputs. Analysis of the meteorological data for the two years (2015 & 2016), revealed major differences, especially the amount and distribution of rainfall during the growing season (April-Sept). According to Nagy (2006a) the most critical period of the growing season in Hungary is between flowering and fertilization (15th July – 15th August) and a period of 3 to 4 days of severe moisture stress at this time can easily reduce final grain yields by 30 percent (Lamm, 2003).

Rainfall in 2015 (303.8 mm) was below the 30-year mean (345.1mm) and specifically in the month of July when the reproductive phase commenced (Figure 1). Unlike 2015, there was adequate rainfall throughout the growing season of 2016, as a result, the yield gain in 2016 was 4.07 t ha^{-1}. Significant effects of the year on the yield and its components were observed very often in long-term field studies due to differences in precipitation and grow degree days accumulation during the vegetative period of maize (Wilhelm



	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
▭ 30 year mean	37,0	30,2	33,5	42,4	58,8	79,5	65,7	60,7	38,0	30,8	45,2	43,5
—○— 2015	39,3	18,6	10,2	21,9	52,9	60,5	35,6	84,0	48,9	86,6	43,2	19,3
- ·◆- · 2016	58,6	78,8	51	14,7	69,2	146,3	84,6	71,7	63,4	92,1	55,5	4,0

Figure 1. Monthly precipitation for the examined period (mm) (Debrecen 2015-2016).

and Wortmann, 2004; Boomsma et al. 2010). Weather regulates heat and moisture supply of the crop environment and therefore has an effect on material transformation, fertilizer efficiency and nutrient uptake by plants (Kovács, 1982; Nagy, 1996).

Based on results of long-term experiment, Nagy, (2006b) observed that higher yields were always accompanied by higher precipitation but low yield was not always accompanied by the lowest amount of precipitation. It was evident that the effect of the year (Figure 2) and fertilization (Table 2) on yield of maize was highy significant compared to tillage.

The effect size of tillage on yield of maize was 2.2% for the examined period(2015-2016), compared to fertilizer and year effect with 43.6 and 53.4% respectively. Among the three tillage treatments, ripper tillage (RT) had the highest average yield (10.37 t ha^{-1}) followed by mouldboard plowing (MT) and strip tillage(ST) with 10.22 and 9.60 t ha^{-1}, respectively. Yield difference between RT and MT was not statistically significant (p>0.05), as compared to ST (Table 3).

Although the average yield of strip tillage(ST) was lowest over the two-year period, in 2015, a relatively dry year, the yield of ST treatment (8.27 t ha^{-1}) varied less than 2% when compared to the MT (8.41 t ha^{-1}) and RT(8.42 t ha^{-1}) treatments. It is evident that yield of maize in ST can reach comparable level as maize in the conventional MT, especially under drier condition. Roger et al. (2007) reported that in persistently dry conditions, strip-tilled maize performed better than maize planted with conventional tillage because of better soil moisture conservation. Similar observations were made by Birkás (2010) and Ratónyi et al. (2014).

According to Nagy (1996) & Fenyves (1997) the depth and method of tillage do not significantly influence the yield of maize on well structured soil. It can be inferred based on the results of our tillage treatments, that soil at the experimental site possess good attributes and may not be the ideal location to evaluate the effectiveness of various tillage treatments. Schneider et al., (2014) , found that the yield gains of deep tillage were strongly dependent on site-specific condition and sites with root-restricting soil layers, deep tillage effects were generally 20% higher than at sites without such layers.

A holistic approach will be needed when selecting the most appropriate tillage method since fuel consumption , machine productivity, time and labour are all likely to be varied, inaddition to the yield, and therefore the method with the highest yield gain may not

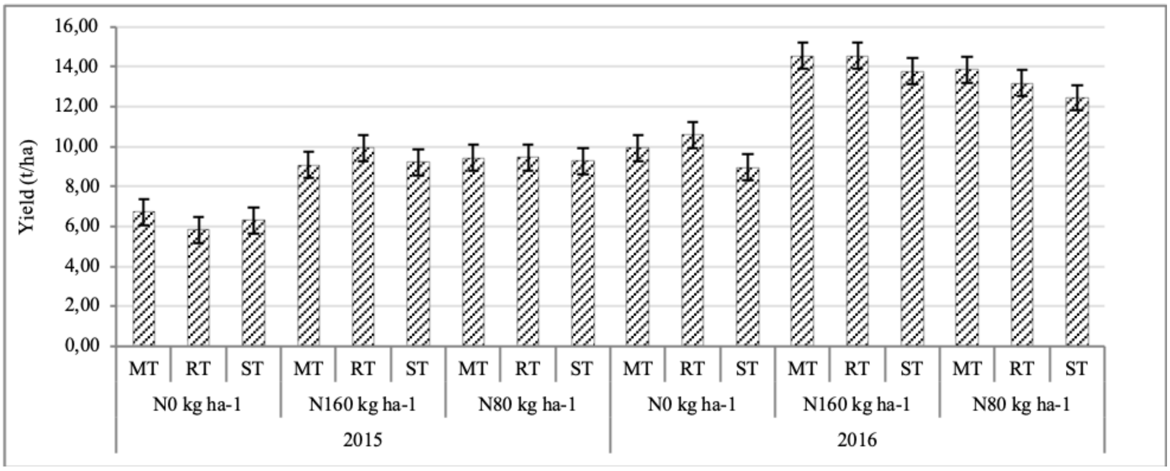


Figure 2. Yield of maize for the tillage and fertiliser treatments (2015-2016) (MT-Mouldboard tillage; RT-Ripper tillage; ST-Strip tillage).

Table 2. Analysis of variance: Tests of Between-Subjects Effects.

Dependent Variable: Yield

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	6255.982[a]	35	178.742	48.380	.000
Intercept	93539.493	1	93539.493	25318.145	.000
Year	3484.418	1	3484.418	943.120	.000
Fertilization	2526.592	2	1263.296	341.934	.000
Tillage	52.366	2	26.183	7.087	.001
Hybrid	10.991	1	10.991	2.975	.085
Year * Fertilization	60.586	2	30.293	8.199	.000
Year * Tillage	44.888	2	22.444	6.075	.002
Year * Hybrid	4.260	1	4.260	1.153	.283
Fertilization * Tillage	13.674	4	3.418	.925	.449
Fertilization * Hybrid	3.359	2	1.679	.455	.635
Tillage * Hybrid	5.975	2	2.988	.809	.446
Year * Fertilization * Tillage	30.408	4	7.602	2.058	.085
Year * Fertilization * Hybrid	.558	2	.279	.076	.927
Year * Tillage * Hybrid	11.290	2	5.645	1.528	.218
Fertilization * Tillage * Hybrid	1.614	4	.404	.109	.979
Year * Fertilization * Tillage * Hybrid	5.001	4	1.250	.338	.852
Error	3059.099	828	3.695		
Total	102854.574	864			
Corrected Total	9315.081	863			

a. R squared = .672 (Adjusted R squared = .658)

necessarily be the most economical one. It is the expressed views of several researchers, that the effects of tillage systems on yield cannot be evaluated on the basis of a single season and that long-term experiments of many years are required.

Table 3. Analysis of variance: Tests of Between-Subjects Effects.

Dependent Variable: Yield
LSD

(I) Tillage	(J) Tillage	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
MT	ST	.60*	.130	.000	.35	.86
	RT	.00	.130	.995	-.25	.26
ST	MT	-.60*	.130	.000	-.86	-.35
	RT	-.60*	.130	.000	-.86	-.35
RT	MT	.00	.130	.995	-.26	.25
	ST	.60*	.130	.000	.35	.86

Based on observed means.
The error term is Mean Square(Error) = 3.660.
*. The mean difference is significant at the .05 level.

Table 4. Analysis of variance - Fertilizer treatments.

Dependent Variable: Yield
LSD

(I) Fertilization	(J) Fertilization	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
0 kg N/ha	80 kg N/ha	-3.22*	.130	.000	-3.48	-2.97
	160 kg N/ha	-3.79*	.130	.000	-4.05	-3.54
80 kg N/ha	0 kg N/ha	3.22*	.130	.000	2.97	3.48
	160 kg N/ha	-.57*	.130	.000	-.83	-.32
160 kg N/ha	0 kg N/ha	3.79*	.130	.000	3.54	4.05
	80 kg N/ha	.57*	.130	.000	.32	.83

Based on observed means.
The error term is Mean Square(Error) = 3.660.
*. The mean difference is significant at the .05 level.

Fertilizer application significantly increased the yield of maize and accounted for 43% of yield variances. Yield differences between fertilizer treatments was highly significant (Table 4). The highest yield (11.88 t ha^{-1}) was obtained with N_{160} kg ha^{-1} treatment, followed by N_{80} kg ha^{-1} (10.83 t ha^{-1}), while the lowest yield (7.48 t ha^{-1}) was recorded in the nonfertilised plots(N_0 kg ha^{-1}).

A positive interaction between tillage and fertilisation was observed, with higher yield variation (CV=40.07) in the non-fertilised (N_0) tillage plots, compared to those which received the N_{80} and N_{160} kg ha^{-1} treatments (CV=22.42). Similar observations were made by Győrffy, (1976) who postulated that effects of tillage depth as well as the number of interventions are reduced or compensated for by fertilization.

In 2016, higher yield was obtained with increase fertiliser dosage due to favourable growing condition which allowed for better fertiliser utilisation. However, 2015 being a relatively dry year with less than optimum water supply, there was no yield increasing effect with higher fertiliser dosage (N_{160} kg ha^{-1}). Similar observation was made by

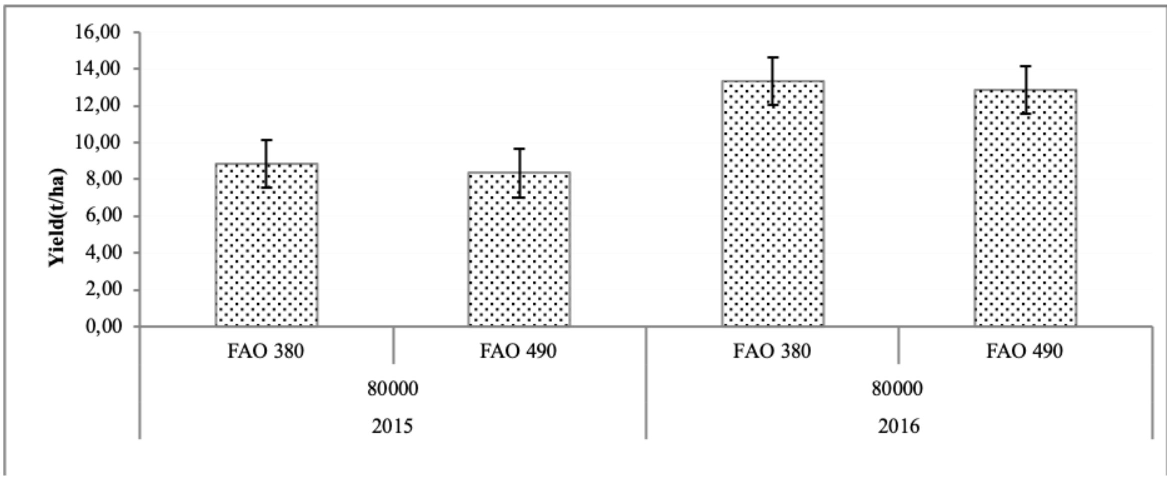


Figure 3. Yield of maize hybrids for the three fertiliser treatments (2015-2016).

Nagy (2006a, 2007) who concluded that a positive correlation exists between fertiliser and water suppy and both factors must be increased or decreased simulataneously inorder to realise optimum benefits. According to Berzsenyi & Dang (2003) in dry years, lower fertiliser dosage had higher stability while in wet years higher fertiliser dosage results in more stable yield.

Maize has high productivity, but it is very sensitive to the agroecological and agrotechnical conditions. When these conditions are optimal, the amount of yield is determined by the differences between the hybrids; but in the case of unfavourable weather conditions or shortcomings in the agrotechnique, the most important factor is the adaptability of the hybrids (Gardner et al. 1990; Marton et al. 2005).

Loupiac (FAO 380) was the better performing of the two hybrids, with an average yield of 11.09 t ha^{-1} compared Armagnac (FAO 490) with 10.60 t ha^{-1} . The adaptability traits of the two hybrids appears to be very similar, since the yield differential between the two hybrids was almost constant (0.48 vs 0.49) in both years , despite the vast variation in weather condition (Figure 3).

Conclusions

Loupiac (FAO 380) was the better performing hybrid compared to Armagnac (FAO 490). Lower dosage of fertiliser produced optimum results in drier year with limited water supply. Ripper tillage and strip tillage can be suitable alternatives for the conventional mouldboard tillage, especially in drier conditions. The best combination of treatments for optimum yield was Lopiac (FAO 380), cultivated under rip tillage (RT) with N_{80} kg ha^{-1} fertiliser.

Acknowledgements

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References

Berzsenyi, Z. (2010): Significance of the 50-year-old Long-term Experiments in Martonvasar. Acta Agronomica Hungarica, 58(Suppl.), pp. 23–34 (2010) https://doi.org/10.1556/AAgr.58.2010.Suppl.1.4

Berzsenyi, Z., Dang, Q. L. (2003): Effect of N-fertilisation on the grain yield and N-fertiliser response of maize (*Zea mays* L.) Növénytermelés, 52, 389-408.

Berzsenyi, Z., Arendas,T., Bonis, P., Micskei, G., Sugár, E.(2011): Longterm effect of crop production factors on the yield and yield stability of maize (*Zea mays* L.) in different years. Acta Agronomica Hungarica. 2011. 59: 3, 191-200. p. https://doi.org/10.1556/AAgr.59.2011.3.1

Birkás, M. (2010). Long-term experiments aimed at improving tillage practices. Acta Agronomica Hungarica. 58. 75-81. https://doi.org/10.1556/AAgr.58.2010.Suppl.1.11

Birkás, M., Kisic, I., Mesic, M., Jug, D., Kende Z.(2015) Climate induced soil deterioration and methods for mitigation. Agriculturae Conspectus Scientificus, 80 (1), 17. https://hrcak.srce.hr/151055

Boomsma, C.R., Santini, J. B., West, T.D., Brewer, J. C., McIntyre, L.M., Vyn, T.J. (2010): Maize grain yield responses to plant height variability resulting from crop rotation and tillage system in long-term experiment. Soil and Tillage Research, 106: 227-240. https://doi.org/10.1016/j.still.2009.12.006

Borchert, H.(1975): Relations of soil loosening effect to soil structure and Climate. German Soil Science Society 22, 201-206.

Eck, H.V., Unger, P.W.(1985): Soil profile modification for increasing crop production. In:Stewart, B.A. (Ed.), Advances in Soil Science. Springer, New York, pp. 65–100.

Fenyves,T. (1997): Effect of soil cultivation and fertilization on soil status and maize yields on a brown forest soil in Gödöllő. Növénytermelés, 46:289-298.

Gardner, F.P., Valle, R., McCloud, D.E. (1990): Yield characteristics of ancient races of maize compared to a modern hybrid. Agron. J., 82: 864-868 p. https://doi.org/10.2134/agronj1990.00021962008200050003x

Győrffy, B. (1976): Evaluation of maize yield in crop production factors. Releases of Agricultural Sciences. 35: 239-266.

Hungarian Central Stats Office (2018): www.ksh.hu. (2019.01.16.)

Iqbal, M., Khan, A. G., Hassan, A., Islam, K. R. (2013): Tillage and Nitrogen Fertilization Impact on Irrigated Corn Yields, and Soil Chemical and Physical Properties Under Semiarid Climate. Journal of Sustainable Watershed Science & Management 1 (3): 90-98. https://doi.org/10.5147/jswsm.v1i3.140

Khan, F.U.H., Tahir, A.R., Yule, I.J. (2001): Intrinsic implication of different tillage practices on soil penetration resistance and crop growth. Int. J. Agric. Biol., 1: 23–6.

Khurshid, K., Iqbal, M., Arif, M. S., Nawaz, A.(2006): Effect of tillage and mulch on soil physical properties and growth of maize. Int. J. Agri. Biol., 8: 593-596.

Kirkegaard, J.A., Lilley, J.M., Howe, G.N., Graham, J.M.(2007): Impact of subsoil water use on wheat yield. Aust. J. Agric. Res. 58, 303–315. https://doi.org/10.1071/AR06285

Kovác, G. J. (1982): Critical ecophysical relationship of water and nutrient dynamics of maize. Növénytermelés, 31. 4. 355- 365.

Kristo, I., Matusek, N., Máte, I. (2013): Effect of cultivation systems on maize productivity and production profitability. Review on Agriculture and Rural Development 2013. vol. 2. (1) 28-32 pp.

Kuhlmann, H., Barraclough, P.B., Weir, A.H.(1989): Utilization of mineral nitrogen in the subsoil by winter wheat. Zeitschrift für Pflanzenernährung und Bodenkunde 152: 291–295. https://doi.org/10.1002/jpln.19891520305

Lamm F.R., Aiken, R.M., Abou, A.A. (2009): Corn yield and water use characteristics as affected by tillage, plant density, and irrigation. Transactions of the ASABE, 52: 133–143. https: //doi.org/10.13031/2013.25954

Lamm, F.R., (2003): Corn Production in the Central Great Plains as Related to Irrigation Capacity. Proceedings of the Central Plains Irrigation Conference. 151-165 p.

Marton L., Szundy, T., Hadi, G., Pintér, J. (2005): Practical results of the selection to improve the adaptability of maize In.: Adaptability and crop safety of maize hybrids, Debrecen, 139-146 p.

Memon S.Q., Mirjat, M.S., Mughal, A. Q., Amjad, N. (2012): Effects of different tillage and fertilizer treatments on growth and yield components of maize. Pak. J. Agri., Agril. Eng., Vet. Sci., 2012, 28 (2): 160-176.

Nagy, J.(2007): Evaluating the effect of year and fertilisation on the yield of mid ripening (FAO 400-499) maize hybrids. Cereal Research Communations.2007. 35.3. 1497-1507.

Nagy, J.(1996): Evaluating the effect of soil cultivation and fertilization on the yield of maize (*Zea mays* L.). Agrokémia és Talajtan, 45. 1-2. 113-124.

Nagy, J.(2003): Effect of Irrigation on Maize Yield (*Zea mays* L.). Acta Agraria Debreceniensis, 1-6.

Nagy, J.(2006a): Maize Production. Akadémiai Kiadó, Budapest.

Nagy, J.(2006b): Evaluation of the effect of crop year on maize yield. Novenytermeles 55(5):299-308.

Pepó, P.(2007): Evaluation of ecological conditions and agrotechnical elements in maize (*Zea mays* L.) production. Acta Agronomica Ovariensis. 2007. 49: 2(1), 169-175. p.

Pepó, P., Csajbók,J.(2013): Integrated Crop Production 1. University of Debrecen. https://www.tankonyvtar.hu.(2019.02.20.)

Rashidi, M., and F. Keshavrzpour. 2007. Effect of different tillage methods on grain yield and yield components of maize (*Zea mays* L.). Intl. J. Agric. Biol., 9 (2): 274-277.

Rátonyi T., Ferencsik S., Sulyok D., Hagymássy Z., Harsányi E. (2015). Conventional and conservation tillage effects on soil conditions and yield of maize. Növénytermelés, 64:(Suppl.) 27-30.

Rogers, D., Leikam, D., Mengel, D. (2007): Corn Production Handbook. Kansas State University Agricultural Experiment Station and Cooperative Extension Service. Accessed: March, 10th , 2019: http://www.ksre.ksu.edu/bookstore/pubs/c560.pdf

Sárvári, M., Bene, E. (2012): Maize production factors. Hungarian agriculture 67 (18): 18-21p.

Schneider, F., Don, Axel., Hennings, I., Schmittmann, O., Seidel, S. (2014). The effect of deep tillage on crop yield – What do we really know? Soil and Tillage Research. 174: 193-204. https://doi.org/10.1016/j.still.2017.07.005

Simić, M., Videnović, Ž., Dolijanović, Ž., Jug, D, Dumanović, Ž. (2009): Maize growing under different tillage systems. 2nd International Scientific/Professional Conference, Agriculture in Nature and Environment Protection, Vukovar, 4th - 6th June, 62-67.

Széles, A., Ragán, P., Nagy, J. (2013): The effect of natural water supply and fertilization on maize (*Zea mays* L.) yield in the case of different crop year. Növénytermelés. 62:Suppl. 119-122 p.

Wilhelm, W.W., Wortmann, C.S.(2004): Tillage and rotation interactions for corn and soybean grain yield as affected by precipitation and air temperature. Agronomy Journal, 96: 425-432. https: //doi.org/10.2134/agronj2004.4250

OBITUARY

Attila BARCZI
(1964–2020)

Dr. Attila Barczi, habilitated associate professor of the Department of Nature Conservation and Landscape Management at the Faculty of Agriculture and Environmental Sciences of Szent István University; editor of Columella - Journal of Agricultural and Environmental Sciences, died unexpectedly on March 14 2020, at the tragically young age of 56.

Dr. Barczi was born in 1964 in Budapest. He completed his primary and secondary schools in Siófok. He began his university studies in 1983 at the József Attila University, Department of Biology, and went on to Gödöllő University of Agricultural Sciences in 1985. He started his work as a graduate agricultural engineer in 1989 at the Gödöllő University of Agricultural Sciences (later: Szent István University).

To further develop his professional knowledge, he obtained a degree in soil science and soil fertility on post-graduate engineering (1991) and one in agricultural-environmental engineering (1995). He defended his doctoral thesis (PhD) in 1997 with highest honors, and habilitated in 2004.

He began to work for the university at the Department of Soil science and Agro-chemistry as a staff member, then continued as a junior assistant professor and later as an assistant professor. He then became an associate professor at the Department of Landscape Ecology (later the Department of Nature Conservation and Landscape Ecology), where he oversaw the work of the department for several terms as head of department. During his rich professional career, he was, among others, the president of the Scientific Students' Associations at the university, the developer and supervisor of several study programs, the acting head of the Doctoral School of Environmental Sciences, and vice-dean for science and research of the Faculty of Agriculture and Environmental Sciences.

Dr. Barczi's subjects, taught in Hungarian and English, played a significant role in all levels of education, including undivided, BSc and MSc programs, post-graduate engineering and doctoral programs. His courses were primarily related to soil science and soil conservation, Hungarian landscapes and landscape management, as well as knowledge on landscape ecology. In his educational activities, he always considered field education tasks, field practical training and complex field landscape analyses to be of paramount importance. As a guest lecturer, he participated in the training of several Hungarian colleges and universities. He also engaged in significant science propagation activities, was frequently invited to speak at farmer forums, events and exhibitions, undertook editorial work in TV programs such as Telecke and Heuréka, regularly talked on professional programs, and undertook professional set of several educational films. The hallmarks of his teaching work lay in the high number and excellent performance of students who wrote their undergraduate, graduate or doctoral thesis under his supervision, while his outstanding talent management activity with Scientific Students' Associations is characterized by national first places, ministerial diplomas, and special awards. In recognition of his teaching and university work, he was eight-time winner of the Gold Medal for Best Performer awarded by students, while his work with Scientific Students' Associations was honored with a commemorative medal, the Páter Károly

Memorial Medal, the SZIE Laurel Wreath Gold Degree and the SZIE Silver Medal.

His research activities were extremely versatile. In addition to the examination and qualification of organic manures, compost and humus materials, he also dealt with agricultural land suitability studies, natural and agricultural landscape analyses, as well as landscape and agricultural assessments. Dr. Barczi's complex approach in education was most prominently evident in landscape analyses – which, based on information in geology, geography, biology, soil science, landscape history, land use and landscape processes – helped to perform sustainable land use and landscape conservation tasks and to plan farming activities that are aligned with landscape conditions. Investigations of the interaction of soil types and soil-forming factors, soil-plant correlation analyses and the study of the time of soil formation were also part of his work, as were soil compaction research, the effects of agricultural and industrial use on soils, and monitoring the effectiveness of recultivation procedures. His research on soil protection initiatives, his erosion databases and his studies on soil degradation were not only currently relevant, but also forward-looking by international standards. His research of the buried soil layers of kurgans and their paleo-ecological and paleosol properties extended over the Carpathian Basin to other Eastern European mounts, which contributed to his international recognition and the expansion of his scientific relations.

The results of his scientific work were published in more than 75 Hungarian and international scientific journal articles, 5 books and 22 book excerpts, 27 professional studies, 55 domestic and international conference volumes and 95 conference abstracts, 22 textbooks, textbook chapters and notes, 25 science propagation publications and 16 works. The recognition of his publications is also indicated by more than 500 independent citations.

Written and edited with scientific rigor but also with a view to popularize, Attila Barczi's colorful educational booklets on the landscapes of Hungary will help theoretical and practical training for many years to come.

During his professional career, he participated in 21 research proposals as a leader or participant. He was a member and official of several Hungarian and international scientific societies, as well as the founder and editor-in-chief of the scientific journal Tájökológiai Lapok, and also helped the work of the editorial boards of several domestic and international journals. The recognition of his research activities was marked by conference awards and excellent research scholarships (Széchenyi and Bolyai Scholarships).

In him, we have lost an erudite, open-minded, professionally recognized, very popular educator, a kind and helpful man and a good friend.

Source of the graphics

Front cover:
Gallo-Roman harvesting machine, called Vallus. Source: U. Troitzsch - W. Weber (1987): Die Technik : Von den Anfangen bis zur Gegenwart

Rear cover:
Portrait of Columella, in Jean de Tournes, Insignium aliquot virorum icones. Lugduni: Apud Ioan. Tornaesium 1559. Centre d'Études Supérieures de la Renaissance - Tours

HELTAI Miklós, editor-in-chief

DSc /wildilfe biology and management/, dean of the the Faculty of Agricultural and Environmental Sciences of the Szent István University, Gödöllő, Hungary, Deputy Director of the Institute for Wildlife Conservation, member of the Committee on Forestry and the Sub-Committee on Wildlife Management of the Hungarian Academy of Sciences. Professional fields: wildliffe biology and management, urban wildlife management, monitoring and management of meso and large carnivores, wildlife habitat management.

Lucius Junius Moderatus Columella

(AD 4 – 70) is the most important writer on agriculture of the Roman empire. His De Re Rustica in twelve volumes has been completely preserved and forms an important source on agriculture. This book was translated to many languages and used as a basic work in agricultural education until the end of the 19th Century.